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The Influence of Fruticose Soil Lichens Upon the Mycorrhizae and Seedling Growth of Forest Trees

Jäkälien vaikutuksesta puiden mykoritsoihin ja taimien kasvuun

Robert T. Brown and Peitsa Mikola

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THE INFLUENCE OF FRUTICOSE SOIL LICHENS UPON THE MYCORRHIZAE AND SEEDLING GROWTH OF FOREST TREES

ROBERT T. BROWN and PEITSA MIKOLA

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PREFACE

This study was conducted at the Department of Silviculture of Helsinki University in 1971—72 when the senior author worked at this Department as a Fulbright scholar during his sabbatical leave from Michigan Technological University. The authors wish to thank all the people who, with expert guidance, helpful suggestions and skillful assistance, made this study possible.

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Houghton, Michigan, and
Helsinki, October 1973

ROBERT T. BROWN and PEITSA MIKOLA

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INTRODUCTION

Inhibiting or stimulating interactions between different members of plant communities are widely distributed, and much research has been conducted in this area (e.g. Intern. Biol. Progr. 1971). Brown (1967), when studying the influence of associated vegetation on the germination of jack pine *(Pinus banksiana)* seeds, determined that twelve out of 56 plants tested inhibited germination at least part of the time and five stimulated it part of the time. *Prunus pumila, P. serotina, Solidago uliginosa, S. juncea* and *Salix pellita* severely limited or completely inhibited germination. All *Cladonia* species tested except *C. cristatella* had no influence upon germination. *C. cristatella*, a small lichen which grows primarily on rotten wood, did partially inhibit germination.

As a continuation of these studies, the growth of jack pines planted with nearly pure field stands of various ground cover species was recorded. Germination corresponded closely with laboratory results, but once germinated, all seedlings grew more or less normally except those in association with reindeer lichens *(Cladonia* subgenus *Cladina)*. The seedlings grown in reindeer lichen cover attained an average height of only 7 cm in 7 years. When some of them were dug and examined for mycorrhizae, very few (10 to 20 per seedling) were found while on similar seedlings grown with orher kinds of cover, many (over 100 per seedling) were found on each small tree. Handley (1963) found similar inhibition of mycorrhizae which he concluded was caused by *Calluna vulgaris*. Ectomycorrhizal fungi, in particular, are known to be very sensitive to substances released by plant roots or leached out from dead plant material, as has been shown by Melin and his associates (e.g. Melin 1946, 1953, 1963).

When continuing the above research, Brown and Hooker (1971) conducted a greenhouse experiment by using a water extract of mixed reindeer lichens once a week to moisten the soil on which small jack pine seedlings were growing; pure water was used the rest of the time. A reduction in the number of mycorrhizae present and in growth was evident on the seedlings which had received the reindeer lichen extract when they were compared with the control which received only water.

These observations suggested that jack pine growth is to some extent inhibited if the mycorrhizae are inhibited by some water soluble substances in reindeer lichens. Leibundgut (1952) observed a somewhat similar inhibition of mycorrhizae of *Pinus silvestris, P mugo*, and *Picea abies* when he grew them with water extracts of *Cladonia* spp.

These observations gave good reason for more detailed studies on the effects of reindeer lichens on the growth of tree seedlings and in particular on mycorrhizal fungi. This paper reports results of these studies. Special attention is given to the effects of different species of *Cladonia*, as well as to possible differences between various species of mycorrhizal and other fungi in their reaction to lichen extracts. Experimental studies which were conducted with pure cultures of fungi and with synthetic mycorrhizae under axenic conditions, have been complemented with nursery plantings and some field observations in northern Finland where reindeer lichens are abundant in the forests. Since reindeer lichens are dominant ground cover plants in vast areas of the northern forests, their possible adverse influence on mycorrhizal fungi or tree seedlings may have a great practical importance for forestry of these regions.

THE EFFECT OF LICHEN EXTRACTS ON FUNGI IN PURE CULTURE

Method

Water extracts of *Cladonia alpestris*, *C. arbuscula (sylvatica)*, *C. rangiferina*, *C. pleurota*, *Cetraria islandica*, and *Stereocaulon paschale* were prepared by blending an amount corresponding to 10 grams oven dry weight of undried fresh lichens in a Bühler homogenizer with 100 ml of distilled water. In addition, humus from beneath *Cladonia alpestris*, *C. arbuscula*, *C. rangiferina*, and *Cetraria islandica* was also extracted by the same technique. These mixtures were filtered and centrifuged to remove solids. Then the centrifugate was put through a millipore filter to remove bacteria and fungus spores. This final filtrate was used as a portion of the liquid in Hagem agar (Modess 1941):

KH_2PO_4	0.5 g
NH_4Cl	0.5 g
$MgSO_4.7H_2O$	0.5 g
$FeCl_3$ (1 % solution)	0.5 ml
Glucose	5.0 g
Malt extract	5.0 g
Agar	15.0 g
H_2O	1000 ml

The agar was prepared with 1/2 enough water and cooled to 60° C; the other 1/2 of the water was then added as millipore filtered extract. This procedure was necessary to prepare sterile agar with a heat-labile extract (boiling denatured the inhibitor). In addition to the extracts, a saturated water solution of usnic acid was included since it is the most abundant lichen acid.

A total of 17 fungus species were used to inoculate petri dishes prepared with the above agar. Of the fungi tested, *Fomes annosus* is a well-known tree parasite. *Collybia butyracea*, *C. dryophila*, *Marasmius androsaceus* and *Lepiota procera* are litter-decomposing saprophytes, whereas all the other species are mycorrhizal. E-57 indicates an unidentified mycorrhizal species which commonly forms ectendotrophic mycorrhizae in forest nurseries (Mikola 1965). The strain was isolated from a spruce mycorrhiza in 1962. *Corticium bicolor*, a fungus forming bright yellow mycrorrhizae in raw humus (Mikola 1962), was isolated from a spruce mycorrhiza in 1960. *Cenococcum graniforme* was isolated from a sclerotium in 1964 and *Fomes annosus* from infected spruce wood in 1964. All the other fungi were isolated from sporocarps with the usual tissue culture technique between 1959 and 1971. The strains belong to the culture collection of the Department of Silviculture.

Inoculation of the petri dishes was performed by placing three 5 mm diameter agar inoculation pieces cut with a cork borer in each petri dish. Six or more replications were made. The control was a standard Hagem agar. The plates were incubated at room temperature from one to four weeks, depending on the growth rate of the fungus.

Growth of the fungi was measured by area of the colony outside the original inoculation piece. The results subjected to Student's »t» test are shown in Table 1. The levels of significance shown illustrate the degree of inhibition: .001 level indicates complete or almost complete inhibition while .01 or .05 indicate some growth of the fungus.

Results

Cladonia alpestris was the most effective growth inhibitor of mycorrhizal fungi (Table 1). The lichen itself is a more effective inhibitor than is the associated decaying humus (Table 2). *Cladonia arbuscula*, *C. rangiferina*, *Cetraria islandica*, and *Stereocaulon paschale* were less effective inhibitors than *C. alpestris*. *Cladonia pleurota* seems about equal to *C. alpestris* in effectiveness but its occurrence in the forest is very limited as compared to that of *C. alpestris* and consequently it can exert little influence.

In order to determine whether different geographic or climatic varieties of the lichen

Table 1. Effect of lichen extracts on the growth of fungi on Hagem agar. Figures show significance levels of inhibition or (+) stimulation (Student's test). X indicates no influence, — indicates no test.

Fungus sp. and year of isolation	Lichen extracts					
	Cladonia alpestris	*C. arbuscula*	*C. pleurota*	*C. rangiferina*	*Cetraria islandica*	*Stereocaulon paschale*
Amanita muscaria 1960	.001	X	X	X	X	.01
A. rubescens 1960	.001	X	.001	X	.01	.01
Boletus bovinus..... 1963	.05	.05	—	X	X	—
B. luteus 1963	X	X	.001	X	X	.05
B. variegatus 1971	.05	X	X	+.05	X	X
Cenococcum graniforme 1964	X	X	—	X	X	—
Collybia butyracea 1963	X	X	X	X	X	—
C. dryophila 1959	.001	.01	.001	.001	.01	—
Corticium bicolor 1960	.001	.05	.001	.05	.001	—
E-57 1962	.01	—	.001	.05	.05	—
Fomes annosus 1964	.001	—	—	+.001	.001	—
Laccaria laccata 1963	.01	.01	—	.05	X	—
Lactarius repraesentaneus 1960	.001	—	—	—	—	—
Lepiota procera 1960	.001	.001	.001	.001	.001	—
Marasmius androsaceus 1959	.001	.001	—	.001	.01	—
Paxillus involutus 1968	.001	.001	.001	.01	.001	.001
Tricholoma flavobrunneum 1960	.001	X	.001	X	X	—
T. imbricatum 1965	.001	X	.001	X	.05	—

might have different influences, the tests shown in Table 1 were duplicated with *C. alpestris* and others lichens from several locations up to 500 km apart. In every case, the results were almost identical to the initial observations. Since individual strains of fungus might differ in their reactions to lichen extracts, other isolates of *Boletus variegatus* and *Paxillus involutus* were tested. No significant differences between strains of the same fungus were observed. Therefore, the results obtained can be considered to apply quite broadly since quite similar results were obtained both in northern and southern areas with many species of fungi and different isolates of two fungal species.

This experiment also compared reactions of different mycorrhizal fungi with each other, with humus decomposers, and with a pathogenic species. No consistency of any kind can be found. Among ectomycorrhizal fungi, *Paxillus involutus*, for instance, was inhibited by all lichens and humus extracts and *Cenococcum graniforme* by none of them. Other ectomycorrhizal fungi were intermediate between these extremes; occasionally stimulation could be observed. Strain E-57, an unidentified ectendomycorrhizal fungus (Mikola 1965), was inhibited by several extracts. Among the saprophytic species, *Collybia butyracea* was unaffected or even stimulated while *C. dryophila* was inhibited by all the extracts. Also of interest is *Fomes annosus*, a pathogen, which was almost completely inhibited by

Table 2. Effect of extracts from humus collected beneath lichen cover shown and of saturated usnic acid solution on mycorrhizal fungus growth. Figures show levels of inhibition or (+) stimulation (Student's t test). X indicated no influence, — indicates no test.

Fungus sp.	Humus extracts beneath				Usnic acid solution
	Cladonia alpestris	*C. arbuscula*	*C. rangiferina*	*Cetraria islandica*	
Amanita muscaria	X	X	+.05	X	X
A. rubescens	.01	+.01	+.05	X	X
Boletus bovinus	.05	X	X	X	—
B. luteus	X	+.05	X	X	X
B. variegatus	X	X	X	X	X
Cenococcum graniforme	X	X	X	X	—
Collybia butyracea	+.05	X	X	X	X
C. dryophila	.01	.01	.05	.01	X
Corticium bicolor	X	X	X	.05	+.05
E-57	—	—	—	—	X
Laccaria laccata	X	X	.01	.01	—
Lepiota procera	X	X	.01	.01	—
Marasmius androsaceus	.05	.05	.01	.01	—
Paxillus involutus	.001	.001	.001	.001	.001
Tricholoma flavobrunneum	X	.05	X	X	X
T. imbricatum	X	.05	X	.001	+.05

Cladonia alpestris extract but stimulated by *C. rangiferina*, as was *Boletus variegatus*, a mycorrhizal fungus.

Thus, no generalization regarding relationships between mode of fungus nutrition and inhibition by *Cladonia alpestris* or other lichens can be made. However, it is interesting to note in regard to inhibition and stimulation that HENNINGSSON and LUNDSTRÖM (1970) obtained stronger inhibition of decay fungi in malt agar with *Hypogymnia psychodes* than with *C. alpestris* while GAGNON (1966) found survival of *Picea mariana* improved by the presence of *Lecidea granulosa*. Ammonifying bacteria, according to MALICKI (1970) are inhibited by *C. arbuscula* but not influenced by *C. rangiferina*. According to the experiments of VARTIA (1950), extracts of 75 species of 149 lichens tested contained some substances inhibiting pathogenic bacteria of humans.

Little influence of either lichen humus extracts or usnic acid is evident except on *Paxillus involutus* and to a lesser degree on *Collybia dryophila* and *Marasmius androsaceus* (Table 2). HENNINGSSON and LUNDSTRÖM (1970) showed that usnic acid reduced the activity of *Fomes pinicola* but stimulated *Allescheria terrestris*.

THE EFFECT OF LICHENS ON THE PHOSPHORUS UPTAKE OF MYCORRHIZAE

After the results of the previous experiment had shown that *Cladonia alpestris* and to a lesser extent other lichens inhibited many mycorrhizal fungi grown in pure culture, it became necessary to investigate whether or not the actual symbiotic relationship between fungus and tree was likewise affected. This was done by applying radioactive phosphorus to axenic synthesis cultures.

Method

120 ml test tubes were filled about 1/2 full of sterile silica sand and enough nutrient solution was added to nearly saturate the sand. A small test tube (15 ml) filled with sterile distilled water was placed in the center of each tube, to be used later for moistening the sand as it dried. The nutrient solution was prepared according to the following formula (LAIHO 1970; STEWARD 1963; HOAGLAND 1948):

KH_2PO_4	0.5	g
$(NH_4)_2SO_4$	0.25	g
$(NH_4)_2HPO_4$	0.025	g
$MgSO_4.7H_2O$	0.15	g
$CaCl_2$	0.05	g
NaCl	0.025	g
Fe^{III} citrate (1 % solution)	1.2	ml
H_3BO_3	2.86	mg
$MnCl_2.4H_2O$	1.81	mg
$CuSO_4.5H_2O$	0.08	mg
$ZnSO_4.7H_2O$	0.22	mg
$H_2MoO_4.H_2O$	0.09	mg
Glucose	2.5	g
Thiamin	25	γ*
H_2O	1000	ml

* 25 mg/1000 ml; 1 ml into this formula.

Pinus silvestris seeds were surface sterilized by soaking them for 5 minutes in 2 % H_2O_2. They were then placed on sterile agar plates for germination. Two of the freshly germinated seedlings were placed in each test tube on the sand. To each of 100 test tubes was added 10 ml of millipore filtered *Cladonia alpestris* extract; into each of another 100 test tubes, 10 ml of *C. rangiferina* extract and into 100 more, 10 ml of *Cetraria islandica* extract. 110 controls each received 10 ml of sterile distilled water.

Each group of 100 was then divided into groups of 10 test tubes. Each group of 10 was inoculated with a pure culture of one of ten mycorrhizal fungi. Ten groups of controls were likewise inoculated. The eleventh group was the uninoculated control. Unfortunately, the experiment did not include uninoculated controls with lichen extracts added. All tubes were plugged with cotton.

The test tubes were randomized and placed in the greenhouse for six months. When the sand began to dry, the tube was tipped so that water ran out of the small tube in the center and moistened it again, thus watering the trees without contamination.

After six months, 10 μCi of ^{32}P in 5 ml of sterile distilled water were added to each test tube. After 48 hours, each seedling was carefully removed, washed in running water and taped on herbarium paper. A sheet of Seran Wrap was immediately put over the top and then an X-ray film placed on top and exposed for 20 hours. The area of exposure on the film was taken as a measure for the ^{32}P uptake. Although the correlation between the area of exposure and the actual amount of ^{32}P is not linear, in this case the method was considered sufficient to reveal relative differences in the ^{32}P uptake of different seedlings. The area of exposure on the film for each seedling was measured carefully with a planimeter with the knowledge that the high uptake areas are seriously underestimated.

In addition to the ^{32}P measurements, the length of stem, main root and needles were recorded for each seedling.

All data were tested for significance using Student's t test and .01 was used as the level of significance.

Results

The developed films showed markedly different rates of ^{32}P uptake for the various treatments (Figs. 1 and 2).

Not all of the inoculated seedlings developed mycorrhizae. The results were calculated separately for mycorrhizal and nonmycorrhizal seedlings (Tables 3 and 5). On the average, in 55 % of the inoculated seedlings, mycorrhizae were formed whereas in the others fungal hyphae only grew around the roots. The mycorrhizal root systems were significantly larger than the nonmycorrhizal ones, but stems and leaves were only slightly larger (insignificant) with mycorrhizal infection (Table 3).

Table 3. Growth, ^{32}P uptake, and mycorrhizal development of pine seedlings with different lichen extracts.

Treatment	Stem length, mm		Leaf length, mm		Root length, mm		^{32}P activity, mm^2		% mycorrhizal
	–[1]	+	–	+	–	+	–	+	
Inoculated control	40	40	21	23	38	40	171	162	55
Inoculated + *Cladonia alpestris* extract	37	39	20	24	33	34	101	116	46
Inoculated + *Cladonia rangiferina* extract	38	39	20	21	30	36	77	201	62
Inoculated + *Cetraria islandica* extract	36	37	20	22	28	39	66	227	58
Average	38	39	20	22	33	37	113	179	55
Uninoculated control	42		25		42		228		

[1]) – nonmycorrhizal seedlings; + mycorrhizal seedlings

Increased mineral nutrient uptake of mycorrhizal roots in comparison to nonmycorrhizal ones has been described by numerous investigators (for literature references, e.g., Bowen 1973). Greatly increased absorption of phosphorus, in particular, has been repeatedly shown by the use of ^{32}P (Kramer and Wilbur 1949; Harley and McCready 1950; Mejstrik and Benecke 1969; and others). Likewise in this experiment the ^{32}P content in seedlings was significantly greater in the mycorrhizal than in the nonmycorrhizal plants.

In the mycorrhizal seedlings treated with *Cladonia alpestris* extract the ^{32}P concentration was about the same as in the nonmycorrhizal seedlings and much less than in the mycorrhizal plants of all the other treatments. Seedlings grown with *C. alpestris* extract also showed a significant inhibition of root development when compared to mycorrhizal seedlings treated with the other lichen extracts and to the inoculated controls. Nonmycorrhizal seedlings treated with *C. rangiferina* and *Cetraria islandica* extracts showed an amazingly low ^{32}P uptake.

In the inoculated control series the mycorrhizal seedlings did not appreciably differ from the nonmycorrhizal ones, whereas the uninoculated control seedlings showed significantly larger stems, needles and roots than any of the inoculated treatments. Their ^{32}P absorption was about equal to that of the mycorrhizal seedlings with *Cladonia rangiferina* or *Cetraria islandica*

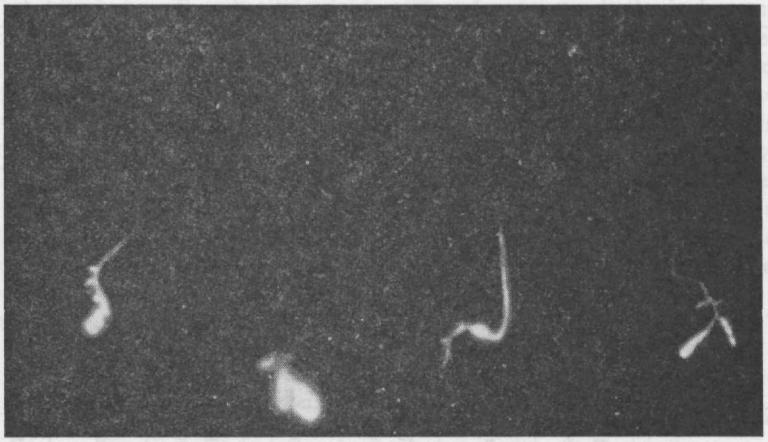

Fig. 1. ^{32}P uptake shown by radioautographs of *Pinus silvestris* seedlings inoculated with *Lactarius repraesentaneus*. From left to right the extracts added to culture solutions: distilled water, *Cladonia alpestris*, *Cladonia rangiferina*, and *Cetraria islandica*.

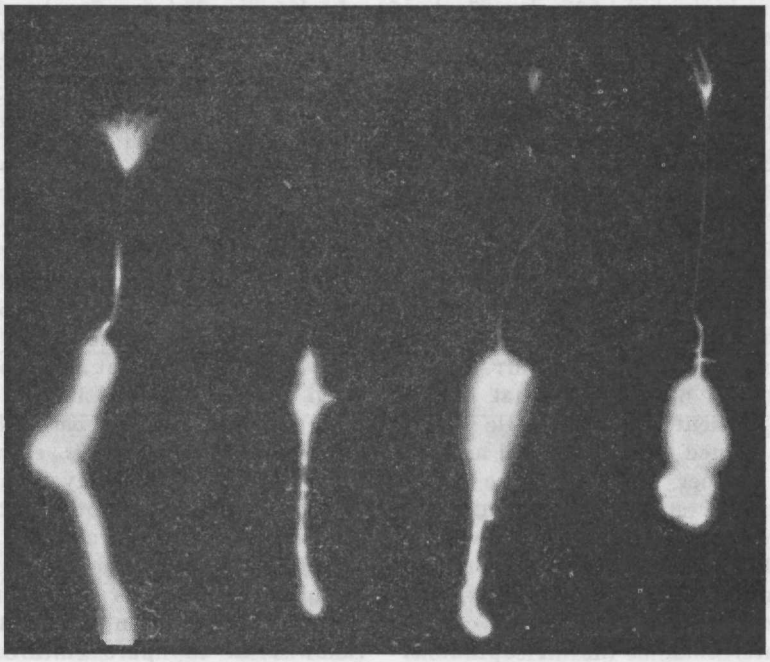

Fig. 2. ^{32}P uptake shown by radioautographs of *Pinus silvestris* seedlings inoculated with *Paxillus involutus*. From left to right the extract added to culture solutions: distilled water, *Cladonia alpestris*, *Cladonia rangiferina*, and *Cetraria islandica*.

Table 4. Mycorrhizal development and ^{32}P absorption efficiency of ten fungi when treated with different lichen extracts.

Mycorrhizal fungus	Lichen extracts						Control	
	Cladonia alpestris		*Cladonia rangiferina*		*Cetraria islandica*			
	Percentage of mycorrhizal seedlings	^{32}P activity, mm^2	Percentage of mycorrhizal seedlings	^{32}P activity, mm^2	Percentage of mycorrhizal seedlings	^{32}P activity, mm^2	Percentage of mycorrhizal seedlings	^{32}P activity, mm^2
Lactarius repraesentaneus .	88	76	57	72	13	54	50	51
Tricholoma flavobrunneum .	22	92	63	116	44	118	57	114
Tricholoma imbricatum	100	130	17	54	71	139	44	149
Boletus variegatus	20	44	88	259	67	164	89	124
Amanita muscaria	29	154	38	95	57	115	26	177
E-57	25	177	50	165	33	76	10	143
Corticium bicolor	50	109	57	181	75	91	100	194
Amanita rubescens	63	50	91	192	75	167	70	186
Boletus luteus	78	147	60	120	88	251	29	185
Paxillus involutus	0	136	80	232	71	458	63	249
Control, no fungi							0	228

extract but significantly higher than that of the inoculated seedlings which remained nonmycorrhizal.

These latter results are in agreement with BJÖRKMAN (1942) and VOIGT (1971), for instance, who have stated that in fertile soils or nutrient solutions, little if any advantage is gained by mycorrhizal association. Here another dimension, the lichen extract, has been added and its influence upon nonmycorrhizal plants is obviously damaging. The ^{32}P uptake by mycorrhizal pine seedlings treated with *C. rangiferina* and *Cetraria islandica* extracts is significantly greater than is the ^{32}P uptake of nonmycorrhizal seedlings. But *C. alpestris* extract had an inhibitory influence throughout all treatments.

Table 4 expands upon some aspects of Table 3. It shows the average ^{32}P uptake for all seedlings grown with each fungus under the influence of each extract. Again the reduction of the ability of seedlings to absorb ^{32}P in the presence of *C. alpestris* extract is well illustrated, but at the same time it is obvious that various mycorrhizal fungi do not behave in the same way. For example, *Boletus variegatus* is much inhibited by *C. alpestris* extract both in the ability to form mycorrhizae and in the ability of its symbiotic partner, the pine seedling, to incorporate ^{32}P. With *C. rangiferina* extract the percentage of mycorrhizal seedlings is more than 4 times higher and absorption of ^{32}P 6 times higher. Likewise in the pure culture experiment, *B. variegatus* was inhibited by *C. alpestris* extract but stimulated by *C. rangiferina* (Table 1).

With some other fungi the results were not so consistent. Thus, *Tricholoma imbricatum* and *Amanita muscaria* show higher

^{32}P uptake with *C. alpestris* extract than with *C. rangiferina* although in pure culture both were inhibited by *C. alpestris* and not influenced by *C. rangiferina* extract (Table 1).

Of particular interest is *Paxillus involutus*, which in pure culture was inhibited by all lichen extracts tested. In the synthesis experiment, however, it formed vigorous mycorrhizae in all the other media, whereas *C. alpestris* completely inhibited the mycorrhiza formation of *Paxillus involutus*.

Lactarius repraesentaneus also deserves special attention. *Lactarius* species, in general, are hard to cultivate and, if successfully isolated, grow poorly in pure culture. Therefore, very little is known about their physiology and symbiotic efficiency. *L. deliciosus* and *L. rufus* can be grown with difficulty in pure culture and have been more often included in synthesis experiments (cf. MELIN 1925; MODESS 1941; PACHLEWSKI 1967). Even the strain of *L. repraesentaneus* used in this investigation showed very poor growth. In spite of that, it seemed to retard the growth of the host plant (Table 5) and the uptake of ^{32}P also was very low (cf. Figs. 1 and 2). The seedlings

Table 5. The average length of needles, stems and roots and the relative ^{32}P uptake of *Pinus silvestris* seedlings inoculated with different mycorrhizal fungi.

Mycorrhizal fungus	Percentage of mycorrhizal seedlings	Average length, mm						^{32}P uptake, mm^2		
		Needle		Stem		Root				
		+[1]	−[2]	+	−	+	−	+	−	All seedlings
Lactarius repraesentaneus	52	22	20	40	34	34	31	81	44	63
Tricholoma flavobrunneum	45	21	17	40	33	47	30	185	47	109
Tricholoma imbricatum	59	24	21	36	40	39	28	158	59	123
Boletus variegatus	60	20	17	38	39	33	24	186	56	134
Amanita muscaria	40	21	20	39	35	38	32	173	120	141
E-57	29	20	20	38	38	37	35	167	136	145
Corticium bicolor	73	28	21	37	35	35	26	173	74	147
Amanita rubescens	76	23	21	41	37	36	35	177	83	154
Boletus luteus	65	20	19	42	39	37	37	176	201	185
Paxillus involutus	58	22	22	41	47	38	44	301	182	249
Average of inoculated seedlings	56	22	20	39	38	37	33	179	113	150
Control, no fungi			25		42		42		228	228
Cenococcum graniforme contamination		28		38		52		619		619

[1]) + = mycorrhizal seedlings
[2]) − = nonmycorrhizal seedlings

inoculated with this fungus were easily distinguishable from most others by their uniformly unhealthy appearance and yellowish color. Probably a balanced symbiosis of pine and *L. repraesentaneus* was not established in this experiment.

The figures in Table 4 suggest that all the mycorrhizal fungi are not equally beneficial. This has previously been shown by numerous laboratory and field experiments, although with somewhat inconsistent results (for literature references and summary of these experiments, see MIKOLA 1973, pp. 396–401). Table 4 also shows

that different mycorrhizal fungi react quite differently to lichen extracts. Thus, several species of mycorrhizal fungi growing with a symbiont would provide a broader range of adaptability to the biotic environment than would a single species. The advisability of introducing several efficient mycorrhizal fungi under conditions such as MIKOLA (1969 a and b), BRAGA and MYERS (1967), VEGA CONDORI (1964) and BJÖRKMAN (1970) describe is substantiated.

Comparisons between different fungi in their ability to form mycorrhizae and to promote the phosphorus uptake and growth of the host plant are made in Table 5. In order to make these comparisons possible, all the different lichen extract treatments have been put together. The mycorrhizal fungi are listed according to their average efficiency of ^{32}P absorption, when both mycorrhizal and nonmycorrhizal seedlings are considered. The same order is used in Table 4.

The ^{32}P absorption of mycorrhizal seedlings is in nearly all cases significantly greater than that of nonmycorrhizal seedling. The only exception is *Boletus luteus* which shows a slight (not significant) decrease of ^{32}P uptake in mycorrhizal seedlings.

When only mycorrhizal seedlings are considered, ^{32}P absorption is quite consistent except for two fungi. The remarkable exceptions are *Lactarius repraesentaneus* and *Cenococcum graniforme*. As was stated previously, *L. repraesentaneus* somehow inhibited the host plant and this was also reflected in the ^{32}P uptake which was less than 50 % of that of most other fungi. *C. graniforme* was not used as inoculum but occurred as a contaminant in six test samples of various treatments, the only contaminating fungus found in any treatment. As can be seen in Table 1, in pure-culture *C. graniforme* was not influenced appreciably by any lichen extract tested. The root systems of the seedlings with *C. graniforme* mycorrhizae were much larger and had more numerous mycorrhizae than did those with any other treatment. The needles were larger, deeper green and more robust looking than were most of those in the other treatments. In total, this small accidental sample of *C. graniforme* gave the best growth of any treatment. The obvious correlation of good growth is the exceedingly high ^{32}P absorption rate of 619 mm^2 as compared to the next highest of 301 by *Paxillus involutus* which grew well or to the lowest of 81 by *Lactarius repraesentaneus* which grew poorly. Although this material is limited, some comparison to the existing knowledge on *Cenococcum graniforme* can be made. MIKOLA (1948) observed that *C. graniforme* is not so sensitive to antibiotic substances as are most other mycorrhizal fungi. Because of its drought resistance and effectiveness in energy utilization it can form mycorrhizae under unfavorable conditions such as in dry soils or under a dense tree canopy (MIKOLA 1948; WORLEY and HACSKAYLO 1959; TRAPPE 1964). Probably it also forms an effective barrier against attack by parasitic fungi, which function has been attributed to mycorrhizal fungi (MARX 1973). Reports on the symbiotic efficiency of *C. graniforme* are somewhat contradictory. SHEMAKHANOVA (1962), PARK (1970), and LAMB and RICHARDS (1971) obtained a remarkable growth promotion by inoculation with *C. graniforme*, whereas in the experiment of LUNDEBERG (1970) the same fungus, through immobilization of exchangeable soil nitrogen, suppressed the growth of pine seedlings.

Paxillus involutus was the most effective fungus ot the species tested in the promotion of both ^{32}P uptake and the growth of seedlings. This particular strain was apparently a very active mycorrhiza former; the average percentage of mycorrhizal plants was as high as 58, in spite of the fact that the *Cladonia alpestris* extract inhibited mycorrhiza formation completely (Table 4). As has shown by LAIHO (1970), there is a great variability in the mycorrhizal affinity of different strains of *P. involutus*. Thus, in the experiment of LUNDEBERG (1970), for instance, *P. involutus* formed no mycorrhizae. In this experiment, strangely, those seedlings were biggest which grew with the presence of *P. involutus* but without established mycorrhizae. The favorable effect of mycorrhizal fungi on tree seedlings even without true mycorrhizal association has been previously shown by LEVISOHN (1956) and LUNDEBERG (1970), for instance. Mycorrhizal fungi, even without mycorrhizal asso-

ciation, can also protect tree roots against pathogenic fungi (HYPPEL 1968).

Table 5 suggests that various fungal species may effect the growth of different organs of the seedlings in different ways. Thus, the best needle growth was obtained with *Corticium bicolor* and *Cenococcum graniforme* and the best stem growth with *Paxillus involutus* and *Boletus luteus*. Although the data are insufficient for any definitive conclusions, observations such as this suggest the use of mixed populations for mycorrhizal inoculation.

FIELD EXPERIMENT ON THE EFFECT OF GROUND COVER ON THE GROWTH OF THE SEEDLINGS

Method

Since the above experiments were done in the laboratory, field validation of results was considered necessary. The forest nursery at Suonenjoki (Central Finland) was chosen as the site for this experiment.

Paired 1 m^2 plots were laid out in the nursery and divided into 1/25 m^2 plots. *Cladonia alpestris* was gathered from the surrounding forest and placed on one of the paired plots. Nylon fish net placed over the plots held the lichens in place. In each square meter plot, 25 one year old seedlings were planted. This was replicated 4 times for each of three kinds of seedlings, *Pinus silvestris*, *Picea abies*, and *Betula verrucosa*, a total of 100 seedlings of each species planted with lichens and 100 planted without lichens. All plots were sprinkled simultaneusly when they became too dry. Plantings were made in mid-May and growth measurements taken in mid-September after two summers.

Results

Table 6 shows that the growth of both *Pinus silvestris* and *Picea abies* is significantly reduced as a result of the *Cladonia alpestris* treatment. On the other hand, *Betula verrucosa* growth was the same for both treatments. Fig. 4 illustrates the differences in appearance of these seedlings. The yellowish color and poor growth of *Picea abies* grown with *C. alpestris* is better illustrated here than in Table 6. Survival was significantly better for spruce and better for pine on control plots. Again, birch was not influenced.

The poorer growth and survival of pine and spruce seedlings on *C. alpestris* plots can not be attributed solely to some substances leached from the lichen. Although the lichen cover may also affect the moisture, temperature and perhaps even nutrient conditions of the substrate, this field experiment corroborates the results of the laboratory studies on the inhibiting influence of *C. alpestris* on *Pinus silvestris*. *Picea abies* was inhibited as well, but *Betula verrucosa* seemed to be unaffected. This difference deserves further investigation.

Table 6. The average growth and survival of seedlings two seasons after planting on paired nursery plots.

Species	*Cladonia alpestris* plots		Control plots	
	Growth, cm	Survival, %	Growth, cm	Survival, %
Pinus silvestris	22.2	93	25.8	100
Picea abies	21.4	84	31.2	100
Betula verrucosa	105.0	70	108.0	67

COMPLEMENTARY FIELD OBSERVATIONS

Laboratory and nursery experiments were complemented with field observations which were made in natural stands of *Cladonia alpestris* where the influence of reindeer grazing was excluded. Such stands were found in a reindeer exclosure at Kätkäsuvanto, Muonio (Northwest Finland) and in the fenced Finland-Soviet Union border zone at Raja-Jooseppi, Inari (Northeast Finland). Thousands of seedlings per hectare were present in *C. alpestris* cover. As has been previously shown by BROWN (1967), *C. alpestris* has no influence upon the germination of pine seed.

About eight years ago the border crossing highway was graded and the *C. alpestris* along the side, as well as all the other vegetation and pine seedlings destroyed. New seedlings which looked much more robust were growing on this lichen denuded soil. Ten of these, not exceeding 30 cm in height and ten growing with *C. alpestris* were dug and, with a similar *Cladonia* grown seedling sample from Kätkäsuvanto, were taken to the laboratoty where they were sectioned at ground level and basal areas measured, age determined and the number of mycorrhizal root tips counted. The seedlings chosen for study were about average size for the *C. alpestris* cover but amoung the smallest on the denuded roadside. Distance between the Raja-Jooseppi collection points was about 1 meter and no soil differences were observable. The Kätkäsuvanto soil was considerably sandier than that at Raja-Jooseppi.

As is seen in Table 7, a tremendous difference exists in the growth of pine seedlings on a denuded roadside and in a dense *C. alpestris* vegetation. Again, the difference cannot be attributed solely to some antibiotic substances released from the lichen; elimination of competition might be a major factor. Particularly noteworthy is the great difference in the number of mycorrhizae. BJÖRKMAN (1942) suggests that light is a decisive factor for mycorrhizal formation, but because the distance between the two sampling points at Raja-Jooseppi was only about one meter, light conditions vere the same for both. More likely the lichen exerts a direct harmful effect on mycorrhizal fungi. A harmful influence of lichen vegetation on mycorrhizae has been reported by LEIBUNDGUT (1952).

The relationship between the density of *C. alpestris* cover and the growth of pine seedlings was also studied with vegetation analyses in the reindeer exclosure of Kätkäsuvanto. There, reindeer had been excluded for 25 years.

140 sample plots of 1 m² were laid out and the coverage of the dominant species rated on a scale from 0 to 5. The height of

Table 7. The growth and mycorrhizal development of *Pinus silvestris* seedlings with and without *Cladonia alpestris*.

Site	Average age, yrs	Average basal area, mm²	Average basal area growth, mm²/year	Average number of mycorrhizae
Cladonia alpestris ..... Raja-Jooseppi	21.3	10.3	0.51	27
C. alpestris ..... Kätkäsuvanto	14.7	3.7	0.25	45
Denuded roadside ..... Raja-Jooseppi	6.4	18.6	2.91	195

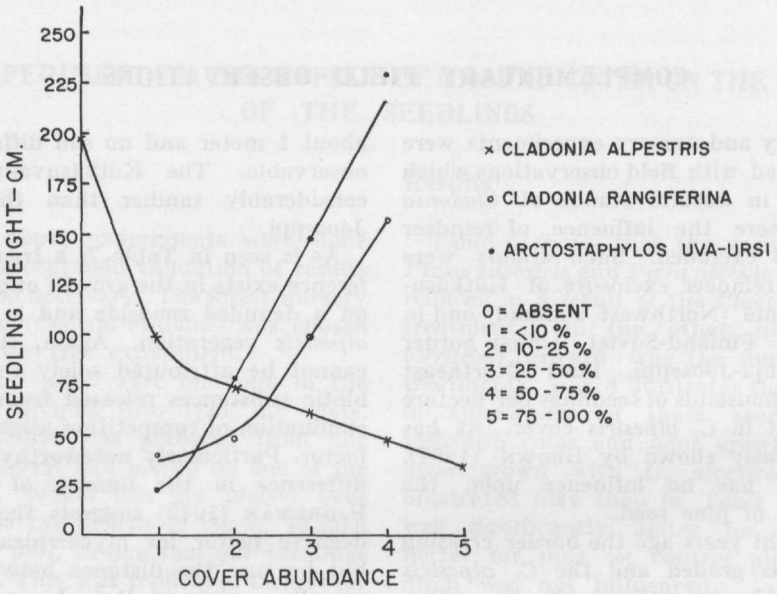

Fig. 3. Growth of *Pinus silvestris* seedlings with different densities of three associated ground cover plants.

all seedlings in the sample plots was recorded. Plots were selected by throwing a 1 meter long stick in a predetermined direction and using it as the near edge of the plot.

Fig. 3 illustrates the relationships between the abundance of *Arctostaphylos uva-ursi*, *C. ragiferina*, and *C. alpestris* and the size of pine seedlings on these 140 plots. Each point on the graph represents at least 4 plots; some points represent up to 20 plots. This figure corroborates the previous data; where *C. alpestris* is abundant, growth of pine seedlings is retarded. While *C. rangiferina* would appear in this graph to be a stimulant to growth, it is not. Actually, it apparently also hinders growth of seedlings although to a smaller degree than does *C. alpestris*. Where *C. rangiferina* or *Arctostaphylos uva-ursi* was abundant, *C. alpestris* was almost or totally excluded. Thus, pine seedlings thrived best with *A. uva-ursi* which had no apparent influence on growth or on germination (Brown 1967).

The effect of elimination of lichens on the growth of pine seedlings is dramatically illustrated in Fig. 5. About 20 years ago a reindeer was tethered to the tree for one day. Pine seedlings are abundant all over the area but only around the tree where the reindeer had removed the lichen do they grow vigorously. Such examples of seedling growth are common in Lapland, as a result of the former practice of keeping female reindeer tethered to trees at the calving season. After removal of the lichen its re-establishment takes a long time (Aakre 1966) during which a dense and vigorous thicket of pine seedlings can develop.

DISCUSSION

The above laboratory and nursery experiments and field observations indicate consistently that many lichens, *Cladonia alpestris* in particular, exert a harmful effect on the growth of pine and spruce seedlings. One of the reasons for such an inhibition is most probably the release from the lichens of some toxic substances which affect adversely the mycorrhizal symbionts of the trees. The sensitivity of different species of mycorrhizal fungi to those substances varies considerably, and many saprophytic and prasitic soil fungi are affected as well. *Paxillus involutus* which in the above experiments was one of the most active mycorrhizal fungi, seems to be particularly sensitive to the antibiotic influence of *C. alpestris*. Since corresponding differences apparently exist in the sensitivity of mycorrhizal fungi against other antibiotic factors in soil, too, a mixed population of fungi may be preferable to a single or a few species for mycorrhizal inoculation (cf. MIKOLA 1970). Then, if some species are inhibited by antibiotic substances present in the soil, others may be more resistant under prevailing contitions and may establish a balanced symbiosis with trees. Such a mixed population also corresponds better to natural conditions where several fungal species usually form mycorrhizae in the root systems of each single tree (ZAK and MARX 1964).

The importance of toxic substances released from lichens for forestry practice is still unknown. Logically, if *C. alpestris* is destroyed or removed, its inhibiting influence will disappear. *Cladonia* can be destroyed, for instance, by fire. Burning is known to be very beneficial for the early growth of tree seedlings and therefore controlled burning has been widely practised as a tool in reforestation. The beneficial effect of fire on seedling growth can be attributed, of course, to several favtors, such as the elimination of growth cover competition, mobilization of nutrients, rise of pH, etc. Destruction of lichens and their inhibiting products can be added to those factors although its relative importance, as compared with the other effects of fire, is a matter of speculation. As was suggested previously, other plants, too, may release toxic substances comparable with lichen antibiotics. According to HANDLEY (1963), *Calluna vulgaris* is one such plant. The high temperature of burning, of course, also kills the mycorrhizal fungi near the soil surface and therefore somewhat delays the commencement of mycorrhizal infection of the seedlings, but once established, the mycorrhizae grow better than in unburned soil (MIKOLA et al. 1964). Neither the relationship of lichens to this phenomenon nor the destruction of inhibitory factors has been investigated.

Reindeer grazing can also destroy lichens effectively and in this respect is comparable with fire. Its strong promotion of seedling growth, such as is seen in Fig. 6, is hard to explain solely on the basis of elimination of competition; some other harmful effect of the lichen may have been eliminated at the same time. Again, the importance of the presence of inhibiting substances in lichens must be considered.

Quite clearly, *C. alpestris* has the strongest inhibitory influence on mycorrhizal fungi and pine seedlings; *C. arbuscula* and *C. rangiferina* have less effect and *Stereocaulon paschale* least. This phenomenon has an interesting correlation with the plant succession. On the dry pine heath-lands of Lapland, *S. paschale* is a typical pioneer species and the first colonizer after fire or heavy grazing whereas *C. alpestris* is a typical climax species; *C. arbuscula* and *C. rangiferina* usually dominate during the middle phase of succession. Thus, along with advancing succession, conditions tend to become more unfavorable for mycorrhizal fungi as well as for pine. Since the substances leached from lichens inhibit saprophytic soil fungi as well, the advancing succession also means retardation of humus decomposition and, consequently, accumulation of soil organic matter. Such a retardation and ultimately stagnation of many

biological processes towards the climax is characteristic of plant successions in colder regions. Even the growth of *C. alpestris* is much slower than that of *C. arbuscula* and *C. rangiferina*.

An interesting analogy is found in the development of moist *Hylocomium*-spruce forests of northern Finland. There as well, with the advancing succession, biological processes slow down, organic matter accumulates and the growth of successive spruce generations gets poorer and poorer (SIRÉN 1955; HÄRMA 1961). It is not known, however, whether toxic excretions of some plants play any role in this process.

Stagnation of biological processes is, of course, contrary to the principles of sound forest management and should be avoided. Fire is known as an effective tool with which to accelerate soil biological processes through release of organically bound nutrients and, perhaps, also through destroying antibiotic factors. As is well known, controlled burning has been practiced with good success in the regeneration of old *Hylocomium*-spruce forests of northern Finland.

The above findings have also their application in the lichen-dominated pine forests of northern Finland, although corresponding growth stagnation, as is known in moist spruce forests, has not been noticed in pine forests. A quite natural reason for that is the fact that wild fires have been much more frequent on dry pine heaths than in moist spruce forests and, therefore, succession in the former has never advanced far enough. Effective control of fires, however, may change the situation. Precribed burning does not belong to the silvicultural practices in lichen-pine forests, neither can it be recommended there in the future. Instead of that, reindeer grazing can be effectively used to combat the possible harmful effects of the lichens. Old pine forests with lichen vegetation should be heavily grazed immediately before final cutting. Then the ground would be effectively prepared for natural reproduction and the inhibiting effects of lichen on mycorrhizal fungi and tree seedlings would be greatly reduced. This system would also reduce the damage of young seedlings by reindeer, because after the cutting there would be very little food to attract reindeer to the area during the early years of seedling growth.

This subarctic forest biome well illustrates the continuity which must exist in any ecological system. A dynamic balance exists among the major components. Lichens apparently hold trees in check by retarding seedling growth. Reindeer, in turn, destroy dense lichen cover making rapid growth of mycorrhizal fungi and trees possible. Humans and other predators control reindeer population. Serious damage to any one component will adversely influence the others. Thus, sound reindeer husbandry and proper forestry practices are integral parts of the multiple-use management of this fragile natural system.