Mycorrhiza (1995) 5:267–272

ORIGINAL PAPER

J. Baar · F.W. de Vries

Effects of manipulation of litter and humus layers on ectomycorrhizal colonization potential in Scots pine stands of different age

Abstract Effects of manipulation of litter and humus layers (removal, doubling and control treatments) on the colonization potential of ectomycorrhizal fungi were studied in two secondary stands of *Pinus sylvestris* (5 and 18 years old) in The Netherlands. Five-month-old, sterile-grown Scots pine seedlings, inoculated with *Laccaria bicolor*, *Paxillus involutus* or *Rhizopogon luteolus* and noninoculated seedlings were used as baits. The seedlings were harvested after one growing season. For comparison, sporocarps of ectomycorrhizal fungi were also investigated. Genus composition on the seedlings was independent of initial inoculum, but determined by both treatment and age of the stands. In both stands, removal of litter and humus layers increased, and addition of organic material decreased the number of ectomycorrhizal types on the seedlings. Not all indigenous genera were observed by either outplanting seedlings or sporocarp surveys.

Key words Colonization potential · Ectomycorrhizal fungi · Litter and humus layer · *Pinus sylvestris*

Introduction

In a previous study, the effects of removal of litter and humus layers on ectomycorrhizal fungi were assessed (Baar and Kuyper 1993). Removal of litter, humus and the herbaceous vegetation by sod cutting (removal of the organic top soil and vegetation) was found to raise the number of species and sporocarps compared to the control plots in young (<15-year-old) and middle-aged (15- to 50-year-old) secondary Scots pine forests. In the same forests, addition of ectorganic layers by adding sods, simulating thick litter and humus layers of old (>50 years) forests, reduced the numbers of species and sporocarps compared to the control plots.

Ectomycorrhizal fungi, however, are not observed when they do not form sporocarps, so sporocarp surveys must be repeated for several years. In the present study, pine seedlings were used as baits to investigate the colonization potential of ectomycorrhizal fungi including nonsporocarp-forming species, as affected by soil treatments (Stenström 1990; Arnebrant 1991; Dahlberg and Stenström 1991).

The objectives of the present study were (1) to determine effects of manipulation of litter and humus layers on the ectomycorrhizal colonization capacity, (2) to investigate replacement of inoculated fungi by indigenous fungi, and (3) to compare the results of baiting with those of sporocarp surveys.

J. Baar (✉) · F.W. de Vries
Biological Station, Agricultural University,
Kampsweg 27, NL-9418 PD Wijster, The Netherlands
Fax: (31) 59-36-2786

Materials and methods

Study sites

The study was carried out in two secondary stands (5 and 18 years old) of *Pinus sylvestris* L. both situated in the forest Dwingeloo in the northeastern part of The Netherlands 52° 49′ N, 6° 28′ E and 52° 50′ N, 6° 25′ E, respectively) in 1992. Until 1939, the site of the 5-year-old stand was heathland. Then *Picea sitchensis* (Bong.) Carr. mixed with *Castanea sativa* Miller, *Fagus sylvatica* L., *Betula* spp. and *Prunus* spp. was planted. In 1988, this stand was felled and replanted with *Pinus sylvestris*. The canopy of the 5-year-old stand covered about 25% in 1992. Until 1920, the site of the 18-year-old stand was also heathland. Then *Pinus sylvestris* mixed with *Quercus rubra* L. and *Picea abies* (L.) Karsten was planted. In 1974, the stand was felled and replanted with *Pinus sylvestris*. The canopy of the 18-year-old stand covered 90% in 1992. The soils of both stands are podzolic. In May 1990, litter and humus layers of four plots (15 × 15 m) per stand were removed ("sod cutting"). At the same time, ectorganic material was added to four plots ("sod adding"), simulating ageing of the humus profile, and four control plots were selected. The few naturally regenerated deciduous trees were chopped and Scots pine seedlings were removed every year. In May 1992, the average thickness of the humus layers of the control and sod-added plots in the 5-year-old stand were 8 and 11 cm, respectively. The herbaceous understory

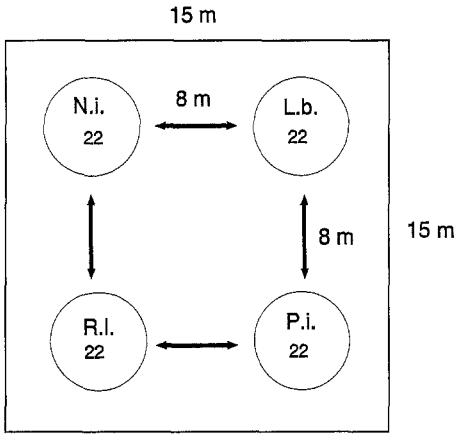

Fig. 1 Experimental design of one plot (*N.i.* noninoculated seedlings, *L.b.* inoculated with *Laccaria bicolor*, *P.i.* inoculated with *Paxillus involutus*, *R.l.* inoculated with *Rhizopogon luteolus*, *22* number of outplanted seedlings)

vegetation of all plots in the 5-year-old stand consisted of *Deschampsia flexuosa* (L.) Trin., *Chamérion angustifolium* (L.) Holub and *Calluna vulgaris* (L.) Hull. The average thickness of the humus layers of the control and sod-added plots in the 18-year-old stand were 10 and 9 cm, respectively. The control and sod-added plots in the 18-year-old stand were dominated by *Deschampsia flexuosa*, *Ceratocapnos claviculata* (L.) Lidén and *Molinia caerulea* (L.) Moench. The herbaceous vegetation of the sod-cut plot consisted of *Calluna vulgaris*, *Carex pilulifera* L. and *Deschampsia flexuosa*.

Baiting procedure

Seeds of *Pinus sylvestris* originating from forest Junne in the northeastern part of The Netherlands were sterilized in 15% H_2O_2 for 30 min. Sterilized seeds were germinated on sterile water agar containing 0.5% glucose in December 1991. After germination, seedlings were inoculated or not with isolates of *Laccaria bicolor* (Maire) P.D. Orton, *Paxillus involutus* (Batsch: Fr.) Fr. or *Rhizopogon luteolus* Fr. *Laccaria bicolor* had been collected in the autumn of 1990 in a 50-year-old secondary stand of *Pinus sylvestris*, located in the northeastern part of The Netherlands. *Paxillus involutus* and *Rhizopogon luteolus* had been collected in the autumn of 1990 in a 15- to 20-year-old primary Scots pine stand, located in the central part of The Netherlands. These fungi were chosen because they reacted strongly on removal of litter and humus layers or accumulation of ectorganic material as shown by observations on sporocarps (Baar and Kuyper 1993), and because these species can easily be cultured and inoculated. *Laccaria bicolor* and *Rhizopogon luteolus* were positively affected by sod cutting, whereas sporocarps of *Paxillus involutus* were mainly found in the control plots.

Inoculated seedlings were grown in vitro on twice autoclaved (20 min, 110° C) peat, vermiculite and MMN solution (at 4:10:7 by volume) in closed glass tubes in a greenhouse from January to May 1992. Peat, vermiculite and MMN solution was used as substrate to stimulate ectomycorrhizal development. Noninoculated seedlings were grown in small pots with sandy soil originating from the 15- to 20-year-old primary Scots pine stand. Nonectomycorrhizal seedlings developed better in sandy soil than in peat, vermiculite and MMN solution. Day temperature was kept at 21° C and night temperature at 8° C for 3 months, then the day temperature became the outdoor temperature while the night temperature was kept at a minimum of 10° C.

In May 1992, 88 seedlings were planted out in one control plot, 88 seedlings in one sod-cut plot and 88 seedlings in one sod-added plot in the 5-year-old stand. Four groups consisting of 22 seedlings each were planted at four sites in each plot. The distance between two sites was 8 m (Fig. 1). Each group of seedlings was covered with wire netting to protect them from grazing by animals. The same procedure was carried out in the 18-year-old stand.

Five months later, 10 surviving seedlings per site were harvested (or fewer when fewer seedlings survived). The root systems were cut off and stored in a glutaraldehyde buffer (Alexander and Bigg 1981) until further analysis.

Determining ectomycorrhizal incidence

The root systems were studied using a dissecting microscope. The ectomycorrhizas were divided in two groups on the basis of appearance. Well-developed ectomycorrhizas with a smooth, relatively thick mantle were categorized as vital. Poorly developed ectomycorrhizas with a dented and more or less wrinkled mantle or no distinct mantle were categorized as non-vital. Only well-developed ectomycorrhizas could be identified on the basis of colour and morphology (Agerer 1987; Ingleby et al. 1990). Types thus recognized generally correspond to fungal taxa at the genus level except for ITE3. Therefore, the terms below-ground ectomycorrhizal types and above-ground fungal genera will be treated as equivalent.

Root length per seedling was determined according to the line intersect method of Newman (1966). Total numbers of root tips, numbers of vital and nonvital ectomycorrhizal and nonectomycorrhizal root tips per seedling were determined. The frequencies of ectomycorrhizal (vital and nonvital) and nonectomycorrhizal root tips were calculated from number of ectomycorrhizal root tips/number of ectomycorrhizal root tips + number of nonectomycorrhizal root tips. The frequencies of vital ectomycorrhizal root tips of each ectomycorrhizal type were also calculated, as well as the total number of root tips/cm root.

Survey of sporocarps

From the beginning of September until the end of October 1992, the plots of the 5- and 18-year-old stand were surveyed three times for sporocarps of ectomycorrhizal fungi. The caps of sporocarps were removed in order to avoid double counting.

Statistical analysis

The main effects of treatments and stand ages were tested with one-way ANOVA when the variables did not show interaction (Sokal and Rohlf 1981). Data not normally distributed, even after log transformation, were analysed with the Kruskal-Wallis test (Siegel and Castellan 1988).

Results

Mycorrhizal types on the seedlings

The initial inoculum did not affect the composition of types on the seedlings after one growing season. However, the composition of ectomycorrhizal types of the seedlings differed between the two stands. *Hebeloma*, *Rhizopogon* and *Thelephora* were only observed on seedlings in the 5-year-old stand and *Lactarius* and *Russula* were found only on seedlings planted in the 18-year-old stand (Table 1).

In the 5-year-old stand, seedlings in all treatments had mycorrhizal root tips dominated by *Laccaria* (Ta-

Table 1 Average frequency of ectomycorrhizal types (in %) of seedlings planted in 5- and 18-year-old secondary Scots pine stands. The ectomycorrhizal types on the seedlings similar to the initial inoculum are printed in bold. The species are abbreviated as follows: Aman *Amanita* spp., Ceno *Cenococcum* spp., Hebe *Hebeloma* spp., ITE3 ITE3, Lacc *Laccaria* spp., Lact *Lactarius* spp., Paxi *Paxillus* spp., Rhiz *Rhizopogon* spp., Russ *Russula* spp., Thel *Thelephora* spp. (*O* control, *R* removal of litter and humus layers, *A* addition of organic material, *n* number of seedlings harvested)

Initiated inoculum	n	Ectomycorrhizal root tips			Vital root tips								
		Non	Non-vital	Vital									
5-year-old stand					Aman	Ceno	Hebe	ITE3	**Lacc**	**Paxi**	**Rhiz**	Russ	Thel
O: Noninoculated	10	28.1	29.1	43.0	—	.6	—	—	43.0	—	—	—	—
Laccaria bicolor	3	39.1	22.8	38.1	—	—	—	—	38.1	—	—	—	—
Paxillus involutus	7	16.1	13.9	70.0	—	—	—	—	66.4	**12.6**	—	—	—
Rhizopogon luteolus	10	14.7	15.6	69.7	—	—	—	—	69.7	—	—	—	—
R: Noninoculated	10	25.7	19.3	56.9	—	—	—	—	46.0	—	58.2	—	26.4
Laccaria bicolor	3	13.0	32.8	54.1	—	—	31.9	—	**47.8**	—	7.8	—	—
Paxillus involutus	8	18.0	23.9	58.1	24.7	—	—	—	47.8	—	16.5	—	—
Rhizopogon luteolus	5	13.9	45.8	40.3	—	.2	33.4	—	16.7	—	**8.8**	—	—
A: Noninoculated	10	59.3	8.3	33.3	—	—	—	17.7	22.3	—	—	—	—
Laccaria bicolor	10	32.8	13.7	53.6	—	—	—	5.8	**50.7**	—	—	—	—
Paxillus involutus	10	11.4	22.7	65.9	—	—	—	—	65.9	—	—	—	—
Rhizopogon luteolus	9	14.5	7.5	78.9	—	—	—	—	78.9	—	—	—	—
18-year-old stand					Aman	Ceno	Hebe	ITE3	**Lacc**	Lact	**Paxi**	**Rhiz**	Russ
O: Noninoculated	10	91.8	5.5	2.7	—	—	—	4.5	—	4.6	—	—	—
Laccaria bicolor	10	79.1	17.3	3.6	—	—	—	—	**8.6**	8.6	—	—	1.8
Paxillus involutus	10	58.9	41.1	—	—	—	—	—	—	—	—	—	—
Rhizopogon luteolus	10	64.0	34.0	11.3	—	—	—	—	—	11.3	—	—	—
R: Noninoculated	10	52.7	15.4	31.8	—	31.8	—	1.3	24.1	11.6	—	—	13.4
Laccaria bicolor	10	51.3	30.9	27.0	—	2.9	—	—	**32.9**	35.1	—	—	—
Paxillus involutus	10	53.5	41.8	4.7	4.9	—	—	—	—	1.0	**4.0**	—	3.9
Rhizopogon luteolus	10	53.5	40.3	6.2	—	—	—	—	—	5.1	—	—	52.0
A: Noninoculated	10	89.0	10.5	5.0	—	5.0	—	—	—	—	—	—	—
Laccaria bicolor	10	68.2	30.9	8.3	—	—	—	—	**8.3**	—	—	—	—
Paxillus involutus	10	58.5	41.5	—	—	—	—	—	—	—	—	—	—
Rhizopogon luteolus	10	73.1	26.7	1.3	—	1.3	—	—	—	—	—	—	—

ble 1). The highest number (6) of ectomycorrhizal types was found in the sod-cut plot (Fig. 2). The lowest number of types (2) was observed in the sod-added plot.

In the sod-cut plot of the 18-year-old stand, *Laccaria*, *Lactarius* and *Russula* were the most frequent types (Table 1). In the sod-added plot, only two types were found, *Cenococcum* and *Laccaria*. The highest number (7) of ectomycorrhizal types was observed in the sod-cut plot, the lowest number (4) in the sod-added plot (Fig. 2).

Of the total number of seedlings, 14% and 2.3% contained two or three ectomycorrhizal types, respectively, on the roots of the same seedling. No ectomycorrhizal root tips were found on 3.3% of the total number of surviving seedlings.

Root parameters

The root length, the number of root tips per seedling and the frequencies of vital ectomycorrhizal root tips of the seedlings were significantly higher ($P<0.001$) in the 5-year-old stand than in the 18-year-old stand (Tables 1, 2). The numbers of root tips/cm root of the noninoculated seedlings in the 5-year-old stand were significantly higher ($P<0.001$) than in the 18-year-old stand (Table 1). The frequencies of occurrence of *Laccaria* were significantly higher ($P<0.001$) in the 5-year-old stand than in the 18-year-old stand.

Sporocarp survey

The number of species and genera over all treatments was higher in the younger stand than in the 18-year-old stand (Table 3). The highest number of genera (6) was found in the control and sod-cut plots of this stand (Table 3). In the 18-year-old stand, the highest number of genera (5) was recorded in the sod-cut plot.

Comparison between baiting and sporocarp survey

Higher numbers of ectomycorrhizal genera were observed in control and sod-added plots in the 5-year-old stand by sporocarp survey than by baiting (Fig. 2). *Cortinarius*, *Gomphidius* and *Suillus* were only observed by

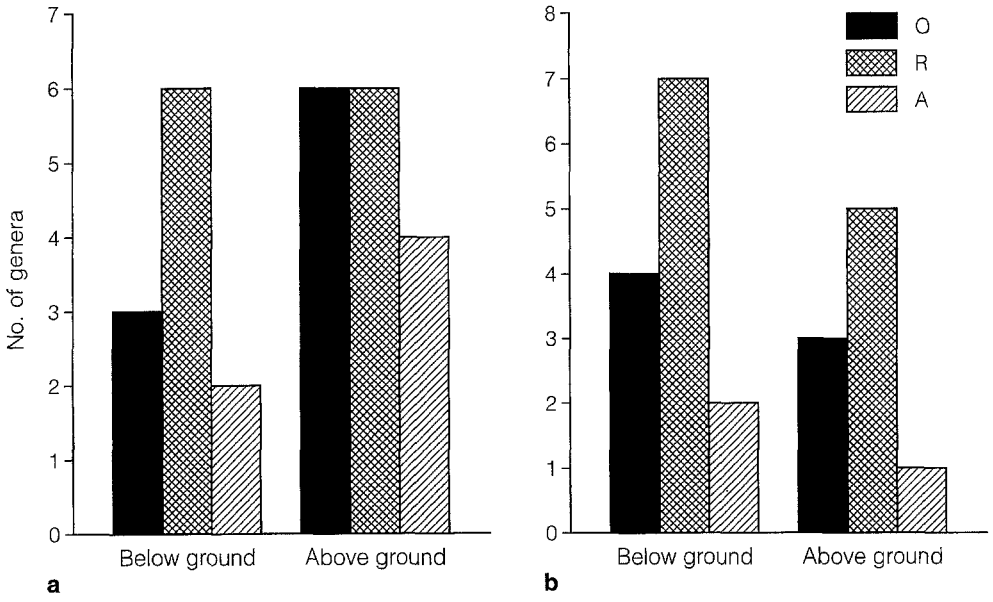

Fig. 2 Number of ectomycorrhizal genera observed on the roots of the seedlings (*below ground*) and as sporocarps (*above ground*) in the (**a**) 5- and (**b**) 18-year-old stands (*O* control, *R* removal of litter and humus layers, *A* addition of organic material)

Table 2 Average root length and total number of roots of the seedlings in the 5- and 18-year-old stands (*O* control, *R* removal of litter and humus layers, *A* addition of organic material, *n* number of seedlings harvested, *Rl* root length, *NrRt* number of root tips per seedling, $Rt \cdot cm^{-1}$ number of root tips/cm root per seedling)

Initial inoculum	5-year-old stand				18-year-old stand			
	n	Rl	NrRt	$Rt \cdot cm^{-1}$	n	Rl	NrRt	$Rt \cdot cm^{-1}$
O: Noninoculated	10	169±63	556.9±230.1	3.4±0.6	10	34±17	87.1±55.2	2.4±0.9
Laccaria bicolor	3	68±15	151.5± 29.5	2.3±0.1	10	34±17	87.1±55.2	2.4±1.0
Paxillus involutus	7	110±40	399.7±194.0	3.5±0.5	10	20± 4	60.6±31.3	2.9±1.0
Rhizopogon luteolus	10	103±34	445.6±176.6	4.3±0.9	10	28± 8	82.7±52.9	2.9±1.6
R: Noninoculated	10	87±28	393.0±159.1	4.5±1.0	10	39±15	129.2±39.2	3.5±0.7
Laccaria bicolor	3	68±14	170.3± 65.6	2.5±0.5	10	54±31	132.6±96.3	2.2±0.8
Paxillus involutus	8	70±29	224.1± 98.4	3.2±0.7	10	36±13	128.5±69.3	3.4±1.2
Rhizopogon luteolus	5	72±32	271.0±113.0	3.8±0.7	10	41± 7	96.0±42.0	2.3±0.7
A: Noninoculated	10	104±40	279.5±137.8	2.5±0.3	10	40±19	83.2±57.6	1.9±0.4
Laccaria bicolor	10	94±52	287.0±201.1	2.9±0.6	10	53±20	139.1±59.3	2.5±0.5
Paxillus involutus	10	100±31	319.2±152.7	3.0±0.8	10	33±13	105.4±67.0	3.3±2.1
Rhizopogon luteolus	9	100±39	266.8±102.7	2.6±0.5	10	36±11	105.7±51.5	2.9±1.2

sporocarp surveys and *Cenococcum*, *Hebeloma* and ITE3 only below ground (Tables 1, 3). Higher numbers of ectomycorrhizal genera were found in all treatments in the 18-year-old stand below ground than by investigating sporocarps (Fig. 2). The ectomycorrhizal genera *Amanita*, *Inocybe* and *Russula* were only observed by sporocarp surveys and Cenococcum and ITE3 only below ground (Tables 1, 3).

Discussion

Ectomycorrhizal genus composition on the seedlings was independent of initial inoculum (Table 1). However, in two cases *Paxillus* was observed in low amounts which might be either inoculated or indigenous. In the 5-year-old stand *Paxillus* was mostly replaced by *Laccaria*, which can be attributed either to direct competition among the fungi or to environmental factors like the nutrient content of the soil (Stenström 1990) differentially affecting the fungi. It is unclear whether inoculated or indigenous *Laccaria* and *Rhizopogon* were observed. We did not attempt to distinguish between inoculated and indigenous genera.

Manipulation of the litter and humus layers affected the ectomycorrhizal types on the seedlings. The highest numbers of vital ectomycorrhizal types were observed in the sod-cut plots (Table 1). These observations are in accordance with observations above ground (Table 3, Fig. 2). Earlier investigations showed that ectomycorrhizal sporocarps and species were positively affected by removal of litter and humus layers (Grosse-Brauckmann and Grosse-Brauckmann 1978; Termorshuizen 1990; Tyler 1991; Baar and Kuyper 1993), probably because of removal of large amounts of nitrogen and phenolics with the litter and humus (Baar et al. 1994b).

The lowest number of ectomycorrhizal types was observed in the sod-added plots (Table 1). Addition of ec-

Table 3 Number of ectomycorrhizal sporocarps in control (*O*), sod-cut (*R*) and sod-added (*A*) plots of 5- and 18-year-old Scots pine stands

Species	5-year-old stand			18-year-old stand		
	O	R	A	O	R	A
Amanita rubescens Pers.: Fr.					2	
Cortinarius croceus (Schaeff.) Fr.		3				
Cortinarius semisanguineus (Fr.) Gill		2	2			
Gomphidius roseus (L.: Fr.) Fr.	1					
Inocybe brevispora Huijsman					7	
Inocybe lacera (Fr.: Fr.) Kumm					33	
Laccaria bicolor	2	3	202		1301	
Laccaria proxima (Boud.) Pat.	1648	943	723	2		
Lactarius hepaticus Plowr.				14	74	1
Lactarius rufus (Scop.: Fr.) Fr.				2	40	
Paxillus involutus	9	2				
Rhizopogon luteolus	2	6				
Russula ochroleuca Pers.				8	3	
Suillus bovinus (L.: Fr.) O. Kunze	13	39	1			
Suillus variegatus (Sw.: Fr.) O. Kuntze	5					
Thelephora terrestris Willd.: Fr.	889	414	294			
Total number of sporocarps	2569	1412	1222	26	1460	1
Number of species	8	8	5	4	7	1
Number of genera	6	6	4	3	5	1

torganic material led to thick humus layers with high amounts of nitrogen and humus components like phenolics. A negative effect of thick humus layers on the ectomycorrhizal types *Dermocybe*, *Hebeloma* and *Piloderma croceum* was observed by Markkola and Ohtonen (1988), who suggested that this was partly due to high amounts of nitrogen in the humus layers. Arnebrant and Söderström (1992) showed that large amounts of nitrogen negatively affected seedling ectomycorrhizas. A negative effect of the presence of organic layers on ectomycorrhizas of seedlings of *Abies concolor* (Gord. et Glend.) Lindl.) was likewise noticed by Alvarez et al. (1979). Schoeneberger and Perry (1982) found a negative effect of forest litter on ectomycorrhizal root tips of seedlings of *Pseudotsuga menziesii* (Mirb.) Franco.

The genus composition of the ectomycorrhizal fungi below and above ground differed among the two stands in all treatments (Table 1). *Laccaria*, presumably mainly consisting of *Laccaria proxima* as shown by the results of the sporocarp survey (Table 3), dominated in all treatments in the 5-year-old stand. Dominance of *Laccaria proxima* in the 5-year-old stand can be explained by establishment from spore inocula when colonizing previously unforested sites (Newton 1992). *Laccaria* ectomycorrhizas in the sod-added plot in the 5-year-old stand may also belong to *Laccaria bicolor* as the results of the sporocarp survey show (Table 3). *Laccaria bicolor* probably benefited from nitrogen present in litter (Baar et al. 1994a). An increase of sporocarps of *Laccaria bicolor* was recorded after fertilization in pine forests (Ohenoja 1988). The most abundant mycorrhizal types on the seedlings in the 18-year-old stand were *Laccaria*, *Lactarius* and *Russula*. *Laccaria* ectomycorrhizas in the 18-year-old stand presumably belong to *Laccaria bicolor* as sporocarps of *Laccaria bicolor* were mainly recorded.

Lactarius and *Russula*, only found in the 18-year-old stand, were able to form ectomycorrhizas with the outplanted seedlings (Tables 1, 3). Fleming et al. (1986) likewise observed in a birch wood that young seedlings largely colonized by fungi were usually found near mature trees. A consequence of this means of infection is that young plants are rapidly integrated into an absorptive network of ectomycorrhizal mycelia, which is likely to be of considerable ecological significance (Read 1991). A similar proposal was made by Arnebrant (1991) who observed the same ectomycorrhizal types on seedlings and on mature trees.

Cenococcum was found on the roots of noninoculated seedlings in the sod-cut plot in the 18-year-old stand (Table 1). This observation is in accordance with findings of Dahlberg and Stenström (1991), who found *Cenococcum* on seedlings planted in mineral soil without humus in former Scots pine forests, but contradicts results of Meyer (1987) and Markkola and Ohtonen (1988), who noticed that ectomycorrhizas of *Cenococcum* were abundant in thick nitrogen-rich humus layers.

The number of root tips/cm root was in accordance with findings by Arnebrant and Söderström (1992). The low numbers of root tips, the low root lengths (Table 2), the low frequencies of vital ectomycorrhizal root tips, high frequencies of nonectomycorrhizal roots (Table 1) and low number of root tips/cm root in all treatments in the 18-year-old stand are probably due to phenolic compounds in the ectorganic layer and to inorganic ammonium in the soil (J. Baar and F.W. de Vries, unpublished work).

Several ectomycorrhizal fungi were not observed by either baiting or sporocarp survey. Genera such as *Cenococcum* and ITE3 never form sporocarps. And no sporocarps of *Hebeloma* were found although ectomycorrhizas were observed. *Hebeloma* might have either

colonized the baits and disappeared after harvesting the outplanted seedlings, or it might also have formed ectomycorrhizas with mature trees and established permanently. Slow-growing fungi might not colonize the seedlings within one growing season even though they were present at high relative frequencies on the roots of mature trees (Arnebrant and Söderström 1991). Dahlberg and Stenström (1991) noted that the number of ectomycorrhizal roots of outplanted seedlings increased 2.5 times within 2 years. An alternative explanation is that some species that may grow rapidly but have just established in the sod-cut plots have too little mycelia to colonize the seedlings (Dahlberg and Stenström 1991) in 5 months. Sporocarps of *Inocybe lacera* were recorded in low numbers in sod-cut plots in Scots pine stands and none in control plots 18 months after sod cutting (Baar and Kuyper 1993). This indicates colonization of the sod-cut plots by *I. lacera* by spores after sod cutting. At the time our seedlings were planted only small sized mycelia of *I. lacera* would be expected.

Baiting is a good method to determine the colonization potential of ectomycorrhizal fungi due to different treatments (Arnebrant and Söderström 1992). Ectomycorrhizal genera of which no sporocarps were observed (no sporocarp formation, hypogeous genera) are also recorded by outplanting seedlings. Although it is possible to identify the ectomycorrhizal types of *Pinus sylvestris* to the genus level, in most cases it is still impossible to identify these types to the species level. A combination of observing sporocarps and roots of outplanted seedlings is a good way to study the effects of different treatments on physiologically active ectomycorrhizal fungi in the field. However, it would be worthwhile to leave the baits in the field for more than one growing season, enabling slower-growing and just established fungi to colonize the seedlings.

The results of the present study demonstrate that manipulation of litter and humus layers strongly affects the ectomycorrhizal colonization capacity. What Termorshuizen (1991) concluded for the occurrence of ectomycorrhizal sporocarps in Scots pine forests of different ages also holds for mycorrhization of seedlings: it is not ageing of the trees but ageing of the forest soil which is likely to be the main factor determining ectomycorrhizal infection.

Acknowledgements This is communication 529 of the Biological Station, Wijster. The investigations were supported by the Foundation for the Life Sciences (SLW), which is subsidized by the Netherlands Organization for Scientific Research (NWO). We thank Dr. E. Arnolds, Dr. L. Brussaard and Dr. T.W. Kuyper for critical reading of the manuscript.

References

Agerer R (1987) Colour atlas of ectomycorrhizae. Einhorn, Schwäbisch Gmünd

Alexander IJ, Bigg WL (1981) Light microscopy of ectomycorrhizas using glycol methacrylate. Trans Br Mycol Soc 77:425–429

Alvarez IF, Rowney DL, Cobb FW Jr (1979) Mycorrhizae and growth of white fir seedlings in mineral soil with and without organic layers in a California forest. Can J For Res 9:311–315

Arnebrant K (1991) Effects of forest fertilization on soil microorganisms. PhD thesis, University of Lund

Arnebrant K, Söderström B (1992) Effects of different fertilizer treatments on ectomycorrhizal colonization potential in two Scots pine forests in Sweden. For Ecol Manag 53:77–89

Baar J, Kuyper TW (1993) Litter removal in forests and effect on mycorrhizal fungi. In: Pegler DN, Boddy L, Ing B, Kirk PM (eds) Fungi of Europe: investigation, recording and mapping. Kew Gardens, London, pp 275–286

Baar J, Ozinga WA, Kuyper TW (1994a) Spatial distribution of *Laccaria bicolor* genets reflected by sporocarps after removal of litter and humus layers in a *Pinus sylvestris* forest. Mycol Res 98:726–728

Baar J, Ozinga WA, Sweers IL, Kuyper TW (1994b) Stimulatory and inhibitory effects of needle litter and grass extracts on the growth of some ectomycorrhizal fungi. Soil Biol Biochem 26:1073–1079

Dahlberg A, Stenström E (1991) Dynamic changes in nursery and indigenous mycorrhiza of *Pinus sylvestris* seedlings planted out in forest and clearcuts. Plant Soil 136:73–86

Fleming LV, Deacon JW, Last FT (1986) Ectomycorrhizal succession in a Scottish birch wood. In: Gianinazzi-Pearson V, Gianinazzi S (eds) Physiological and genetical aspects of mycorrhizae. INRA, Paris, pp 259–264

Grosse-Brauckmann H, Grosse-Brauckmann G (1978) Zur Pilzflora der Umgebung von Darmstadt vor 50 Jahren und heute (ein Vergleich der floristischen Befunde Franz Kallenbachs aus der Zeit von 1918 bis 1942 mit dem gegenwärtigen Vorkommen der Arten). Z Mykol 44:257–269

Ingleby K, Mason PA, Last FT, Fleming LV (1990) Identification of ectomycorrhizas. ITE research publication no 5, London, pp 1–112

Markkola AM, Ohtonen R (1988) The effect of acid deposition on fungi in forest humus. In: Jansen AE, Dighton J, Bresser AHM (eds) Ectomycorrhizae and acid rain. Commission of the European Communities, Brussels, pp 122–126

Meyer FH (1987) Extreme Standorte und Ektomykorrhiza (insbesondere *Cenococcum geophilum*). Angew Bot 61:39–46

Newman EI (1966) A method of estimating the total length in a root sample. J Appl Ecol 3:139–145

Newton AC (1992) Towards a functional classification of ectomycorrhizal fungi. Mycorrhiza 2:75–79

Ohenoja E (1988) Effect of forest management procedures on fungal fruitbody production in Finland. Acta Bot Fenn 136:81–84

Read DJ (1991) Mycorrhizas in ecosystems. Nature's response to the "law of the minimum". In: Hawksworth DL (ed) Frontiers in mycology. CAB International, Wallingford, UK

Schoeneberger MM, Perry DA (1983) The effect of soil disturbance on growth and ectomycorrhizae of Douglas-fir and western hemlock seedlings: a greenhouse bioassay. Can J For Res 12:343–353

Siegel S, Castellan NJ (1988) Nonparametric statistics for the behavioral sciences. McGraw-Hill, New York

Sokal RR, Rohlf FJ (1981) Biometry, 2nd edn. Freeman, San Francisco

Stenström E (1990) Ecology of mycorrhizal *Pinus sylvestris* seedlings. Aspects of colonization and growth. PhD thesis, Swedish University of Agricultural Sciences, Uppsala

Termorshuizen AJ (1990) Decline of carpophores of mycorrhizal fungi in stands of *Pinus sylvestris*. PhD thesis, Agricultural University Wageningen

Termorshuizen AJ (1991) Succession of mycorrhizal fungi in stands of *Pinus sylvestris* in the Netherlands. J Veg Sci 2:555–564

Tyler G (1991) Effects of litter treatments on the sporophore production of beech forest macrofungi. Mycol Res 95:1137–1139