Ectomycorrhizae in Scots pine seedlings at different trophic levels of a drained mire. A preliminary study

Tytti Sarjala and Seppo Kaunisto

Tytti Sarjala & Seppo Kaunisto, Finnish Forest Research Institute, Parkano Research Station, Kaironiementie 54, FIN-39700 Parkano, Finland (e-mail tytti.sarjala@metla.fi, seppo.kaunisto@metla.fi)

The mycorrhizal infection of the roots of four-year-old Scots pine seedlings was studied by analysing root ergosterol, endogenous polyamines, nitrogen, phosphorus and potassium concentrations after two years in the field. The seedlings had been planted in a 25-year-old pine plantation on an originally treeless mire representing a wide peat nitrogen gradient. Common mycorrhizal types with Scots pine, such as *Cenococcum*, *Piloderma* and *Boletaceae* types, and a number of unidentified types were found in the roots. The roots contained quite normal or a little lower levels of ergosterol than reported elsewhere on tree roots in mineral soil forests. After two growing seasons in the field a positive correlation (r = 0.584**) was found between the root ergosterol and peat nitrogen concentrations. The root ergosterol and spermidine concentrations correlated positively. This may be due to a more abundant physical presence of the fungal tissue which contains more spermidine than the other polyamines, or it may be due to a positive effect of the mycorrhizal fungi on the metabolic activity of the roots.

Key words: Ectomycorrhiza, ergosterol, peat nitrogen, *Pinus sylvestris*, polyamines

INTRODUCTION

Nitrogen is an important determinant of productivity in boreal ecosystems. In acid organic soils such as in drained peatland forests, the mineralisation rate is low and a high proportion of the total N is present in organic forms. Ectomycorrhizal symbiosis improves the ability of the trees to take up nutrients, and the ability of the ectomycorrhizal fungi to use organic nitrogen has been appreciated for some time (Smith and Read 1997). Paavilainen (1966) studied the effect of drainage intensity on the occurrence of short roots in peat and later (Paavilainen 1968) the effect of fertilisation (nutrient combination and amount) on the occurrence of short roots, but mycorrhizal symbiosis has not been very widely studied in drained peatland forests. However, it can be assumed that ectomycorrhizal fungi have a fundamental role in the nutrition of the trees in peatland forest ecosystems where soluble organic N compounds constitute a significant pool as primary sources of nitrogen.

Polyamines are a part of the overall metabolism of nitrogenous compounds although they do not seem to function in the normal nitrogen nutrition (Altman & Levin 1993). Among them putrescine, spermidine and spermine are essential for the normal growth and development of plants, animals and micro-organisms (Galston and Kaur-Sawhney 1990). Putrescine has been used as an indicator for nutrient imbalance, especially for

latent potassium deficiency (Sarjala & Kaunisto 1993, 1996, Kaunisto & Sarjala 1997).

Mycorrhizal symbiosis is also known to affect the polyamine contents of plants (Kytöviita & Sarjala 1997), and the source of nitrogen in pure culture conditions affects the endogenous polyamine concentrations of the ectomycorrhizal fungal mycelium (Sarjala 1999). Johnson & McGill (1990) used ergosterol, a membrane component of the fungal cells, and biosynthetic enzymes of polyamines, arginine decarboxylase and ornithine decarboxylase, to indicate the metabolic activity and hyphal growth of an ectomycorrhizal fungus, *Hebeloma crustuliniforme.*

Most pine growing peatland sites are known to have imbalance between different nutrients, especially between mineral nutrients and nitrogen. On the other hand*,* mycorrhizal symbiosis is known to be of crucial importance for tree nutrition. Even so, the knowledge of mycorrhizal infection in drained peatland forests is limited (Paavilainen 1966, 1968). Several studies have shown that pine growth on peatland sites is closely related to the peat total nitrogen concentration if the other nutrients are in balance (e.g. Kaunisto 1982, 1985, 1987). Therefore we focused our study on the abundance and activity of mycorrhizae in Scots pine seedlings on a drained peatland site having a wide peat total N gradient. This study is a preliminary work in a larger project focusing on nitrogen dynamics in drained peatland forests in Finland.

Arnebrandt (1991) and Dahlberg and Stenström (1991) have used baiting plants for studying ectomycorrhizal colonization potential in Scots pine forests in Sweden. A similar approach was used in this investigation by using Scots pine seedlings planted inside young pine stands on a drained mire. A rough identification of the mycorrhizal types was made. Root ergosterol was analysed to indicate the fungal biomass in the roots. The endogenous polyamines, nitrogen, phosphorus and potassium concentrations of the seedlings were analysed to indicate the nutrient conditions and metabolic activity of the roots.

MATERIAL AND METHODS

Nursery grown two-year-old Scots pine (*Pinus sylvestris* L.) seedlings were used as baits for mycor-

rhizal fungi in young Scots pine stands on a drained mire (62°9´N, 22°52´E). Site types on this, originally treeless mire, varied from low-sedge bogs to sedge-rich fens. The mire had been drained in 1969 and sown and planted with pine in 1974. Two similar experiments involving several different fertilisation and site improvement treatments such as liming and site preparation, were established on the site in 1974 (Alkkia Exp. 109a + b, Kaunisto 1982, Kaunisto et al. 1986). Twenty-five PK- or NPK- broadcast fertilised plots from these experiments were chosen for this study. PK fertiliser, a mixture of Moroccan rock phosphate and KCl, amounting to 42 kg ha^{-1} of elemental P and 50 kg ha^{-1} of K were spread on the peat surface. Nitrogen was applied as ammonium nitrate fertiliser amounting to 100 kg ha^{-1} of elemental nitrogen. Fertilisation treatments were repeated in 1989. The plots for this study were selected on the basis of the earlier results in order to create a wide peat total nitrogen gradient. The total nitrogen concentration in peat ranged from 0.76 to 2.58% in the peat layer 5–10 cm from the surface (Kaunisto 1982).

Thirty two-year-old pine seedlings were planted on each plot between the tree rows (about 3.2 m apart) on unprepared peat surface in May 1997 and some extra seedlings were taken for visual observations and ergosterol analyses. After the first and the second growing seasons (September 1997 and October 1998) ten pine seedlings per plot were collected for analyses.

The fungal biomass in the roots from three seedlings per plot in 1997 and from one to three seedlings per plot in 1998 was estimated by using ergosterol analyses performed with HPLC (Nylund and Wallander 1992).

A visual examination of the roots from five seedlings per plot was carried out under a dissection microscope to make a rough estimation of the fungal types classified according to their colour and structure (Laiho et al. 1987). The occurrence of some distinctive types of mycorrhizae was recorded from the plots: yellow *Piloderma*- and black *Cenococcum*-types, a group of types having an outlook typical of *Boletaceae*, rhizomorphous type, light brown and dark brown smooth-types with clusters, and a thick smooth type.

All needles and roots of the sampled seedlings

within one plot were pooled to represent that plot. The nutrients in the needles and roots were analysed with the methods routinely used at the Finnish Forest Research Institute (Halonen et al. 1983). Nitrogen was analysed with a CHN-analyzer (LECO CHN-600). Phosphorus was measured spectrophotometrically from dry-ashed material with the vanado-molybdate method. The analyses of potassium were performed by a flame atomic spectrophotometer (Varian AA-30). The polyamine concentrations of the roots were analysed with HPLC (Merck-Hitachi) after $HClO_4$ extraction and dansylation (Sarjala & Kaunisto 1993).

Regression and correlation analyses were used to analyse the relationships of the needle and root nutrient and root ergosterol concentrations with the peat total nitrogen concentration and the relationships between the root polyamine and ergosterol concentrations. Differences between the slopes of regression lines of ergosterol plotted against peat N between the years 1997 and 1998 were also tested.

RESULTS

Mycorrhizal types and fungal biomass

All of the nursery grown Scots pine seedlings were mycorrhizal before planting in the forest. The initial mycorrhizal roots were mainly of brown and smooth types. *Boletaceae*-types were observed in 50% of the seedlings. After one or two growing seasons in the forest several mycorrhizal types common to Scots pine roots were found (Table 1). *Piloderma*- and *Cenococcum*-types, which were not observed in the nursery seedlings, were found to colonise the roots in the forest, *Piloderma* mainly on the low N plots and *Cenococcum* in the majority of the plots. Dark and light brown smooth types with clusters were found on all the plots

Table 1. Mycorrhizal types of the seedlings on the twenty five experimental plots representing peat N gradient from 0.8 to 2.6% N in peat (5–10 cm) in 1997 and 1998. 1 = found, 0 = not found.

| N% in peat	Cenococcum		Thick smooth		Boletaceae-type		Dark brown		Light brown		Rhizomorphs		Piloderma	
	97	98	97	98	97	98	97	98	97	98	97	98	97	98
0.8	1	1	1	1	1	1	1	1	1	1	1	1	1	0
1.0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1.0	1	1	1	1	1	1	1	1	1	0	1	1	1	1
1.1	1	1	1	1	1	1	1	1	1	1	1	1	0	0
1.2	0	1	1	0	1	1	1	1	0	1	1	1	0	0
1.2	1	0	1	1	1	1	1	1	1	1	1	1	1	1
1.3	1	1	1	1	1	0	1	1	1	1	1	0	1	1
1.4	1	1	1	1	1	1	1	1	1	1	1	1	1	0
1.5	1	0	1	1	1	1	1	1	1	1	1	1	0	1
1.6	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1.6	1	1	1	0	1	1	1	1	1	1	1	1	0	0
1.8	1	1	1	1	1	1	1	1	1	1	1	0	0	0
1.8	1	1	1	1	1	0	1	1	1	1	1	0	1	0
1.8	1	0	1	1	1	0	1	1	1	1	1	0	1	0
1.8	1	0	1	0	1	0	1	1	1	1	1	0	0	0
1.9	1	1	1	1	1	0	1	1	1	1	0	0	1	1
1.9	1	1	0	1	1	1	1	1	1	1	1	1	0	0
2.0	1	1	1	1	1	0	1	1	1	1	1	0	0	0
2.0	1	1	1	1	1	1	1	1	1	1	1	0	0	1
2.0	0	1	1	0	1	0	1	1	1	1	1	1	0	0
2.1	1	1	1	0	0	0	1	1	1	1	0	0	0	0
2.2	1	1	1	1	0	1	1	1	1	1	0	1	0	0
2.3	1	1	1	0	1	0	1	1	1	1	1	0	0	0
2.3	1	0	1	0	1	0	1	1	1	1	1	0	0	0
2.6	1	1	1	1	1	0	1	1	1	1	1	0	0	0
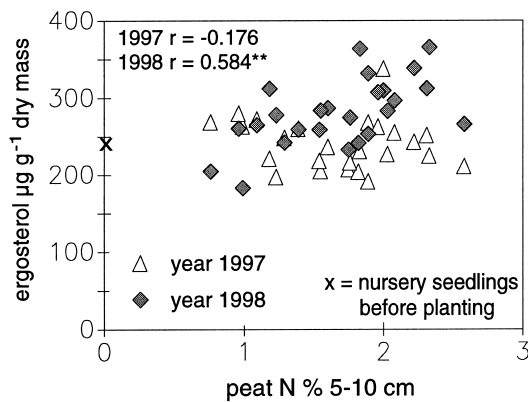
Fig. 1. Correlation between the root ergosterol and the peat total nitrogen concentrations in the 5–10 cm peat layer in 1997 and 1998.

throughout the N gradient. Increases in *Boleta-ceae*-types and types with rhizomorphs were also observed, when compared with the initial colonization of the nursery seedlings. After the second year *Boletaceae-* and *Piloderma*-types and those with rhizomorphs were found more frequently on low than high nitrogen plots. In fact, *Piloderma* was not found on the plots with the peat N concentration > 2.0% (Table 1). The Scots pine seedlings did not survive very well on some plots and after the second year some of the seedlings had died.

The peat total nitrogen concentration did not correlate with the root ergosterol concentration after the first growing season. After two growing seasons a positive correlation (r = 0.584**) was found between the root ergosterol and peat nitrogen content (Fig. 1). No significant difference was, however, found between the slopes of regression lines in 1997 and 1998. The nursery seedlings were mycorrhizal before planting with the average ergosterol concentration 249 ± 45 mg g^{-1} dry mass. The ergosterol concentrations in the roots of the transplanted seedlings were slightly higher on average in 1998 (281 ± 44 mg g^{-1}) than in 1997 (240 ± 33 mg g^{-1}) (p < 0.01).

Nutrients

The average N concentrations in the needles and roots were 11.6 mg g^{-1} dry mass and 9.8 mg g^{-1} dry mass respectively in 1997 and they showed a

significant increase in 1998 being 13.7 mg g^{-1} dry mass in the needles and 10.6 mg g^{-1} dry mass in the roots. No significant differences in the needle P or K concentrations could be found between 1997 (P 1.28 mg g^{-1}, dry mass, K 4.47 mg g^{-1} dry mass on average) and 1998 (P 1.33 mg g^{-1} dry mass, K 4.53 mg g^{-1} dry mass), whereas the average P and K concentrations in the roots decreased significantly from 1997 (P 1.47 mg g^{-1} dry mass, K 3.29 mg g^{-1} dry mass) to 1998 (P 1.28 mg g^{-1} dry mass, K 2.85 mg g^{-1} dry mass) (Fig. 2). The needle N/P ratio was 9.1 on average in 1997 and slightly higher (10.3) in 1998. There was no difference in the needle and root nitrogen concentrations between PK and NPK fertilised seedlings in 1997 but the concentrations were slightly higher on the NPK fertilised plots in 1998.

A significant positive correlation between the root and peat total nitrogen and between the needle and peat total nitrogen concentrations (Fig. 2) and also between the needle N/P ratio and peat total nitrogen concentration (r = 0.474, p = 0.019) were found in 1997 but not in 1998. No correlation was found between the potassium or phosphorus concentrations of the seedlings and the peat nitrogen concentration.

Polyamines

The putrescine concentrations of the roots varied from 126 to 398 nmol g^{-1} fresh weight, spermidine from 25 to 81 nmol g^{-1} fresh weight and spermine from 1 to 5 nmol g^{-1} fresh weight.

The polyamine and the main nutrient (N, P, K) concentrations in the roots did not correlate with each other. The polyamine levels of the needles were not measured.

Putrescine and spermine concentrations had a positive but non-significant correlation with the ergosterol concentrations of the roots (Fig. 3), whereas a significant correlation was found between spermidine and ergosterol (r = 0.440, p < 0.05 in 1997 and r = 0.634, p < 0.001 in 1998).

DISCUSSION

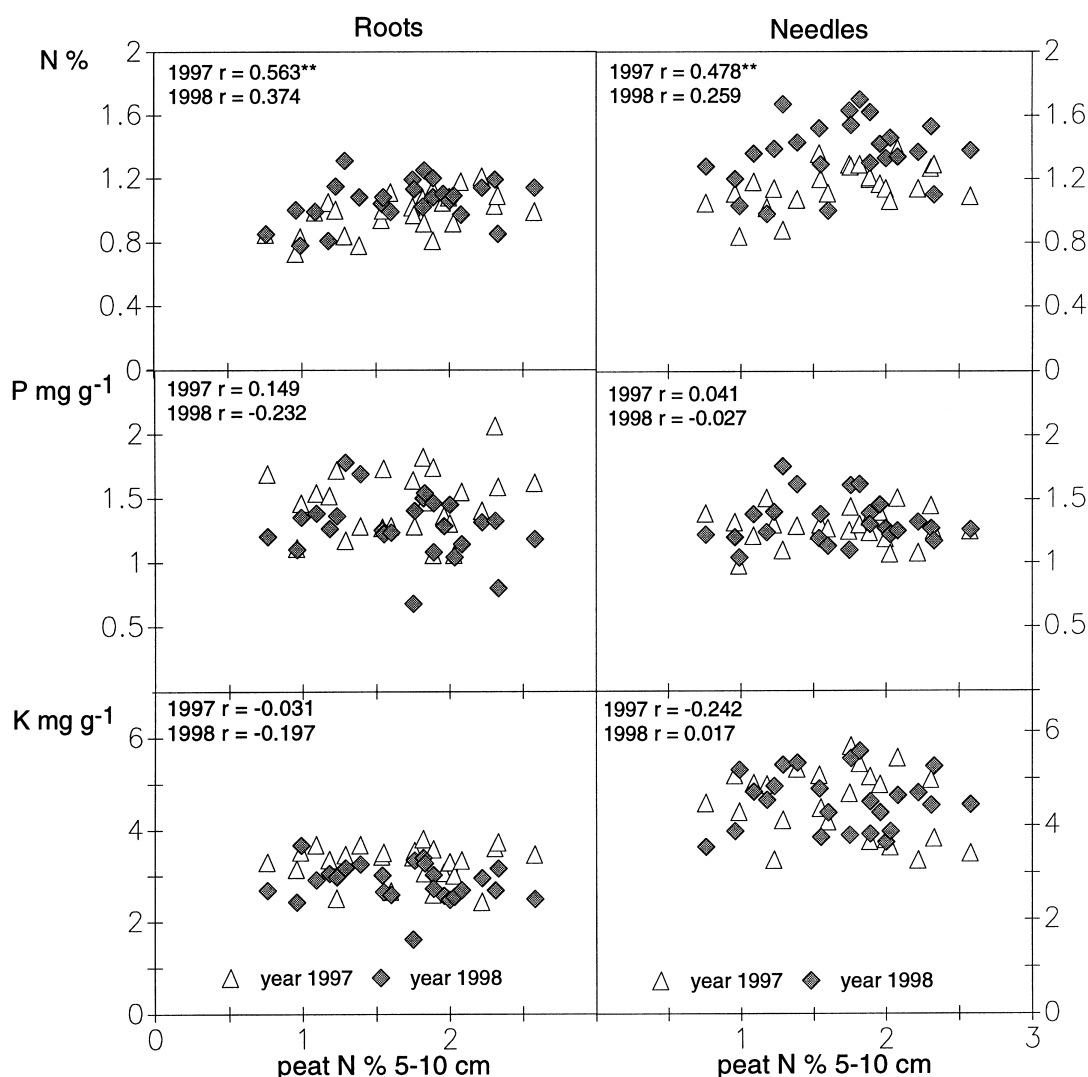The roots of Scots pine seedlings grown in drained peatland forest stands were mycorrhizal on all the

Fig. 2. Correlations between the nitrogen (% dry mass), phosphorus and potassium concentrations (mg g⁻¹ dry mass) of the roots and needles and the peat total nitrogen concentration in the 5–10 cm peat layer in 1997 and 1998.

plots and had common mycorrhizal types and a number of unidentified types. They contained similar or slightly lower concentrations of ergosterol than reported in the pine roots in mineral forest soils (Markkola et al. 1995).

Dahlberg and Stenström (1991) reported that indigenous mycorrhizal species gradually suppressed the initial dominance of nursery mycorrhizae within two years after planting of Scots pine seedlings in the forest. In this study, the average ergosterol concentration of the roots decreased first from the levels found in the nursery seed-

lings but increased again in the following year. This suggests a temporary decrease in the root fungal biomass after planting or it may also be due to changes in the mycorrhizal fungal species in the roots. Changes in the mycorrhizae after planting were observed in *Piloderma-*, *Cenococcum-* and *Boletaceae*-types and types with rhizomorphs, which all increased in frequency.

Interestingly, the nitrogen concentration of the seedlings and the needle N/P ratio were more dependent on the peat total nitrogen content after the first growing season when the ergosterol con-
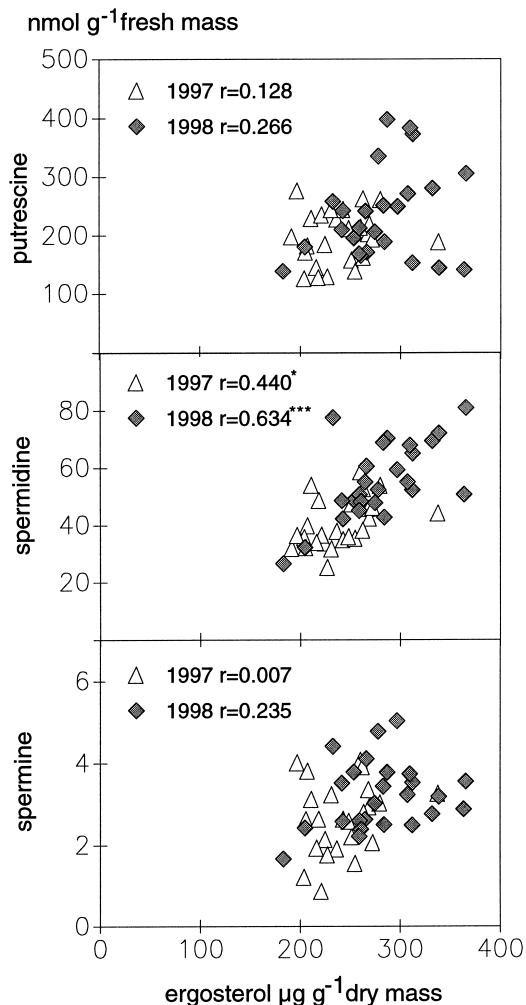
nmol g^{-1}fresh mass

Fig. 3. Correlations between the putrescine, spermidine and spermine concentrations and the ergosterol concentration of the roots in 1997 and 1998.

among the mycorrhizal species in the roots.

Almost all seedlings suffered from both nitrogen and phosphorus deficiency (N < 13.1 and P < 1.37 mg g^{-1}, Paarlahti et al. 1971) in the first year and from phosphorus deficiency still in the second. However, the needle N concentrations rose above the limit value in most seedlings in the second year. The root and needle nitrogen concentrations increased quite independently of the peat total nitrogen concentration implying an overall better nitrogen nutrition of seedlings. The polyamine concentrations in the roots represented quite normal levels found in earlier studies in Scots pine seedling roots under controlled nutrient and temperature conditions (Sarjala et al. 1997). The potassium concentrations of the pine roots were about 3 mg g^{-1} dry mass being at about the same level as in the heavily fertilised sapling stands in the study by Paavilainen (1968). No accumulation of putrescine was observed in these cases, as in K deficient Scots pine and Norway spruce needles (Sarjala & Kaunisto 1993, 1996, Kaunisto & Sarjala 1997) and non-mycorrhizal Scots pine roots (Sarjala 1996). In mycorrhizal roots polyamine profiles are affected by the mycorrhizal fungus and are not determined only by the K status of the roots. The ergosterol and spermidine concentrations of the roots correlated positively. This may be caused by the physical presence of the fungal tissue which contains more spermidine than the other polyamines (Sarjala 1999), or it reflects a positive effect of the mycorrhizal fungi on the metabolic activity of the roots (Johnson & McGill 1990).

When interpreting the results one has to keep in mind that the investigation represents only one case of a peat N gradient in a drained peatland forest and cannot be generalised. Therefore further research on the subject matter is needed. According to Paavilainen (1966) the frequency of mycorrhizal root tips and good mycorrhizal types in peatland increased after drainage. This preliminary study also shows that a drained peatland forest has mycorrhizal inoculum potential in soil. The presence and activity of mycorrhizae are among the factors which affect nutrient uptake and balance of trees in drained peatland forests and should be taken into account in further studies when estimating tree nutrition on varying nitrogen supply in peat.

centrations were still slightly depressed after planting, whereas the root ergosterol concentrations correlated positively with the peat nitrogen concentration in the second year. Kaunisto (1982) showed that in the same experiments mineral nitrogen concentrations in peat increased along with the increasing peat total nitrogen concentration. The results imply that the seedlings were dependent on available mineral nitrogen in peat during the first growing season. After the second year the ergosterol concentrations increased suggesting an increased mycorrhizal infection or changes

REFERENCES

Altman, A. & Levin, N. 1993. Interactions of polyamines and nitrogen nutrition in plants. Physiologia Plantarum 89: 653–658.

Arnebrant, K. 1991. Effects of forest fertilization on soil micro-organisms. Doctoral Thesis. Department of Ecology. University of Lund, Sweden. 133pp.

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

Galston, A. W. & Kaur-Sawhney, R. K. 1990. Polyamines in plant physiology. Plant Physiology 94: 406–410.

Halonen, O., Tulkki, H. & Derome, J. 1983. Nutrient analysis methods. Metsäntutkimuslaitoksen tiedonantoja — The Finnish Forest Research Institute, Research Papers 121: 1–28.

Johnson, B. N. & McGill, W. B. 1990. Ontological and environmental influences on ergosterol content and activities of polyamine biosynthesis enzymes in Hebeloma crustuliniforme mycelia. Canadian Journal of Microbiology 36: 628–689.

Kaunisto, S. 1982. Development of pine plantations on drained bogs as affected by some peat properties, fertilization, soil preparation and liming. (Seloste: Männyn istutustaimien kehityksen riippuvuus eräistä turpeen ominaisuuksista sekä lannoituksesta, muokkauksesta ja kalkituksesta ojitetuilla avosuolla.) Communicationes Instituti Forestalis Fenniae 109: 1–56.

Kaunisto, S. 1985. Lannoituksen, ilman lämpösumman ja eräiden kasvualustan ominaisuuksien vaikutus mäntytaimikoiden kasvuun turvemailla. (Summary: Effect of fertilization, temperature sum and some peat properties on the height growth of young pine sapling stands on peatlands). Folia Forestalia 616: 1–27.

Kaunisto, S. 1987. Effect of refertilization on the development and foliar nutrient contents of young Scots pine stands on drained mires of different nitrogen status. (Seloste: Jatkolannoituksen vaikutus mäntytaimikoiden kehitykseen ja neulasten ravinnepitoisuuksiin typpitaloudeltaan erilaisilla ojitetuilla soilla.) Communicationes Instituti Forestalis Fenniae 140: 1–58.

Kaunisto, S., Kinnunen, K., Lehtinen, S., Nevanranta, K. & Tukeva, J. 1986. Alkkian kenttäkokeet 1961–1986. Metsäntutkimuslaitoksen tiedonantoja — The Finnish Forest Research Institute, Research Papers 236: 1–98.

Kaunisto, S. & Sarjala, T. 1997. Critical needle potassium concentrations indicated by diamine putrescine in Norway spruce growing on peat soils. Silva Fennica 31:

Kytöviita, M.-M. & Sarjala, T. 1997. Effects of defoliation and symbiosis on polyamine levels in pine and birch. Mycorrhiza 7: 107–111.

Laiho, O., Sarjala, T., Hyvärinen, R. & Rautiainen, L. 1987. Lannoituksen vaikutus männikön mykoritsoihin. Folia Forestalia 699: 1–22.

Markkola, A. M., Ohtonen, R., Tarvainen, O. & Ahonen-Jonnarth U. 1995. Estimates of fungal biomass in Scots pine stands on an urban pollution gradient. New Phytologist 131: 139–147.

Nylund, J. E. & Wallander, H. 1992. Ergosterol analysis as a means of quantifying mycorrhizal biomass. Methods in Microbiology 24: 77–88.

Paarlahti, K., Reinikainen, A. & Veijalainen, H. 1971. Nutritional diagnosis of Scots pine by needle and peat analysis. (Summary: Maa- ja neulasanalyysi turvemaiden männiköiden ravitsemustilan määrityksessä.) Communicationes Instituti Forestalis Fenniae 74.5: 1–58.

Paavilainen, E. 1966. Maan vesitalouden järjestelyn vaikutuksesta rämemännikön juurisuhteisiin. (Summary: On the effect of drainage on the root systems of Scots pine on peat soils.) Communicationes Instituti Forestalis Fenniae 61.1: 1–110.

Paavilainen, E. 1968. Juuristotutkimuksia Kivisuon metsänlannoituskoekentällä. (Summary: Root studies at the Kivisuo forest fertilization area.) Communicationes Instituti Forestalis Fenniae 66.1: 1–31.

Sarjala, T. 1996. Growth, potassium and polyamine concentrations of Scots pine seedlings in relation to potassium availability under controlled growth conditions. Journal of Plant Physiology 147: 593–598.

Sarjala, T. 1999. The effect of organic and inorganic nitrogen sources on endogenous polyamines and growth of the ectomycorrhizal fungi in pure culture. Mycorrhiza 8: 277–281.

Sarjala, T. & Kaunisto, S. 1993. Needle polyamine concentrations and potassium nutrition in Scots pine. Tree Physiology 13: 87–96.

Sarjala, T. & Kaunisto S. 1996. Effect of different potassium sources on the seasonal variation of potassium and free polyamines in Scots pine needles. Silva Fennica 30: 387–398.

Sarjala, T., Taulavuori, K., Savonen, E.-M. & Edfast, A.-B. 1997. Does availability of potassium affect cold hardening of Scots pine through polyamine metabolism. Physiologia Plantarum 99: 56–62.

Smith, S. E. & Read, D. J. 1997. Mycorrhizal Symbiosis. Academic Press. Cambridge. ISBN 0-12-652840-3. 605 pp.