

Best wishes

Tarja Laatikainen

Mycorrhizal growth in pure cultures in the presence of pesticides

Tarja Laatikainen, H. Heinonen-Tanski

Department of Environmental Sciences, University of Kuopio, P. O. Box 1627, FIN-70211 Kuopio (Finland)

Accepted: January 16, 2002

Abstract

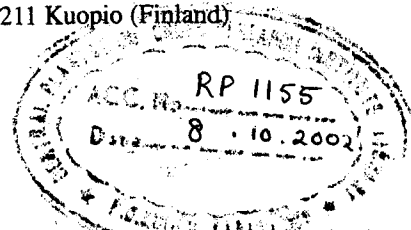
The effects of pesticides on 64 ectomycorrhizal fungi of boreal forest trees were studied *in vitro*. The pesticides (fungicides: benomyl, chlorothalonil, copper oxychloride, maneb and propiconazole; herbicides: chlorthiamid, glyphosate, hexazinone, linuron and terbuthylazine; insecticide: cypermethrin) were selected as those commonly used in Nordic forest nurseries and afforestation sites. In general, the fungicides proved to be more toxic to ectomycorrhizal fungi than the herbicides and cypermethrin. The fungicides, chlorothalonil and propiconazole, had the clearest inhibitory effect on growth of mycorrhizal fungi. Conversely, maneb, glyphosate and terbuthylazine stimulated the growth of some mycorrhizal fungi. *Leccinum versipelle* and *L. scabrum*, *Paxillus involutus* and *Cenococcum geophilum* were the most sensitive ectomycorrhizal fungi to the various pesticides.

Key words: ectomycorrhizal fungi – fungicide – herbicide – insecticide cypermethrin – pesticide side effect

Introduction

Pesticides have been widely and regularly used in forests and forest nurseries against fungal diseases (fungicides), weeds (herbicides) and herbivores (insecticides). According to a questionnaire conducted in 1996 about 1000 kilograms of pesticides (as active ingredient, a.i.) have been used each year in Finnish forest nurseries, of which 42% were herbicides, 40% fungicides and 18% insecticides (Juntunen 2001). The most commonly used herbicides were terbuthylazine (Gardoprim-Neste®) and glyphosate (Roundup®) and fungicides chlorothalonil (Bravo 500®), maneb (Maneba®) and propiconazole (Tilt 250 EC®).

Corresponding author: T. Laatikainen
e-mail: Tarja.Laatikainen@uku.fi



Pesticide usage has been more common in agricultural sites than in forests and forest nurseries in Finland (Londesborough *et al.* 2000) as is the case in other European countries (Brouwer *et al.* 1994). The trend of pesticide consumption in Finland had been declining during 1990's (Fig. 1). This decline is partly due to the better selectivity and higher efficiency of new pesticides. Thus, a lower dose of these new products can achieve the same or even better effect than was earlier possible. Nonetheless, many of these pesticides are persistent, especially in soils with a low microbial activity to degrade pesticides (Fomsgaard 1995), and they can affect the growth of tree seedlings if agricultural land is later to be afforested. This might be a greater problem in Scandinavia than in lower latitudes. The colder climate means that the half lives of pesticides may be prolonged (Heinonen-Tanski 1989).

Transportation and deposition of pesticides by air are known to be important sources of contamination of non-agricultural areas (Torstensson 1995, Dubus *et al.* 2000). For example, atrazine and lindane have been detected in the precipitation also in Finland (Hirvi and Rekolainen 1995), although the sale and usage of atrazine has been prohibited since 1991 and lindane was banned in already 1987 (Rokkanen J., personal communication). Therefore, these deposits have clearly foreign origins. These transported pesticides may have a contributory influence on the total pesticide load on soils and surface waters. Since forests cover about 75% of the Finnish land area (OECD 1999), this deposition of foreign pesticides can have a major impact in Finnish forest soils.

Rhizosphere microorganisms, especially mycorrhiza, are very important for the growth of plants. Most forest trees live in symbiotic associations with ectomycorrhizal fungi, which include members of the families of basidiomycetes and some of ascomycetes (Smith and

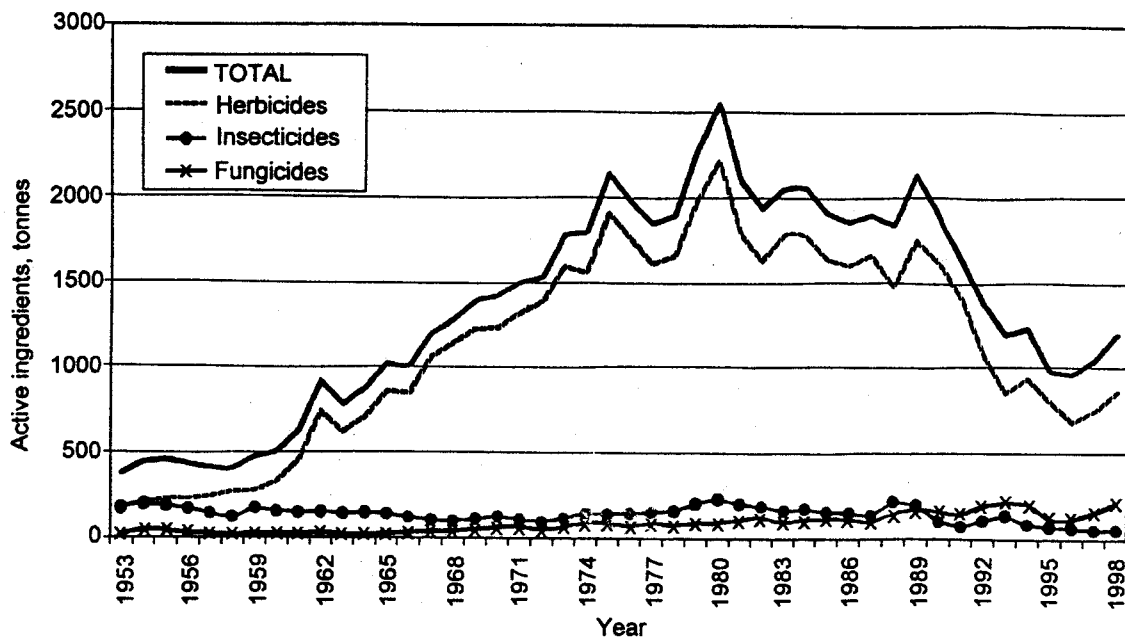


Fig. 1. The total sales of agricultural and forest pesticides as active ingredients in Finland 1953–1998 (redrawn from Hynninen and Blomqvist 1999)

Read 1997). Ectomycorrhizal fungi enhance the growth and development of tree seedlings by increasing their mineral nutrient and water uptake, by synthesizing vitamins, amino acids, auxins and gibberellins, all of which stimulate plant growth (Harley and Smith 1983), and by protecting the seedlings against potential plant pathogens (Unestam and Beyer-Ericson 1990).

Active mycorrhizal fungi are important for the thriving of forest tree seedlings in forest nurseries and for a successful start after plantation (Mikola 1973, Halonen and Laiho 1991, Marx 1991). The inoculation of ectomycorrhizal fungi to the soil of nurseries (Marx *et al.* 1986, Atlas and Bartha 1992) or soil transfer from well-stocked plantation into old non-reforested clear-cut sites (Amaranthus and Perry 1987) has increased seedling growth and mycorrhizal formation and improved considerably the survival rate of the seedlings.

Pesticide effects on soil microbes have been tested mostly in agricultural soils (e.g. Atlas *et al.* 1977, Schüepp and Bodmer 1991, Tu 1992, Tu 1993) and plants with VA-mycorrhizae (Plenchette and Perrin 1992), but there are only a few studies examining their effects on forest soils (Ingham *et al.* 1986, Colinas *et al.* 1994). The herbicides, 2,4-D and trifluralin (Iloba 1980) and the fungicide triadimefon (Marx *et al.* 1986) affected the development of ectomycorrhizae with pine seedlings in forest nurseries. Furthermore, Manninen *et al.* (1998) showed in a field trial with pine seedlings that the fungicides copper oxychloride and propiconazole could both reduce soil microbial activity and impair the development of ectomycorrhiza.

There is very little information available about the side effects of various forest pesticides on non-target ectomycorrhizal fungi. We have studied the effect of forest pesticides on ectomycorrhizal fungi of Scots pine (*Pinus sylvestris* L.), Norway spruce (*Picea abies* L.) and European silver birch (*Betula pendula* Roth.) in cultivation tests. The selected pesticides are now or have been in common use in forest nurseries and at afforestation sites in Finland (Table 1).

Materials and methods

Ectomycorrhiza. 64 ectomycorrhizal fungal strains were used for these pesticide tests (Table 2). The strains originated from isolates from our personal collections in the University of Kuopio (UKU), and from the collections of Professor Veikko Hintikka (VH) (Finland), University of Helsinki (isolates of Professor Peitsa Mikola by Hambi and Dr. Tytti Sarjala) (UH) (Finland), Swedish Agricultural University, Uppsala (SLU) (Sweden), Lund University (LU) (Sweden), and Centraalbureau voor Schimmelcultures (CBS) (Netherlands). New inoculations from slant cultures were first grown in modified Hagem's agar at $18 \pm 1^\circ\text{C}$.

Pesticides. The following pesticides were used in our experiment: fungicides, benomyl (Benlate 500 g/kg, Du Pont), chlorothalonil (technical, Sareko), copper oxychloride (Copper oxychloride 588 g/kg, Kemira), maneb (Maneba 800 g/kg, Kemira) and propiconazole

Table 1. Concentrations, application rates (per one application) and target application of studied pesticides as recommended for forest nurseries and afforestation sites. (s = solid and l = liquid formulate)

Active ingredient	Pesticide trade name in Finland (formulate)		Concentration of active ingredient [g/kg] or [g/l]	Application rate in forest nursery [kg/ha]	Target of application in forestry	References
<i>Fungicides</i>						
benomyl ¹	Benlate	(s)	500	0.2–0.6	<i>Botrytis cinerea</i> , <i>Fusarium</i> sp., <i>Altenaria</i> sp. ect.	Lilja <i>et al.</i> 1997 Du Pont 1999
chlorothalonil	Bravo 500	(l)	500	1.5–2.0	<i>Gremmeniella abietina</i> (Lagerb.), <i>Lophodermium seditiosum</i> (Minter, Stanley & Millar)	Lilja <i>et al.</i> 1997 Berner 1998
copper oxychloride	Kuprijauhe	(s)	588	0.249	various fungal diseases in seedlings	Kemira 1998
maneb	Maneba	(s)	800	0.96–1.92	<i>Gremmeniella abietina</i> (Lagerb.), <i>Lophodermium seditiosum</i> (Minter, Stanley & Millar)	Kemira 1992 Lilja <i>et al.</i> 1997
propiconazole	Tilt 250 EC	(l)	250	0.125	<i>Gremmeniella abietina</i> (Lagerb.), <i>Phacidium infestans</i> (P. Karst.), ect.	Lilja <i>et al.</i> 1997 Kemira 1998
<i>Herbicides</i>						
chlorthiamid ²	Rikkaruohontuho Prefix	(s)	75	3.75–6.0	weeds in leaf tree sapling stand	Mäkinen 1998 ⁶
glyphosate	Roundup Bio	(l)	360	1.44–2.16	weeds at afforestation site	Kemira 1998
hexazinone ³	Velpar L	(l)	240	0.24–0.48	weeds at afforestation site of coniferous trees and in pine sapling stand	Du Pont 1999
linuron	Afalon-neste	(l)	450	0.90–1.35	weeds in ornamental tree sapling stand	AgrEvo 1999
terbuthylazine	Gardoprim-neste ⁴	(l)	500	1.0–1.2	weeds at afforestation site and in forest nursery	Kemira 1996
<i>Insecticide</i>						
cypermethrin	Ripcord ⁵	(l)	100	0.017–0.027	insects in coniferous tree seedlings	Kemira 1996

¹ Expired 31. 12. 1997

² Expired 31. 12. 1996

³ Expired 31. 12. 1999

⁴ Out of sale 31. 12. 1998

⁵ Out of sale 31. 12. 1999

⁶ Personal communication

(technical, Ciba); herbicides, chlorthiamid (Prefix 75 g/kg, Kemira), glyphosate (Rodeo 480 g/l, Monsanto), hexazinone (technical, Du Pont), linuron (technical, Du Pont) and terbuthylazine (Gardoprim 500 g/l, Ciba); and an insecticide, cypermethrin (Ripcord 100 g/l, Kemira).

Pesticides were first dissolved in acetone to a concentration of 1 mg active ingredient/1 ml acetone and added to liquefied agar media to final concentrations of 1 ppm for herbicides, and 1 ppm and 10 ppm for fungicides and an insecticide, cypermethrin. Additional, tests

with propiconazole were carried out with a pesticide concentration of 0.1 ppm.

Cultivation and treatments. Tests were made with two different liquefied agar culture media, modified Hagem's medium (Modess 1941) and modified Melin-Norkrans' medium (Marx 1969). No antibiotic or fungicidal components were added to these test media (Heinonen-Tanski and Holopainen 1991). The cultivations were carried out in 90 mm diameter Petri dishes. The pesticides at various concentrations were added to

Table 2. Origin, number and average period of growth of strains of ectomycorrhizal fungi, and number of tested pesticides per one strain

Ectomycorrhizal fungi	Origin of strain	No. of tested strains	No. of tested pesticides	Period of growth [in weeks]
<i>Amanita muscaria umbria</i>	UH	1	10	2.4–9.4
<i>Amanita regalis</i>	UH	1	2	2.7
<i>Boletus edulis</i>	UKU	1	4	7.4
<i>Cantharellus cibarius</i>	UKU	1	11	3.2–5.9
<i>Cenococcum geophilum</i>	UKU	8	9–11	3.1–6.4
	SLU	1	11	3.3
<i>Corticium bicolor</i>	UH	1	11	4.7–5.6
<i>Lactarius rufus</i>	UKU	3	1, 1, 2	4.0–9.1
<i>Leccinum scabrum</i>	UKU	1	11	4.0–6.3
<i>Leccinum versipelle</i>	UKU	1	8	1.0–6.1
<i>Paxillus involutus</i>	UKU	2	2, 10	2.2–2.9
	VH	1	9	2.6
	UH	1	8	1.7–2.6
	SLU	1	10	2.6
	LU	2	6, 10	1.9–2.6
<i>Piloderma crocecum</i>	UH	1	11	3.9–7.9
<i>Pisolithus tinctorius</i>	SLU	1	7	7.8
<i>Suillus bovinus</i>	UKU	7	1–11	2.3–8.4
	VH	1	11	2.3–9.6
	LU	2	10, 11	5.0–6.0
	CBS	1	10	2.6–10.4
<i>Suillus luteus</i>	UKU	1	11	2.1–3.1
	SLU	1	11	2.1–5.2
	CBS	1	11	2.1–7.4
<i>Suillus variegatus</i>	UKU	11	2–11	2.3–6.2
	VH	1	10	2.3–6.3
	SLU	1	10	2.3
	CBS	1	8	2.3
Unidentified ectendotrophic fungus	UH	1	7	2.4
Unidentified ectomycorrhizal fungi	UKU	7	11	1.0–4.4
Total number		64	11	

Origins of isolates: University of Kuopio (UKU), Prof. Veikko Hintikka (VH), University of Helsinki (Prof. Peitsa Mikola/Dr. Tytti Sarjala) (UH), Swedish Agricultural University (SLU), Lund University (LU) and Centraalbureau voor Schimmelculturen (CBS).

liquefied agar media. Inoculations were performed in a laminar flow-chamber with sterile apparatus. Three cubic pieces with dimensions of 1–3 mm of mycelium tested were placed on duplicate pesticide and control agar dishes. Petri dishes were sealed with parafilm to avoid drying of the agar. The inoculated fungi were incubated at $18 \pm 1^\circ\text{C}$ for 2–8 weeks.

Observation. The fungal growth was compared with non-pesticide-controls after the colony of inoculated fungi at control dishes were grown to a diameter of 1 cm. The growth was estimated as (a) no pesticide effect (growth as in control), (b) inhibition by pesticide (growth less than in control), (c) strong inhibition by pesticide (no growth), and (d) stimulation by pesticide (growth better than in control) (Heinonen-Tanski *et al.* 1982).

Analysis of data. The effects of pesticides on the growth of ectomycorrhizal fungi were tested by using one-way ANOVA combined with Tukey's Multiple Range Test of the SPSS for WIN package.

Results

Fungicides

Chlorothalonil had the highest toxicity of any of the tested pesticides (Fig. 2a). Only one *Cenococcum geophilum* (UKU) and one *Suillus variegatus* (UKU) strain could tolerate chlorothalonil at the concentration of 1 ppm, and at the concentration of 10 ppm this pesticide strongly inhibited the growth of 90–100% of strains.

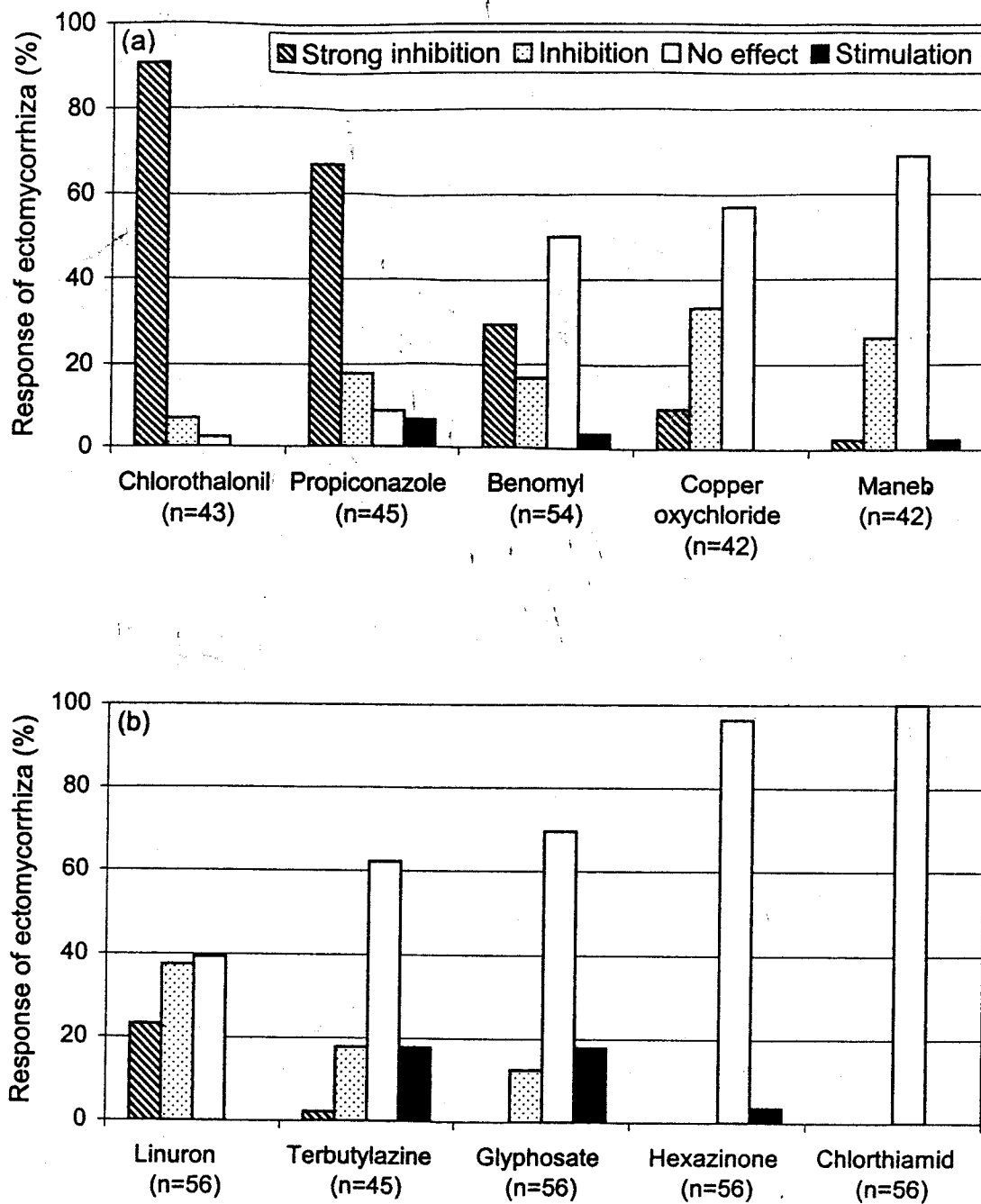


Fig. 2. Effects of fungicides (a) and herbicides (b) on the growth of ectomycorrhizal fungi at the concentration of 1 ppm on modified Melin-Norkrans' agar media. n = number of ectomycorrhizal strains tested

Inhibition was stronger on modified MMN-agar than on Hagem's agar.

Propiconazole had also clear toxicity to the tested ectomycorrhizal fungi (Fig. 2a). The inhibition effect was a slightly stronger on modified Hagem's agar. Surprisingly, at concentrations of 0.1 ppm and 1 ppm, the growth of some ectomycorrhizal fungi was stimulated. At the concentration of 0.1 ppm on modified MMN-agar, propiconazole stimulated the growth of three *C. geophilum* (UKU) (Fig. 3a) and *Cantharellus*

cibarius (UKU) (Fig. 3c) strains. Furthermore, three other strains of *C. geophilum* (UKU), and one strain of *S. variegatus* (UKU) and *Amanita muscaria umbria* (UH) could tolerate propiconazole. At the concentration of 1 ppm on modified Hagem's agar, the growth of one *Suillus bovinus* (UKU) and two *S. variegatus* (UKU) strains (Fig. 3b) were stimulated by propiconazole.

Benomyl had a rather similar inhibitory effect on the fungal strains at both concentrations and on both agars: about 20% of fungal strains experienced some and

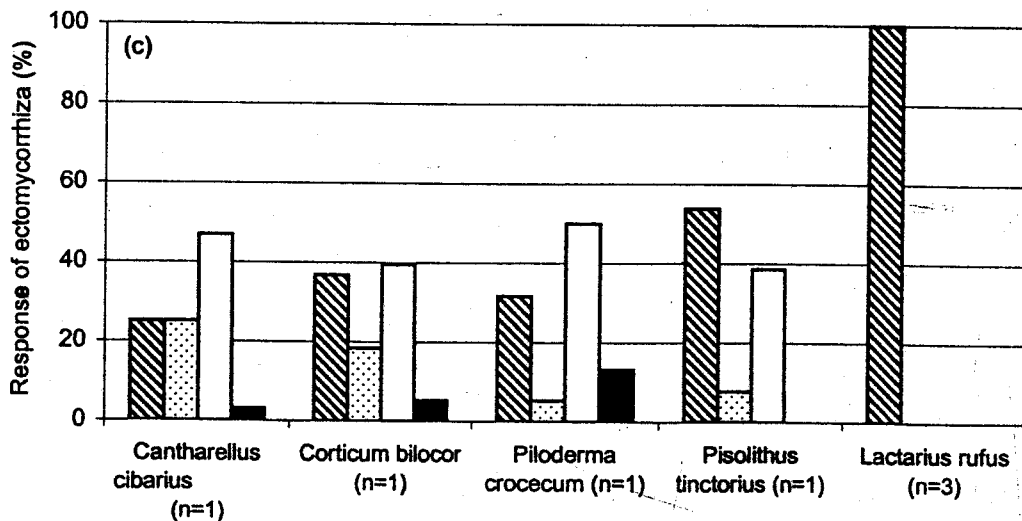
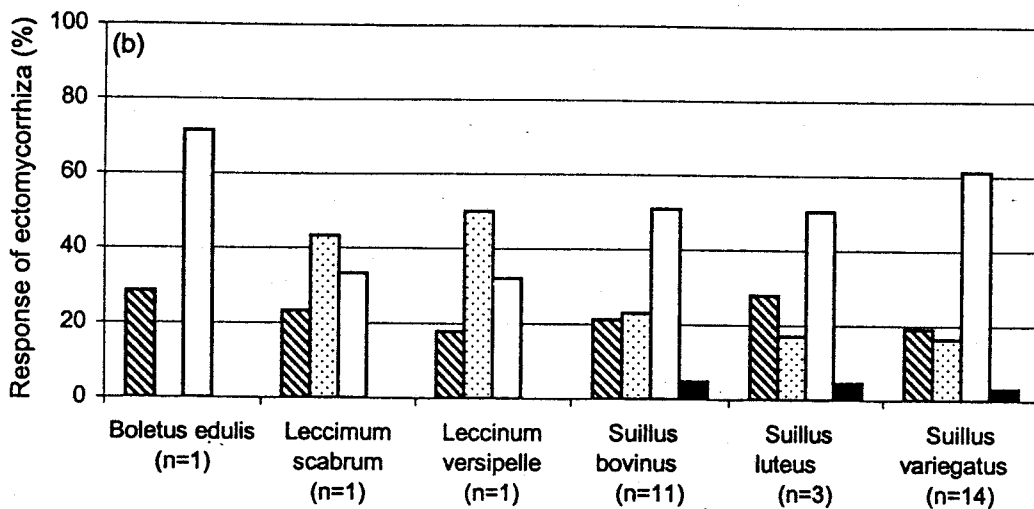
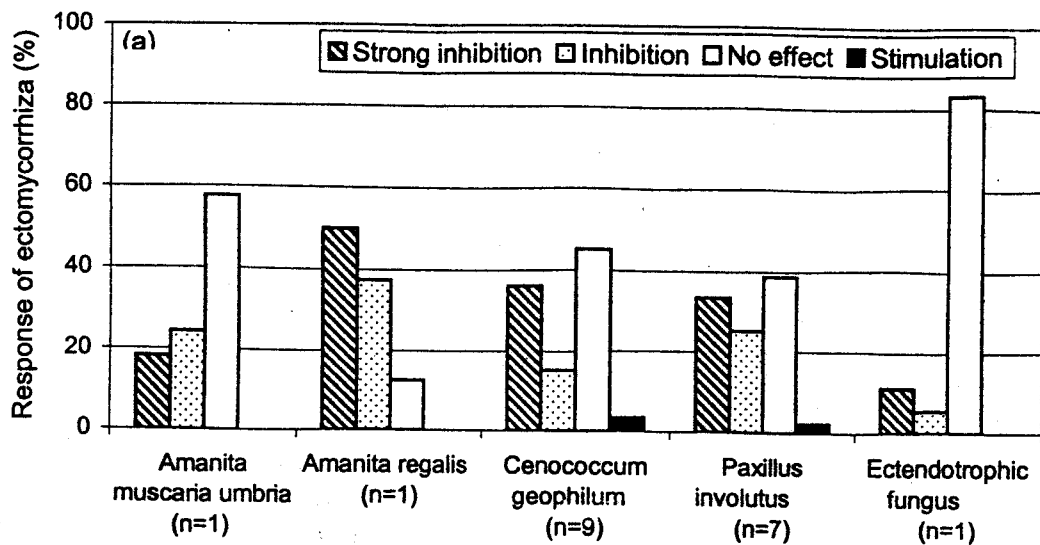


Fig. 3. Responses of some ectomycorrhizal fungi to different pesticides at the concentration of 1 ppm on modified Melin-Norkrans' agar media. n = number of ectomycorrhizal strains tested

Table 3. Estimated concentrations of studied pesticides (a.i.) in one application in top-layer of soil and repetition of applications during one growing season

Pesticide (active ingredient)	Concentration in top-layer ¹		Repetition of application [times a ⁻¹]
	in mineral soil [ppm]	in organic soil [ppm]	
<i>Fungicides</i>			
benomyl	0.8–2.4	8–24	4–6
chlorothalonil	6–8	60–80	4–6
copper oxychloride	1	10	5
maneb	3.8–7.7	38–77	4–8
propiconazole	0.5	5	4
<i>Herbicides</i>			
chlorthiamid	15–24	150–240	1
glyphosate	5.8–8.6	58–86	1
hexazinone	1.0–1.9	10–19	1
linuron	3.6–5.4	36–54	2
terbuthylazine	4.0–4.8	40–48	1
<i>Insecticide</i>			
cypermethrin	0.07–0.11	0.7–1.1	1 ²

¹ Concentration of active ingredient in one application. Pesticide is assumed to be incorporated in top-soil layer of 5 cm depth. Bulk densities used in calculations were 0.5 g/cm³ for mineral soil and 0.05 g/cm³ for organic soil (Heiskanen and Tamminen 1992).

² Bunch of coniferous seedlings is dipped in pesticide solution before out planting.

about 30% of fungal strains showed a strong inhibition on growth (Fig. 2a). Benomyl inhibited strongly the growth of all *C. geophilum* and unidentified ectomycorrhizal fungi. There was some inhibition to the growth of *C. cibarius*, *A. muscaria umbria*, both *Leccinum* strains and some strains in the genes of *Suillus* and *P. involutus*. At the concentration of 1 ppm benomyl, some stimulation of the growth was observed on both agars with strains of *P. involutus* (VH) (Fig. 3a), *S. variegatus* (SLU) and *S. bovinus* (UKU) (Fig. 3b).

Copper oxychloride (Fig. 2a) inhibited the growth of *C. cibarius*, *Corticium bicolor*, and nearly all of the *P. involutus* and *Suillus* strains tested. Maneb inhibited the growth of most of the tested *P. involutus* and *S. bovinus* strains, with few exceptions. The strains of *C. geophilum* and unidentified ectomycorrhizal fungi tolerated copper oxychloride and maneab best of all.

Herbicides

The herbicide, linuron, inhibited strongly the growth of *Boletus edulis* (Fig. 3b), *C. geophilum*, unidentified ectendotrophic fungus, *P. involutus* and *Pisolithus tinctorius*, (Fig. 3c) and to some extent all of the tested *Leccinum* and *Suillus* strains. Most of the unidentified ectomycorrhizal fungi belonged to those linuron-sensitive strains (Fig. 2b). The inhibition effect was similar on both agar media.

Glyphosate and terbuthylazine caused a slight stimulation of growth of some ectomycorrhiza e.g. one third of strains belonging to various *Suillus* species (Fig. 3b) and *C. bicolor* (Fig. 3c). Terbuthylazine stimulated also two strains of *C. geophilum* and *Piloderma croceum* (Fig. 3c), and glyphosate two of the unidentified ectomycorrhizal fungal strains (Fig. 2b). Hexazinone stimulated the growth of one *S. bovinus* (LU) and one unidentified ectomycorrhizal fungal strain, but otherwise hexazinone and chlorthiamid had no effect on mycorrhizal growth on both agar media (Fig. 2b).

Insecticide cypermethrin

At the concentration of 10 ppm, the insecticide cypermethrin inhibited the growth of 25% of all fungi tested including half of the *C. geophilum* strains and unidentified ectomycorrhizal fungal strains and one third of the *S. bovinus* strains. Additionally, cypermethrin had a slight stimulatory effect on the growth of one unidentified ectomycorrhizal fungus at the concentration of 1 ppm.

Sensitive and tolerant ectomycorrhizal fungi

Amanita regalis, *P. tinctorius* (Fig. 3a) and *Lactarius rufus* (Fig. 3c) seemed to be the most sensitive of all tested ectomycorrhizal fungi but there was only one

strain of each kind of fungi tested. Strains from genus *Leccinum* (Fig. 3b), *P. involutus* and *C. geophilum* (Fig. 3a) were sensitive to various fungicides, and to the herbicides linuron (especially *P. involutus*) and glyphosate (*Leccinum versipelle* and *P. involutus*). Half of *Suillus* strains were sensitive to various fungicides and some were also sensitive to the herbicide linuron (Fig. 3b).

The ectendotrophic fungus tested was much more tolerant to different pesticides than the other tested fungi (Fig. 3a). Only linuron caused strong inhibition and maneb evoked some inhibition to the growth of this fungal strain.

Discussion

The pesticide concentrations used in these tests (1 ppm for herbicides, 1 and 10 ppm for fungicides and the insecticide cypermethrin) were at the same levels as those found in the 5 cm top-soil layer of forest nursery soil or in peat-pot containers. We have estimated the concentration of pesticides (*a.i.*) after one application, which would leach into a soil top-layer of 5 cm depth in mineral and in organic soils (Table 3). As can be seen, in most cases the pesticide concentration exceeds 1 ppm for different pesticides and 0.1 ppm for propiconazole. Pesticide concentrations may be even higher on the surface of plant roots, because the pesticides are generally sprayed on seeds or seedlings (fungicides, insecticides) or on weeds (herbicides), or seedlings may even be dipped into pesticide solution (insecticides). Pesticide solution or residues can leach into the container or soil either during the application or afterwards due to irrigation or rainwater.

The depths to which a pesticide can leach into the soil are dependent on the organic matter content of soil and the physico-chemical properties of pesticide. In peat-pots, pesticides can be bound to the peat medium and only a small fraction may be detectable in leaching water. For example, in a trial with peat-pots seedlings, less than 1% of applied chlorothalonil, but almost 30% of applied propiconazole leached through the peat medium (Juntunen and Kitunen 200x). Soil microbial activity can release soil-bound pesticides back to undergo environmental interactions (Levanon *et al.* 1994, Musumeci and de Barros Ostiz 1994), and thus, the mycorrhiza and mycelium, which are mainly present in this organic top-layer of the soil (Smith and Read 1997), can be exposed to pesticides.

The fungicides tested in this study proved to be toxic to ectomycorrhizal fungi, presumably due to their general mode of actions. Fungicides can inhibit fungal cell division (benomyl), impair ergosterol biosynthesis (propiconazole), inactivate fungal cell thiols (chlorotha-

lonil), cause protein damage (copper oxychloride) or bind to cell copper compounds (maneb), (Pesticide Manual 1991). For example, chlorothalonil is viewed as an effective fungicide against a broad spectrum of plant pathogens. Propiconazole, which is a systemic fungicide, belonging to group called sterol synthesis inhibitors also has a broad range of activity. The side effects of propiconazole on soil fungi have been studied by Elmholt (1991), who reported that propiconazole had significant inhibitory effects on *Cladosporium* while *Penicillium* spp. was not affected.

In addition to the studies performed by Marx *et al.* (1986), there are some other experiments examining the side effects of pesticide on ectomycorrhizal fungi. Sobotka (1970) tested *Suillus variegatus* in pure cultures with different pesticides including the fungicide maneb, which proved to be very toxic to tested *S. variegatus* strain. In a field experiment propiconazole and copper oxychloride reduced ectomycorrhizal growth of Scots pine seedlings growing in sandy-filled-pots (Manninen *et al.* 1998). At higher concentrations, benomyl decreased the growth of ectomycorrhizal fungi *Pisolithus tinctorius* and *Thelephora terrestris* in pure culture tests (Marx and Rowan 1981). On the contrary, in a field trial benomyl increased the development of ectomycorrhizas of nursery-grown *Pinus taeda* seedlings with *P. tinctorius* (Marx and Rowan 1981) and enhanced mycorrhization of laboratory-grown *Pinus strobus* seedlings (De la Bastide and Kendrick 1990).

Benomyl has been reported to inhibit the growth and depolymerize the microtubules in the *Cenococcum geophilum* (Niini and Raudaskoski 1993), which differ from the other tested ectomycorrhizal fungi since it is an ascomycete fungus. This genetical distance from the other ectomycorrhizal fungi could account for its different response from all other identified fungi when tested with these pesticides. In Kuopio we do not use benomyl when isolating the fungal strains (Heinonen-Tanski and Holopainen 1991), thus, our own isolates may be more sensitive to benomyl than strains originating from other collections.

The herbicides, glyphosate and hexazinone, have been tested with various ectomycorrhizal fungi in pure culture tests, e.g. *Hebeloma crustuliniforme*, *Laccaria laccata* and *Suillus tomentosum*, and they inhibited all of the tested fungi at concentrations above 10 ppm (Chakravarty and Sidhu 1987), and in a second experiment they had inhibitory effects on *C. geophilum*, *Hebeloma longicaudum* and *P. tinctorius* at concentrations below 100 ppm (Estok *et al.* 1989), though such high concentrations may not be particularly relevant to the situation in forest nurseries.

In general, *P. involutus* and *C. geophilum* are thought to be relatively resistant to different environmental stress factors (Holopainen *et al.* 1996). In our study

Paxillus involutus, *C. geophilum* and *Leccinum* strains were the most sensitive ectomycorrhizal fungi to many of the pesticides tested. *S. variegatus* has been reported to be rather sensitive to environmental stress factors (Ohtonen *et al.* 1990), but this sensitivity does not seem to extend to pesticides because in our study the growth of various *Suillus* strains was not especially impaired by these pesticides.

The growth stimulation of some ectomycorrhizal fungal strains was caused mainly by herbicides glyphosate, terbuthylazine and hexazinone (*Suillus* species), but in a few rare cases also by the insecticide, cypermethrin and all of the fungicides, except chlorothalonil. The growth stimulation of ectomycorrhizal fungi might indicate that these fungi are able to degrade tested pesticides, but this stimulation noted here in the laboratory may be less likely to occur in field conditions if there is a lack of nutrients such as potassium or phosphorous in the soil (DaSilva *et al.* 1977). In some cases pesticide molecule is not mineralized but can become incorporated into the fungal tissue (Donnelly *et al.* 1993). Ectomycorrhizae having the potential to degrade and mineralize pesticides and other persistent organic pollutants could also be used in bioremediation (Meharg *et al.* 1997a). For example, *Amanita* species, *P. involutus*, and *Suillus* species are known to degrade several organic pollutants (Meharg and Cairney 2000) and *C. geophilum* can immobilise, for example, hexazinone (Donnelly and Fletcher 1994). The growth of ectomycorrhizal fungi in symbiosis with *Pinus sylvestris* has stimulated even greater pollutant mineralization than in pure cultures (Meharg *et al.* 1997b).

Knowledge on the capabilities of ectomycorrhizal fungi to tolerate pesticides might be useful in deciding which pesticides would be less harmful to mycorrhizal fungi when used in forest nurseries and in afforested fields. Pesticides may affect directly seedlings roots, and thus, pure culture tests alone are not recommended when estimating pesticide effects on mycorrhizae, and deciding the usefulness of different pesticides in the nursery or new plantations (Unestam *et al.* 1989). Clear phytotoxicity of some fungicides, e.g. chlorothalonil, on forest nursery seedlings (James and Woo 1984) and some other plants (Chlorothalonil 1996), has been detected. It is also claimed that most herbicides and some other pesticides will affect the metabolism of mycorrhizal fungi and can interfere with the establishment of symbiosis between fungi and the plant (DaSilva *et al.* 1977, Marx and Rowan 1981). In some cases, pesticide toxicity in pure culture test has been confirmed with field experiments in nurseries (Marx *et al.* 1986, Marx 1987, Chakravarty and Sidhu 1987, Chakravarty and Chatarpaul 1988).

In this experiment chlorothalonil and propiconazole were particularly toxic to different ectomycorrhizal

fungi in pure culture tests. Due to their intensive use both in forest nurseries and agricultural land, it is recommended that the toxicity of these fungicides to ectomycorrhizal fungi should be evaluated also with field experiments.

Acknowledgements

This work was financially supported by Finnish Ministry of Education, the Finnish Cultural Foundation and the Finnish Cultural Foundation of Kainuu. We thank Mrs. Mirja Korhonen, Miss Päivi Korhonen (M. Sc.) and Mr. Juha Jäntti for technical assistance. We express gratitude to Dr. Ewen MacDonald for language consultation. We also thank Prof. V. Hintikka, University of Helsinki, Swedish Agricultural University (SLU), University of Lund and Centraalbureau voor Schimmelcultures for the strains of ectomycorrhizal fungi from their collections.

References

- AgrEvo Nordic Finland (1999): Afalon-neste/26. 8. 1999, instructions for use. (in Finnish and Swedish)
- Amaranthus, M. P., Perry, D. A. (1987): Effect of soil transfer on ectomycorrhizal formation and survival and growth of conifer seedlings on old, nonreforested clear-cuts. *Can. J. Forest Res.* **17**, 944–950.
- Altas, R. M., Pramer, D., Bartha, R. (1977): Assessment of pesticides on non-target soil microorganisms. *Soil Biol. Biochem.* **10**, 231–239.
- Atlas, R. M., Bartha, R. (1992): *Microbial Ecology. Fundamentals and Applications*. Third Edition. The Benjamin/Cummings Publishing Company, Inc., 536 p.
- Berner (1998): *Kasvinsuojeluopas 1998–1999*, Berner Oy, 163 p. (in Finnish)
- Brouwer, F. M., Terluin, I. J., Godeschalk, F. E. (1994): Pesticides in the EC. Agricultural Economics Research Institute (LEI-DLO), Onderzoekverslag 121, Hague, 159 p.
- Chakravarty, P., Sidhu, S. S. (1987): Effect of glyphosate, hexazinone and triclopyr on *in vitro* growth of five species of ectomycorrhizal fungi. *Eur. J. Forest Pathol.* **17**, 204–210.
- Chakravarty, P., Chatarpaul, L. (1988): The effects of Velpar (hexazinone) on seedling growth and ectomycorrhizal symbiosis of *Pinus resinosa*. *Can. J. Forest Res.* **18**, 917–921.
- Chlorothalonil (1996): Environmental health criteria 183, World Health Organization, Geneva, 145 p.
- Colinas, C., Ingham, E., Molina, R. (1994): Population responses of target and non-target forest soil organisms to selected biocides. *Soil Biol. Biochem.* **26**, 41–47.
- DaSilva, E. J., Henriksson, L. E., Urdis, M. (1977): Growth responses of mycorrhizal *Boletus* and *Rhizopogon* species to pesticides. *T. Brit. Mycol. Soc.* **68**, 434–437.
- De la Bastide, P. Y., Kendrick, B. (1990): The *in vitro* effects of benomyl on disease tolerance, ectomycorrhiza formation, and growth of white pine (*Pinus strobus*) seedlings. *Can. J. Bot.* **68**, 444–448.

- Donnelly, P. K., Entry, J. A., Crawford, D. L. (1993): Degradation of atrazine and 2,4-dichlorophenoxyacetic acid by mycorrhizal fungi at three nitrogen concentrations *in vitro*. *Appl. Environ. Microb.* **59**, 2642–2647.
- Donnelly, P. K., Fletcher, J. S. (1994): Potential use of mycorrhizal fungi as bioremediation agents. In: *Bioremediation through rhizosphere technology* (Eds: Anderson, T. A., Coats, J. R.). Chapter 8. ACS Symposium Series 563, American Chemical Society, Washington DC, 93–99.
- Dubus, I. G., Hollis, J. M., Brown, C. D. (2000): Pesticides in rainfall in Europe. *Environ. Pollut.* **10**, 331–344.
- Du Pont (1999): Instructions for use (29. 9. 1999, A.-M. Grimm). (in Finnish)
- Elmholt, S. (1991): Side effects of propiconazole (Tilt 250 EC) on non-target soil fungi in a field trial compared with natural stress effects. *Microbial Ecol.* **22**, 99–108.
- Estok, D., Freedman, B., Boyle, D. (1989): Effects on the herbicides 2,4-D, glyphosate, hexazinone and triclopyr on the growth of three species of ectomycorrhizal fungi. *B. Environ. Contam. Tox.* **42**, 835–839.
- Fomsgaard, I. S. (1995): Degradation of pesticides in sub-surface soils, unsaturated zone – a review of method and results. *Int. J. Environ. An. Ch.* **58**, 231–245.
- Halonen, A., Laiho, O. (1991): Metsitettyjen peltojen mykorrhizat. Abstract: Mycorrhizae of afforested fields. In: *Developing methods for afforestation of fields* (Eds: Ferm, A., Polet, K.) Interim report. The Finnish Forest Research Institute, Kannus Research Station, **391**, 86–91. (in Finnish)
- Harley, J. L., Smith, S. E. (1983): *Mycorrhizal Symbiosis*. Academic Press, London and New York, 483 p.
- Heinonen-Tanski, H. (1989): The effect of temperature and liming on the degradation of glyphosate in two arctic forest soils. *Soil Biol. Biochem.* **21**, 313–317.
- Heinonen-Tanski, H., Holopainen, T. (1991): Maintenance of ectomycorrhizal fungi. *Method. Microbiol.* **23**, 413–422.
- Heinonen-Tanski, H., Oros, G., Kecskés, M. (1982): The effect of soil pesticides on the growth of red clover *Rhizobia*. *Acta Agr. Scand.* **32**, 283–288.
- Heiskanen, J., Tamminen, P. (1992): Maan fysikaalisten ominaisuuksien määrittäminen. Metsäntutkimuslaitoksen tiedonantoja 424, Helsinki, 32 p. (in Finnish)
- Hirvi, J.-P., Rekolainen, S. (1995): Pesticides in precipitation and surface water in Finland. In: *Pesticides in precipitation and surface water* (Ed: Helweg, A.). *TemaNord* **558**, 12–18.
- Holopainen, T., Heinonen-Tanski, H., Halonen, A. (1996): Injuries to Scots pine mycorrhizas and chemical gradients in forest soil in the environment of a pulp mill in Central Finland. *Water Air Soil Poll.* **87**, 111–130.
- Hynninen, E.-L., Blomqvist, H. (1999): Pesticide sales in Finland 1998. *Kemia-Kemi* **26**, 498–500.
- Iloba, C. (1980): The use of chemicals for weed control in forest tree nurseries. In: *Tropical mycorrhiza research* (Ed: Mikola, P.). Oxford, Clarendon Press, 121–123.
- Ingham, E. R., Cambardella, C., Coleman, D. C. (1986): Manipulation of bacteria, fungi and protozoa by biocides in Lodgepole pine forest soil microcosms: effects on organism interactions and nitrogen mineralization. *Can. J. Soil Sci.* **66**, 261–272.
- James, R. L., Woo, J. Y. (1984): Fungicide trials to control Botrytis blight at nurseries in Idaho and Montana. *Tree Planter's Notes*, Fall 1984, 16–19.
- Juntunen, M.-L. (2001): Use of pesticides in Finnish forest nurseries in 1996. *Silva Fenn.* **35**, 147–157.
- Juntunen, M.-L., Kitunen, V. (200x): Leaching of propiconazole and chlorothalonil through container peat medium in Scots pine seedling production. (manuscript)
- Kemira (1992): *Torjunta-aineet 1992–93*, Kemira Oy, 95 p. (in Finnish)
- Kemira (1996): *Kasvinsuojeluopas*, Kemira Argo Oy, 97 p. (in Finnish)
- Kemira (1998): *Käsvukirja*, Kemira Agro Oy, 159 p. (in Finnish)
- Levanon, D., Meisinger, J. J., Codling, E. E., Starr, J. L. (1994): Impact of tillage on microbial activity and the fate of pesticides in the upper soil. *Water Air Soil Poll.* **72**, 179–189.
- Lilja, A., Lilja, S., Kurkela, T., Rikala, R. (1997): Nursery Practices and managements of fungal diseases in forest nurseries in Finland. A review. *Silva Fenn.* **31**, 79–100.
- Londesborough, S., Hynninen, E.-L., Blomqvist, H. (2000): Pesticides sales in Finland in 1999. *Kemia-Kemi* **27**, 492–494.
- Manninen, A.-M., Laatikainen, T., Holopainen, T. (1998): Condition of Scots pine fine roots and mycorrhiza after fungicide application and low-level ozone exposure in a 2-year field experiment. *Trees* **12**, 347–355.
- Marx, D. H. (1969): The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. I. Antagonism of mycorrhizal fungi to root pathogenic fungi and soil bacteria. *Phytopathology* **59**, 153–163.
- Marx, D. H. (1987): Triadimefon and *Pisolithus* ectomycorrhizae affect second-year field performance of Loblolly pine. Research note SE-349, December 1987. United States Department of Agriculture, Forest Service, Southeastern Forest Experiment Station, 6 p.
- Marx, D. H. (1991): The practical significance of ectomycorrhizae in forest establishment. *Ecophysiology of ectomycorrhizae of forest trees*. The Marcus Wallenberg Foundation Symposia Proceedings: 7, Stockholm, Sweden, 54–90.
- Marx, D. H., Cordell, C. E., France, R. C. (1986): Effects of triadimefon on growth and ectomycorrhizal development of Loblolly and Slash pines in nurseries. *Phytopathology* **76**, 824–831.
- Marx, D. H., Rowan, S. J. (1981): Fungicides influence growth and development of specific ectomycorrhizae on Loblolly pine seedlings. *Forest Sci.* **27**, 167–176.
- Meharg, A. A., Cairney, J. W. G. (2000): Ectomycorrhizas – extending the capabilities of rhizosphere remediation? *Review. Soil Biol. Biochem.* **32**, 1475–1484.
- Meharg, A. A., Cairney, J. W. G., Maguire, N. (1997b): Mineralization of 2,4-dichlorophenol by ectomycorrhizal fungi in axenic culture and in symbiosis with pine. *Chemosphere* **34**, 2495–2504.
- Meharg, A. A., Dennis, G. R., Cairney, J. W. G. (1997a): Biotransformation of 2,4,6-trinitrotoluene (TNT) by ectomycorrhizal basidiomycetes. *Chemosphere* **35**, 513–521.

- Mikola, P. (1973): Application of mycorrhizal symbiosis in forestry practice. In: Ectomycorrhizae – their ecology and physiology (Eds: Marks, G. C., Kozłowski, T. T.). Academic Press, New York and London, 383–411.
- Modess, O. (1941): Zur Kenntnis der Mykorrhizabildner von Kiefer und Fichte. *Symb. Bot. Upsal.* **5**, 1–147.
- Musumeci, M. R., de Barros Ostiz, S. (1994): Binding of cypermethrin residue in Brazilian soils and its release by microbial activity. *Rev. Microbiol., Sao Paulo* **25**, 216–219.
- Niini, S., Raudaskoski, M. (1993): Response of ectomycorrhizal fungi to benomyl and nocodazole: growth inhibition and microtubule depolymerization. *Mycorrhiza* **3**, 83–91.
- OECD (1999): OECD Environmental Data, Compendium 1999 Edition. 332 p.
- Ohtonen, R., Markkola, A. M., Heinonen-Tanski, H., Fritze, H. (1990): Biological parameters as indicators of changes in Scots pine forests (*Pinus sylvestris* L.) caused by air pollution. In: Acidification in Finland (Eds: Kauppi, P., Anttila, P., Kenttämies, K.). Springer-Verlag, Berlin, Heidelberg, 373–392.
- Pesticide manual: a world compendium. 9th edition (1991). Eds: C. R. Worthing, R. J. Hance. The British Crop Protection Council, Surrey, UK, 1991, 1141 p.
- Plenchette, C., Perrin, R. (1992): Evaluation in the greenhouse of the effects of fungicides on the development of mycorrhizae on leek and wheat. *Mycorrhiza* **1**, 59–62.
- Schüepp, H., Bodmer, M. (1991): Complex response of VA-mycorrhizae to xenobiotic substances. *Toxicol. Environ. Chem.* **30**, 193–199.
- Smith, S. E., Read, D. J. (1997): *Mycorrhizal Symbiosis*. Second edition, Academic Press, London and New York, 605 p.
- Sobotka, A. (1970): Die Testung des Eisflusses von Pestiziden auf die Mycorrhiza-Pilze in Waldböden. *Zbl. Bakt. II Natur.* **125**, 723–730.
- Torstensson, L. (1995): Pesticides in precipitation, Consequences for the terrestrial environment. In: Pesticides in precipitation and surface water (Ed: Helweg, A.). *Tema-Nord* **558**, 84–93.
- Tu, C. M. (1992): Effects of some herbicides on activities of microorganisms and enzymes in soil. *J. Environ. Sci. Heal. B* **27**, 695–709.
- Tu, C. M. (1993): Effect of fungicides, captafol and chlorothalonil, on microbial and enzymatic activities in mineral soil. *J. Environ. Sci. Heal. B* **28**, 67–80.
- Unestam, T., Beyer-Ericson, L. (1990): Diseases of container-grown conifer nursery seedlings in Sweden. In: Proceedings of the first meeting of IUFRO Working Party S2.07–09 (Diseases and Insects in forest nursery) (Eds: Sutherland, J. R., Glover, S. G.). Victoria, British Columbia, Canada, August 22–30, 1990, 105–108.
- Unestam, T., Chakravarty, P., Damm, E. (1989): Fungicides: *in vitro* tests not useful for evaluating effects on ectomycorrhizae. *Agr. Ecosyst. Environ.* **28**, 535–538.