Commission of the European Communities

Air Pollution Research Report 30

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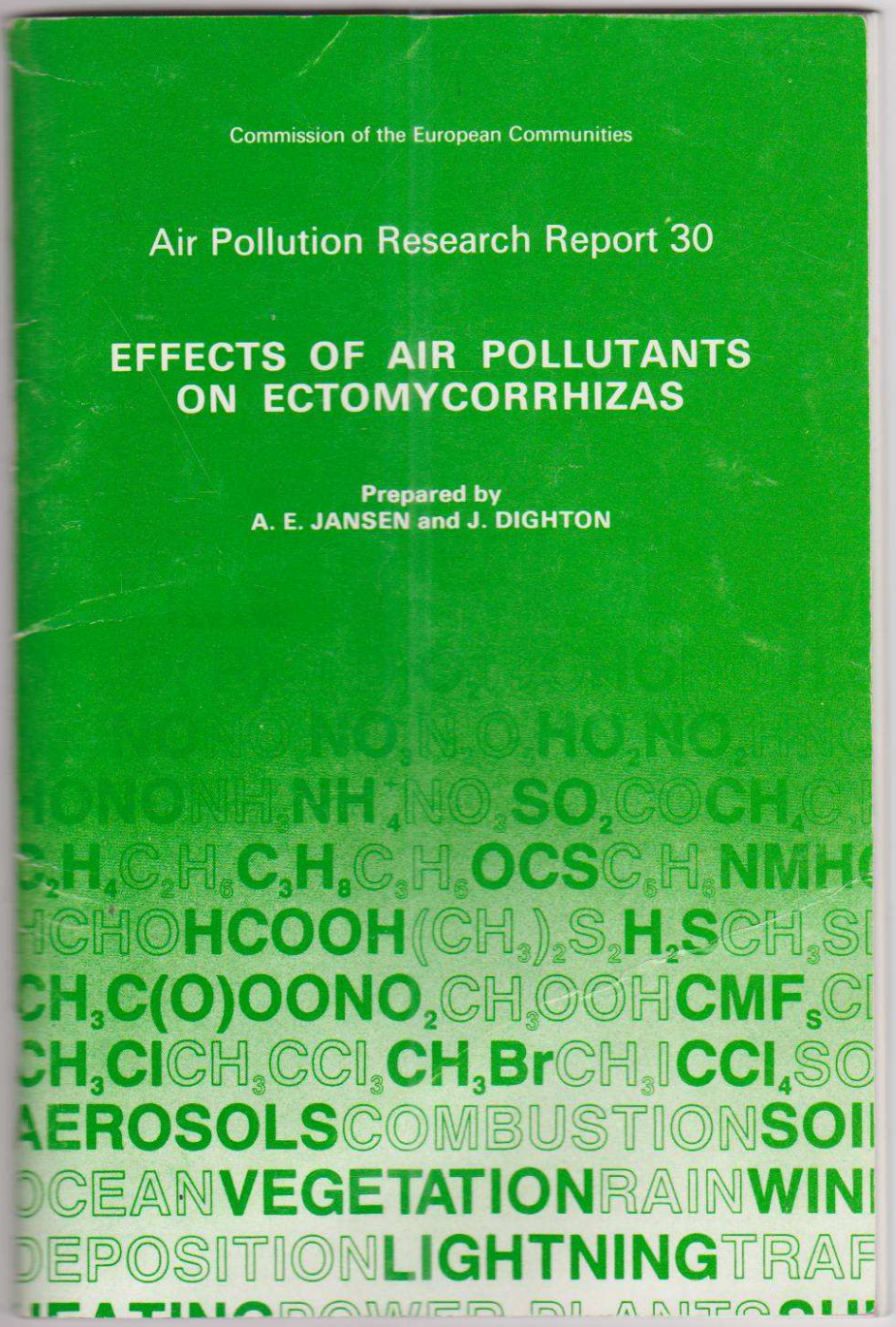
Prepared by
A. E. JANSEN and J. DIGHTON



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Directorate-General for Science, Research and Development
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EFFECTS OF AIR POLLUTANTS ON ECTOMYCORRHIZAS

A Review

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Brussels, October 1990

Effects of air pollutants on ectomycorrhizas. A review.

Edited by: A. E. JANSEN and J. DIGHTON

ISBN 2-87263-046-5 EUR 13035 Dépôt légal: D/1990/0157/S

This is Report No 30 in the Air Pollution Report Series of the Environmental Research Programme of the Commission of the European Communities, Directorate-General for Science, Research and Development.

For more information concerning this series, please contact:
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Printed in Belgium

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To the memory of Jan J. Barkman, who founded the studies on acid rain and ectomycorrhizas in The Netherlands.

SUMMARY

This paper reviews the influences of atmospheric pollution on ectomycorrhizas and ectomycorrhizal fungi. Effects of other pollutants, e.g. of heavy metals, are outside the scope of this review.

Ectomycorrhizal fungi receive their carbon (energy) requirements from the associated tree host, and supply the tree host with water and inorganic nutrients. The gaseous air pollutants O_3 and SO_2 strongly reduce the respiration of mycorrhizal fungi in pure culture and excised mycorrhizal and non-mycorrhizal root tips. However, due to their below ground occurrence, it is unlikely that roots will be exposed to these gasses.

Atmospheric pollutants affect mycorrhizas via changes in soil chemistry and changes in carbon availability. A lowered pH due to atmospheric deposition also results in increased aluminium and manganese concentrations and increased leaching of calcium and magnesium. These changes may reduce the mycorrhizal status and induce a shift in composition of mycorrhizal populations. Especially species with much extramatrical hyphae or strands declined.

Nitrogen addition is discussed separately as it may act as fertilizer. The addition of nitrogen in a balanced nitrogen-phosphorus-potassium (NPK) fertilization may stimulate the production of fruitbodies of certain species. Addition of nitrogen alone may lead to an imbalance of available nutrients, and generally reduces the fructification and the mycorrhizal status. Such effects are strongest on oligotrophic soils.

The carbon demand by mycorrhizal fungi can be considerably: the highest carbon consumption reported equalled $38 \cdot 10^3$ kg ha^{-1} yr^{-1}. In areas in Europe consumption between 1.4 kg ha^{-1} yr^{-1} and $8.5 \cdot 10^3$ kg ha^{-1} yr^{-1} were reported. Gaseous pollutants may reduce both the photosynthetic capacity and the transport of photosynthates to roots and mycorrhizas. Quantitative data are, however, very scarce and interrelations between carbon allocation to roots and mycorrhizas and nutrient uptake are not yet clearly understood.

Seen over the lifetime of forest stands, mainly the species of mycorrhizal fungi that are characteristic for older stands, are affected by air pollution. These can be classed as K-strategists. More opportunistic species, which can be classed as r-strategists, appear to be less susceptible for air pollution.

Relations between effects of atmospheric pollutants and theories of mycorrhizal regulation are discussed.

EFFECTS OF AIR POLLUTANTS ON ECTOMYCORRHIZAS
A Review

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1 INTRODUCTION; AIM AND DELIMITATION

This review aims to discuss the effects of atmospheric pollutants on ectomycorrhizas and ectomycorrhizal fungi. The main questions are: (i) which atmospheric pollutants play a role; (ii) what are the effects on fungi and consequently on trees, and, to a lesser extent, ecosystems; and (iii) what is known about the mechanism of the processes.

The study is restricted to the influence of 'acid rain' and effects due to atmospheric deposition of SO_2, O_3, NO_x, NH_3, although there can be effects on mycorrhizas of other types of pollution. The influence of nitrogen is included only when it originates from atmospheric deposition, not from fertilizer additions. However, nitrogen fertilization is studied because it may give the same effects as nitrogen deposition resulting from atmospheric pollution. We are aware that other pollutants, particularly accumulation of heavy metals in soil, e.g. as a result of mining activity are important in inhibiting mycorrhizal and root physiology, but they are outside the scope of this review.

The review focuses mainly on the situation in the temperate zone in Western Europe. Only ectomycorrhizal fungi and ectomycorrhizal trees are considered as the reaction of Vesicular-Arbuscular mycorrhizal fungi and endomycorrhizal trees to air pollutants is quite different (Meyer 1987, Heijne et al. 1989).

2 MYCORRHIZAS AND THEIR DISTRIBUTION

2.1 What mycorrhizas are; a very brief outline

A mycorrhiza is an association of a living root and a fungus. In trees, the roots involved are the ultimate laterals of fine roots, called short roots or feeder roots. The most common type in tree roots is ectomycorrhiza or sheathing mycorrhiza. In this type a dense sheath or mantle of mycelium around the root is formed by the fungal hyphae (see e.g. Harley & Smith 1983, Jackson & Mason 1984). The fungal hyphae penetrate into the root where they form a network of hyphae between the cortical cells of the root (Hartig net). From the mantle hyphae penetrate into the soil in the form of hairs (short hyphae), extramatrical hyphae and strands (rhizomorphs). The strands can reach considerable distances from the roots, often in excess of 20 cm

(Read 1984), so exploiting a much bigger volume of soil than roots and root hairs can do.

Other types of mycorrhiza include VA-mycorrhiza and Ericoid mycorrhiza. These types lack a sheath of mycelium around the root, and the fungal hyphae penetrate inside the cortical cells of the host plants. They are common in many species of herbs and shrubs, and are less common in temperate forest trees.

Morphology of an ectomycorrhiza is influenced by both tree and fungus. The fungus inhibits linear growth of the root and stimulates the root to form side roots. The manner of branching, e.g. pinnate or dichotomous, is an important characteristic of mycorrhizas, and is tree determined. The fungal mantle determines the outer appearance of the mycorrhiza including colour of the mycorrhiza and surface structure. Structure of the Hartig net is determined by both fungus and tree. A mycorrhiza is usually slightly thicker than a non-mycorrhizal root (e.g. Moore et al. 1989) because of hypertrophy of root cortical cells as a consequence of the mycorrhizal symbiosis (Mudge 1987) and volume of Hartig net and mantle.

In 1921, Melin distinguished 5 types of ectomycorrhiza using characteristics of branching and tree host species, but he was not sure whether these 5 types were really different from each other. Dominik (1956, 1969) distinguished 75 form-genera on macroscopical and microscopical characteristics. Both classifications were too general in the view of the many tree and fungal species involved. Zak (1969) proposed to identify mycorrhizas by naming both the tree species and the fungal species, e.g. *Pseudotsuga menziesii* + *Poria terrestris*. This attempt has won acceptance and since that time many mycorrhizas have been described and knowledge of morphology and anatomy of mycorrhizas is increasing (e.g. Agerer 1987-..). Recently some mycorrhizas were described with unidentified fungal partners, under names as e.g.: "Fagirhiza cystidiophora" (Brand 1988). Such a description may contribute to insight in morphological variation of mycorrhizas. Identification of the fungal partner is, however, necessary for further studies.

Ectomycorrhizas are common in trees belonging to the Pinaceae (e.g. in the genera *Pinus, Picea, Abies, Pseudotsuga*), Fagaceae (e.g. *Fagus, Quercus*), Betulaceae (e.g. *Betula, Alnus*) and Myrtaceae (Trappe 1962). This means that the vast majority of the native and planted trees in temperate Europe are ectomycorrhizal. Most of these tree species are considered obligatory ectomycorrhizal (Meyer 1987), i.e. in vivo they always grow in symbiosis with mycorrhizal fungi. Fungi involved are Basidiomycetes,

Ascomycetes and, more rarely, species of *Endogone* (Trappe 1962). About 24% of the species of the fungus flora in The Netherlands are ectomycorrhizal (Arnolds 1989). Most of these fungi are obligate symbionts, i.e. in vivo they can not grow at all or only to a very limited extend unless they are associated with a host. Some species of mycorrhizal fungi can grow saprophytically under certain conditions (facultative symbionts). Certain ectomycorrhizal fungi can be cultured in vitro, using special nutritional media, but many other species, e.g. species of genera as *Russula* and *Cortinarius*, can not be cultured at present. This limits mycorrhiza studies, especially because it are the declining species which appear to be unculturable.

2.2 The physiology of mycorrhizas related to possible effects of pollution

The association of the mycorrhizal fungus and the root of the host is generally considered a mutualistic symbiosis. The fungus obtains all or most of its carbon (energy) requirements from the host, the host is supplied with water and inorganic minerals taken up from the soil by the fungus.

Trees with mycorrhizas have often a larger content and uptake of nitrogen, phosphate and potassium than non-mycorrhizal trees (Harley 1989, Harley & Smith 1983, Kamminga-Van Wijk & Prins 1988). Niederer & Wieser (1988) showed that excised mycorrhizas of *Picea abies* had enhanced phosphate uptake capacities compared to non-mycorrhizal roots as a result of different uptake kinetics, particularly a mechanism for absorbing phosphorus at high external concentrations.

Similarly, potassium absorption is stimulated by mycorrhizas, but evidence suggested that sulphate absorption is not stimulated. Better growth or higher nutrient uptake is not found in all experiments with seedlings and mycorrhizal fungi. Mycorrhizal species show a large inter- and intra-specific variation in physiology, e.g. in amounts or production of phosphatase, nitrase, IAA, ethylene (e.g. DeVries et al. 1987, Ho 1987). This, and the inter- and intra-specific variation of the tree hosts may well explain differences found in experiments.

Two hypotheses have been put forward to explain the regulation of the mycorrhizal symbiosis: the carbohydrate theory and the hormone theory (recently reviewed by Nylund 1988).

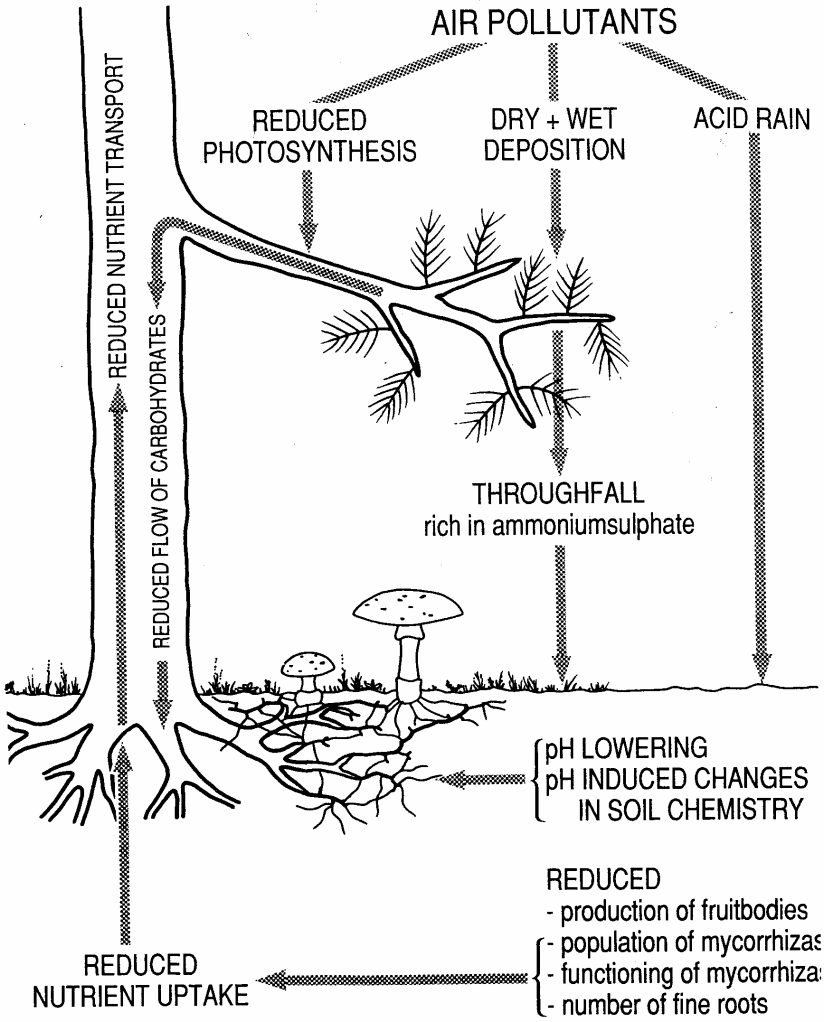


Fig. 1. Diagrammatic representation of the effects of air pollution and the resulting acid rain and throughfall on the soil-mycorrhiza-tree system.

The carbohydrate theory states that formation of mycorrhizas is regulated by the root carbohydrate concentration, as influenced by the amount of carbohydrates formed and transported to the host's root system. This theory was formulated by Björkman (1942), who summarized it as: "Mycorrhizae develop characteristically if the roots of the host plant contain a surplus of soluble carbohydrates" (Björkman 1949, cited by Nylund 1988). However, according to Meyer (1962, 1968, cited by Nylund 1988), mycorrhizas act as a sink for sugars, and a higher carbohydrate concentration in roots is not the cause but the result of mycorrhiza formation.

The hormone theory states that auxin produced by the fungus regulates mycorrhizal formation, influences root carbohydrate status, and may be affected by concentrations of minerals in the soil. This theory, formulated by Slankis (1951, 1974, cited by Nylund 1988) was based on observations that auxin or fungal exudates induced mycorrhiza-like branching in pine roots, and that terminating the supply of auxin or exudates resulted in renewed elongation of roots and formation of root hairs. A high level of nitrogen in the growing medium of ectomycorrhizas terminated the symbiosis, and also suppressed, in vitro, the production of IAA by the mycorrhizal fungus *Suillus bovinus*.

A combined carbohydrate-nitrogen effect was described by Richards (1965). His own data and re-analyzed data from Björkman showed a very significant relation between the proportion of mycorrhizas and the ratio of reducing sugars:nitrogen in the roots. He therefore suggested that the mycorrhiza formation was dependent on the balance between carbohydrate and nitrogen in root tissues. However, his ideas never got the status of a 'theory'.

The mechanism of regulation of mycorrhizal symbiosis is of importance for the understanding of the effects of atmospheric pollutants, and reverse, effects of pollutants can give indications for the mechanism of regulation. There are two major types of effect of pollutants on mycorrhizas (Fig. 1). Firstly, changes in pH and soil chemistry can make toxic elements more mobile (Al, Mn, Mg etc.) and, with the direct input of nitrogen, the availability of nutrient elements is enhanced. Secondly, the effect of pollutants on aboveground parts of the tree can reduce photosynthesis and increase respiration, thus reducing carbon allocation to the roots for root growth and maintenance of the mycorrhizal association. Increase in nitrogen in soil links with the hormone theory of mycorrhizal regulation, as seen above, by reducing IAA production. Similarly reduction in photosynthate transport to the roots links with the carbohydrate theory. Regulation of mycorrhizal formation by both carbohydrate and nitrogen is the basis of

Richard's balance theory. However, the influence of pH and increased mobility of toxic elements do not relate to any of the existing theories of mycorrhizal regulation. At present each of these processes has been studied independently, but it is seen that they are all interrelated and their combined effects should, therefore, be studied.

There are direct links between carbon supply to the mycorrhizal fungus from the plant host and the uptake of nutrients from the soil. For example, nitrogen uptake through the GS (glutamine synthetase) and GOGAT (glutamate synthetase) pathways are dependent upon the supply of energy from stored root sugars via glycolysis (France & Reid 1983). In the same way, energy is required in the building of polyphosphates (a phosphate storage compound unique to mycorrhizal associations) and the fungal reduction of polyphosphates by the polyphosphate glucokinase pathway (Cappaccio & Callow 1982). Thus potential reduction of carbon allocation to the symbiotic partnership may not only reduce the biomass of fungal tissue supported, but may significantly and adversely affect the physiology of the association to the detriment of the tree host.

As forests age, there are changes in the supply and demand of mineral nutrients. Dighton & Harrison (1990) have shown that phosphorus uptake by *Picea sitchensis* increases around canopy closure but declines again in older stands. This supports the hypothesis of Miller (1981, 1984) suggesting increased internal nutrient cycling within the mature tree and, hence, reduced dependence on soil and, possibly, mycorrhizas for nutrition. Similar changes in the internal allocation of carbon within mature trees, compared to young trees, may allow more carbohydrate to be allocated to mycorrhizal maintenance (Dighton & Mason 1985). These authors cite data to show that fungi often associated with older trees (*Amanita muscaria* and *Leccinum* spp) required higher concentrations of glucose to support hyphal growth in culture than *Hebeloma* spp which is regarded as usually associating with younger trees. It is, however, not clear whether internal reallocation and decreased uptake in older trees is a result of a decreased demand, or whether it is a result of shortage in available nutrients in the soil due to insufficient mineralization in older forest floors.

The effects of toxic metal ions on roots have been studied because changes in soil pH induce increased mobility of toxic metals. Using X-ray microprobe analysis on the cortical cells of 4 to 6 weeks old *Picea abies* seedlings grown in 2 mM aluminium (Ca:Al 0.75), Godbold et. al. (1987) showed that the vacuolar content of Mg, K and P declined significantly with respect to control plants. The ratio of phosphorus to sulphur in the vacuol

Table 1. Phosphorus:Sulphur ratio in *Picea abies* roots grown at 2 mM aluminium for 4 to 6 weeks at two different pH values. (After Godbold et al. 1987)

	pH 4		pH 3
	no Al	with Al	with Al
Cortex	11.8	0.14	0.30
Endodermis	10.2	3.3	0.21

Table 2. Response of tree roots to aluminium concentration in solution. (After Eldhurst et al. 1987)

	Al concentration (mM) to cause	
Tree species	Decrease in root growth	Lethal
Picea abies	0.5-1	6-10
Betula pendula	1-3	10-12
Pinus sylvestris	3-5	25-30

was also found to be sensitive to aluminium (Table 1). They ascribe the drop in the phosphorus content to either an inhibition of the phosphate metabolism, or to a reduced storage.

In hydroponic nutrient flow conditions, Eldhurst et al. (1987) showed that different tree species had differing tolerances to soluble aluminium (Table 2). They determined this in terms of the reduction in root growth and the level of aluminium required to cause root death.

The response in *Betula* was found to be the same under optimum nutrition or under nutrient stress and for *Pinus sylvestris* in the presence or absence of *Suillus bovinus* mycorrhizas. It would appear from this single study, therefore, that *Suillus bovinus* had no protective function in reducing aluminium toxicity. The authors, however, state that the concentrations of aluminium used in this study are high, compared with levels reported in the field, which seldom exceed 0.5 mM.

2.3 Observations of mycorrhizal damage in forest stands

The plight of the German forests was brought to the attention of the scientific world by Ulrich in the early 1980's (Pearce 1986). From his studies of the soil chemistry, Ulrich suggested that the decline and death of the forests was attributable to atmospheric pollution, particularly acidification of the soil by dissolved gases, such as SO_2 in the rain (acid rain). His suggestion was that the acid *per se* or the effects of reduced pH on the soil solution chemistry (increased mobility of ions of aluminium, and manganese in soil solution to plant and fungal toxic levels; increased leaching of magnesium and calcium) were inhibiting root growth and physiology and in some instances actually causing root death.

As ectomycorrhizas are an integral part of the functioning of root systems of many conifer and broadleafed tree species, there was interest in understanding the effects of pollution on the survival and functioning of the mycorrhizal fungal component in this symbiotic association. Furthermore, not all tree species appeared to be affected to the same degree. Meyer (1987) observed that trees suffering most from reduction in vitality or growth were obligatorily ectomycorrhizal, e.g. species belonging to the genera *Pinus, Picea, Fagus* and *Quercus*, whereas facultatively ectomycorrhizal or non-ectomycorrhizal trees, suffered less from environmental stress.

Damage to the fungal component of the mycorrhizal association in terms of hypertrophied Hartig net and reduced mantle development of Norway spruce (*Picea abies*) under moderate and severe pollution stress had previously been identified by Sobotka (1964) in the forests of the Ore mountains in Czechoslovakia. He also suggested that the nutrition of the trees would be impaired as a result of this damage. Degradation of the fine root and mycorrhiza system in diseased stands was also reported by German researchers, e.g. by Liss et al. (1984), Blaschke et al. (1985), Meyer (1987), Blaschke (1988).

Independently, there were many observations in Europe on the decline of fruitbodies of ectomycorrhizal fungi. These declines have been reported from ten European countries from which the best documented cases came from the Federal Republic of Germany (Winterhoff 1984, Derbsch & Schmitt 1987, Schmitt 1988), from The Netherlands (Arnolds 1985, 1988, Jansen & Van Dobben 1987) and from Czechoslovakia (Fellner 1988). Many of these authors attributed the decrease of fruitbodies of ectomycorrhizal fungi to air pollution, as they could demonstrate relations between patterns of fungal

decline and patterns of air pollution, e.g. the correlation between the pattern of decline of *Cantharellus cibarius* and several other species of mycorrhizal fungi with patterns of SO_2 pollution in the air (Jansen et al. 1985, Arnolds 1985, 1988, Jansen & Van Dobben 1987). However, not all species of mycorrhizal fungi were affected in the same way, and, before detailed research started, the relation between fruitbody production and mycorrhizal status was unknown.

Of the fungal species occurring in well developed, healthy woods or forest stands 45-50 % is mycorrhizal (Arnolds 1988) determined by fruitbody observations. In affected stands the proportion of mycorrhizal fungi to the total list of species has decreased to 10% and less (De Vries et al. 1985, Jansen own observations).

A problem arose in the interpretation of these phenomena. Two hypotheses were postulated to explain the relation between the decline of trees and of mycorrhizal fungi (see e.g. Asche & Flückiger 1987, Arnolds 1988, Persson 1988).

(i) Effects are soil mediated: Decreased occurrence of mycorrhizas results from changes in soil chemistry attributable to the deposition of air pollutants, e.g. a lower pH, a higher concentration of Al^{3+} ions, changed Al:Ca and Al:Mg ratios, a higher concentration of ammonia or nitrate.

(ii) Effects are tree mediated: Reduced photosynthesis and changes in carbon allocation as consequences of atmospheric pollutants result in reduced carbohydrate transport to roots and mycorrhizal fungi, causing reduced mycorrhizal infection and a lower fruitbody production.

In other words: are mycorrhizas decreasing because the soil is becoming an unsuitable habitat or because trees are diseased? Is the effect on mycorrhizas caused by soil chemical changes, or by reduced carbon supply? Irrespective of the possible mechanism, a disturbed, reduced or impoverished mycorrhizal status will have consequences for the nutrition of the host. Reduced or impaired nutrient uptake will reduce tree growth, and so lead to a "downward life spiral" of the tree (Persson 1988).

These two possible pathways have a direct relation to the two theories on the regulation of mycorrhiza formation: the carbohydrate theory and the hormone theory (see section 2.2). Carbon relations and effects of reduced transport of photosynthates to roots and mycorrhizas are discussed in section 4.3, effects of changed soil conditions in section 4.2.

3 CHANGES IN SOIL CHEMISTRY DUE TO ATMOSPHERIC DEPOSITION

The main air pollutants deposited are SO_x (all sulphur oxides, SO_3^-, SO_4^{2-}), NO_x (all nitrogen oxides, NO_3^-, HNO_3) and NH_x (gaseous NH_3 plus NH_4^+). Direct dry deposition is usually rapidly converted into a liquid phase, as was shown by Wookey (1988) for SO_2. He quotes Babich & Stotsky (1980): "as all cells are covered by a thin film of water and because of high solubility of SO_2 in water, a cell is probably never exposed to gase SO_2". This will also be true for mycorrhizas.

Mycorrhizas may, however, be affected by changes in soil chemistry resulting from direct deposition on the forest floor or soil surface. In forests and heathlands there is an additional influence of throughfall chemistry. Throughfall water can differ significantly in concentrations a chemical composition from bulk deposition. This difference is caused by a much higher dry deposition in the canopy (Van Breemen & Van Dijk 1988) a by leaching and exchange of ions from the foliage. Leaching from the can is dependent on the nature of the dominant tree species (Bergkvist et al 1986).

SO_x, NO_x and NH_x are potential acids and their deposition causes acidification of the rainwater and consequently of the soil, leading particularly in base-poor sandy soils to higher concentrations of potent toxic metals such as Al^{3+} (Van Breemen & Van Dijk 1988). Skeffington & B (1986) showed that in a sandy humoferric podzol treated with either

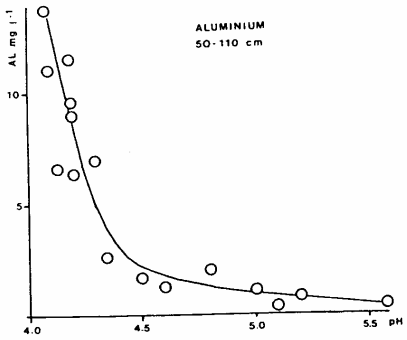


Figure 2. The relationship between the pH and the aluminium concentrati percolating water in the lower horizons of a spruce forest soil. (After Tyler et al. 1987).

distilled water or 0.5 mM H_2SO_4 at pH 3.0, Al and Mg solubility increased due to the acidification and they appeared in the leachates from acidified lysimeters. In addition, NO_3^- increased, particularly from lower soil horizons as a result of increased nitrification. These results were obtained from high inputs of acid (14 keq ha^{-1} yr^{-1} H^+, compared to the normal input in wet deposition of 1 keq ha^{-1} yr^{-1}). Similarly, Brown (1985) showed that a decrease in pH of rain increased the leaching of Ca, Mg, K, Mn and Al from *Pteridium* litter which would, therefore, increase the concentrations of these elements in the rooting zone of the soil.

From natural forest stands in Sweden, under *Fagus sylvatica, Carpinus betulus, Quercus robur* and *Picea abies*, Tyler et al. (1987) showed that the concentration of Al, Mg, Cd and Zn in percolating water was dependent on rain pH. Aluminium concentrations in soil water increased from 1-2 mg l^{-1} at pH 5.5 to 12-13 mg l^{-1} at pH 4.2 (see Fig. 2). In their study of soil chemical changes in a Swedish podzol, Nilsson & Bergkvist (1983) showed that the concentration of aluminium in soil solution due to increased acidification reached 3.3-9.8 μmol l^{-1} in leachates below the A0 horizon and 29.3-47.0 μmol l^{-1} below the A2 horizon.

The acidifying potential of high ammonium deposition (e.g in The Netherlands average 40-80 kg N ha^{-1} yr^{-1} with locally up to more than 200-300 kg N ha^{-1} yr^{-1}), which is deposited together with SO_2 as $(NH_4)_2SO_4$ (Van Breemen & Van Dijk 1988), is an important consequence of the eutrophication with nitrogen. According to Van Breemen & Van Dijk (1988) uptake of NH_4^+ by plants and nitrification of NH_4^+ to NO_3^- are the dominant sources of H^+ in forest soils in The Netherlands. They also report that the nitrification rates in acid forest soils (pH 3.5) is sufficiently high to account for the transformation of all atmospherically deposited NH_4^+ to HNO_3.

4 THE EFFECTS OF POLLUTION ON MYCORRHIZAS

4.1 Direct effects of gaseous pollutants

Just as foliage of trees may be damaged by direct oxidation from O_3 pollution or the acidifying action of SO_2 (CEC 1986), so fungi may also be affected. We are aware of few studies which measured these direct effects. Carney et al. (1978) investigated the activity of mycorrhizal roots exposed to O_3 and SO_2. They exposed excised non-mycorrhizal and roots inoculated with either *Thelephora terrestris* or *Pisolithus tinctorius* to 50 and 500 $\mu l\ m^{-3}$ O_3 or SO_2 in phosphate buffer solution in flasks and determined their respiration as an index of metabolic activity. Mycorrhizal roots showed higher rates of respiration than non-mycorrhizal roots, presumably due to the increased live biomass of the fungal component. Oxygen consumption of excised non-mycorrhizal root fragments was reduced by 74-90% when exposed to O_3 or SO_2. Exposure to SO_2 appeared more detrimental than O_3. Respiration decrease due to the pollutants was less in mycorrhizal roots than non-mycorrhizal roots. From this they hypothesized a protective role of the mycorrhizal association. Although the authors did not know which type of injury is caused by exposure to O_3 or SO_2, they stated that if newly developed fine roots were exposed to these gases, these roots would be unable to develop mycorrhizas because mycorrhizas would never develop in moribund or dead tissue.

In addition, it has been shown by Garret et al. (1982) that exposure to 50 and 500 $\mu g\ l^{-1}$ O_3 and SO_2 for one hour was sufficient to reduce respiration by 65-85% from *P. tinctorius* and *T. terrestris* fungal isolates in pure culture. *Pinus taeda* roots, in association with these fungi, showed a reduction of 30-65% in respiration when exposed to 50 $\mu g\ l^{-1}$ O_3 or SO_2.

As far as we are aware, there appears to be little or no information on the direct effects of NO_x and NH_3 on mycorrhizal fungi and mycorrhizas.

We must be careful in the interpretation of these experiments as they do not really represent the natural system in soil where high concentrations of either gas are unlikely to be encountered by roots. SO_2, NO_x and NH_3 will be readily absorbed into solution on the surface of particles and the roots will be exposed to changes in pH rather than to increased gas concentrations. Similarly, it is equally unlikely that roots will be exposed to O_3. Except for a very few mycorrhizal roots or hyphae occurring in the upper litter layers, it is most unlikely that gaseous pollutants will have a direct effect on their physiology. The direct effects of gaseous

pollutants are probably more important on other groups of fungi, particularly phylloplane fungi (Magan & McLeod 1988, McLeod 1988) and saprotrophic fungi in fresh litter on the soil surface (Wookey 1988).

4.2 Indirect effects: changes in soil chemistry

This section deals with the effects on mycorrhizas of a lowered pH and its consequences in terms of raised aluminium concentration, raised Al:Ca and Al:Mg ratios, and the effects of nitrogen input.

4.2.1 pH

The gaseous pollutants SO_2, NO_x and NH_x are all acidifiers, causing a lowering of soil pH, especially in poorly buffered soils. In addition to induced changes in soil chemistry, changes in soil pH *per se* can have effects on roots and mycorrhizal fungi.

Most fungi have pH optima for growth and mycorrhizal fungi are no exception. This is illustrated by work of Hung & Trappe (1983) who divided their species into five groups according to their growth in culture at pH between 2 and 7. The groups were: (i) growth at optimum pH only e.g. *Amanita muscaria*, (ii) growth increasing with increasing pH e.g. *Hebeloma crustuliniforme*, (iii) best growth spanning 3 pH units e.g. *Laccaria laccata, Piloderma bicolor, Pisolithus tinctorius, Suillus lakei*, (iv) best growth spanning 4 pH units e.g. *Rhizopogon vinicolor, Thelephora terrestris*, (v) best growth spanning 5 pH units e.g. *Cenococcum geophilum, Pisolithus tinctorius*. However, they also showed that there was considerable intraspecific variability in growth response to pH within *Cenococcum geophilum* and *Laccaria laccata*, making generalizations difficult.

It is possible, therefore, that pH *per se* may significantly influence the growth and thus the competitiveness of mycorrhizal fungi under conditions of pollution by acidifying gases. For example, *Pisolithus tinctorius* was found to be more susceptible to reduced pH resulting from sulphuric acid applications to sandy soil in which *Pinus banksiana* were planted, than was *Laccaria laccata* (McAfee & Fortin 1987). The drop of 0.6 pH units in soil as a result of the difference between the pH 5.6 and 2.5 rain treatments added to the soil was enough to induce a change in competitive advantage between the mycorrhizal fungi within 14 weeks. The effect of acid treatment

in this study may not have been entirely due to change in pH *per se*: induced changes in soil chemistry may also have been important, but were not measured. Also Dighton & Skeffington (1987) found a shift in the composition of mycorrhizal types in an experiment with acidified rain. They attributed the effects to elevated aluminium concentrations rather than to lowered pH (see below).

The influence of artificial lowering of the pH has not only been studied in pot experiments but also in forest stands. Blaschke (1988) investigated the effect of simulated acid rain on 80 year old *Picea abies* in permanent plots. Of a variety of treatments given, including acid irrigation at pH 2.8, normal irrigation at pH 5.0, liming (alone or in combination with either rain treatment), it was shown that acidification significantly reduced mycorrhizal infection in humus, but not in mineral soil. Liming in conjunction with acid rain neutralized the effect of acid and liming with normal rain addition stimulated the mycorrhizal formation.

Høiland (1986) studied the fungal fruitbodies in plots (70 years old *Pinus sylvestris* stands) where a watery solution of H_2SO_4 at pH values of 2.5, 3, 4 and 5.6 was applied. Compared to a control (no treatment at all), the total number of species of mycorrhizal fungi decreased (not significant) and the production of fruitbodies increased (significant). Høiland concluded that the acid treatment strongly influenced the fungus flora and made some species much stronger in the competition at the expense of others. However, he did not indicate whether this effect could be an effect of the watering, as their was no control that just got 'clean' water. As he only studied the fruitbodies, this experiment does not give information on the effects of the belowground mycorrhizas.

There are observations in stands under different loading, so not in experiments. In Finland, under different loadings of sulphur and nitrogen, Markkola & Ohtonen (1988) showed that the frequency of occurrence of rhizomorphic species of mycorrhiza associated with *Pinus sylvestris*, such as *Piloderma croceum, Dermocybe, Hebeloma* and "type 03", were significantly reduced with increasing pollutant loading. *Cenococcum geophilum*, however, appeared to increase. They also showed that the number of healthy mycorrhizas declined as did soil respiration with increased pollution. With a parallel increase in both sulphur and nitrogen into the system under study, it is difficult to separate the effects of each pollutant.

Estivalet et al. (1988) showed that there was a reduction in the types of mycorrhiza associated with *Picea abies* stands in France under conditions of tree decline in only one of their sites. This decline was not associated

with root necrosis nor root pathogens. The authors suggested that the reduction in the mycorrhizal infection potential of the soil was caused by soil microbial factors, probably the presence of some species of *Penicillium, Trichoderma, Acremonium* or *Cylindrocarpon*. Similarly, Von Alten & Fischer (1987) suggested that there was no effect of stand health on the mycorrhizal status of *Picea abies* in West Germany. However, they noted that roots and mycorrhizas from damaged stands showed greater deposition of tannins in the tonoplast and vacuoles than those from healthy stands.

4.2.2 pH induced changes in soil chemistry

As pointed out in section 3, there are many changes in soil chemistry due to deposition of atmospheric pollutants. Most pollutants are acidifiers, therefore we can expect them to cause changes in soil chemistry from the addition of a element above ambient levels and changes in soil pH. As a consequence of both, ionic imbalances may occur as a result of redistribution of charges on soil particle interfaces.

Field observations

Much of the emphasis of the Göttingen laboratory (FRG) was centered around the effects of acid rain in increasing the availability of toxic elements in soil, particularly aluminium. In a series of articles the effects of increased aluminium concentrations on the vitality and growth of tree roots and induced reduction of enzymatic competence were demonstrated (Hüttermann 1982; Von Gehrmann & Ulrich 1982; Von Becker 1982). It was Von Becker's article, which demonstrated contorted growth and reduced mycorrhizal development on beech (*Fagus sylvatica*) roots as a result of polluted soil. He observed roots with intact mantles only in the proximal region of the lateral roots. The distal ends of the roots were either reduced in their mycorrhizal development or even reduced to a vascular bundle with no cortical or fungal tissue. Similar necrosis of beech root endodermis and meristematic regions was shown to result from increased aluminium levels at low soil pH (Hüttermann et al. 1983). Blaschke (1986, 1988) also noted a decline in the population of mycorrhizas and abundance of short root tips of *Picea abies* in Höglwald (FRG) when subjected to increased acidification.

Using X-ray dispersive analysis (EDAX) techniques, Bauch (1983) examined the elemental concentration in cortical and xylem cell walls of fine roots of firs and spruce from polluted and non-polluted sites. His data support those of Ulrich in that the Ca:Al ratio of healthy trees is greater than 1 and in diseased trees it is less than one. However the ratio is altered not by increasing the concentration of aluminium in diseased tree roots, but by the reduction of both calcium and magnesium content.

These observations, largely made on roots extracted from mature forests led to an increased interest in the effects of pollutants on roots and mycorrhizas which lagged far behind the physiologists studying the impacts of pollutants on aboveground plant parts. Thus a number of research groups around the world embarked on studies of seedling trees and mycorrhizal fungi in pure culture, manipulated in the laboratory, to investigate the effects of atmospheric pollution on roots, mycorrhizas and mycorrhizal fungi. Although this work, as will be described below, has increased our knowledge considerably, its relevance to forest decline has to be seen in the light of Sobotka's (1964) observations that the effects of pollution were evident on mature trees, but less so on seedling trees.

Fungi in pure culture

There has been some interest in studying the fungal component of mycorrhizal associations in the presence of pollution. In general, the worl has assumed the chemical changes associated with the pollutant (usually ac: rain) increases the mobility of toxic ions such as aluminium. In pure culture, Thompson & Medve (1984) showed that 146 μM aluminium in solution was sufficient to significantly reduce hyphal growth of *Cenococcum*, *Pisolithus* and *Thelephora*, whereas *Suillus luteus* showed no growth reducti until concentrations reached 1000 μM. They also showed that manganese was less toxic than aluminium with *Suillus* and *Cenococcum* showing no growth suppression at the levels used; *Thelephora* growth reduced at 660 μM and *Pisolithus* at 2000 μM. Similarly in the saprotrophic fungus *Aspergillus flavus*, Firestone et al. (1983) showed that concentrations of aluminium greater than 600 μM were needed to suppress germination of spores.

Jongbloed & Borst-Pauwels (1988, 1989) studied the responses of three species of mycorrhizal fungi in pure cultures on increasing aluminium concentrations, on different Al:Ca, Al:Mg and Al:PO4 ratios. Mycelium of *Laccaria bicolor* appears to have optimal dry weight gain at Al:Ca ratio of 5, at lower or higher ratios, growth was restricted. In more sensitive species, *Lactarius hepaticus* and *L. rufus*, growth was restricted at Al:Ca

ratio higher than 1 or 2. The dry weight reduction was accompanied by a strong reduction in lateral growth. The authors concluded that if this occurred in nature too, it could imply a reduction in capacity of the fungus to reach young, growing roots, and thus to form mycorrhizas. It also could imply a reduction in absorbing surface and nutrient uptake capacity. Growth reduction at high aluminium concentrations could not be reversed by raising Ca or Mg concentrations. However, growth was better with lower Al:PO_4 ratios even when the aluminium concentration was high (Jansen et al. 1990, Jongbloed pers. comm.). This is possibly explained by the absorption of aluminium to polyphosphate droplets, as found by Kottke (Kottke & Oberwinkler 1990, I. Kottke pers. comm.)

In addition to the effects of pH and increased concentration of toxic metals on biomass production, their effect on mycorrhizal fungal physiology may also be affected. Apart from significant reduction in growth of *Amanita muscaria* and *Rhizopogon roseolus* occurring at 400 μM and 3700 μM aluminium respectively, Oelbe-Farivar (1985) showed that protein synthesis and OH^- and H^+ transport systems in the fungi were inhibited in the presence of high aluminium concentrations. Such reduction in the mycorrhizal physiological potential could be important even at low levels of aluminium toxicity and is an area which needs further study.

Pot experiments

Schier (1985) grew *Picea rubens* and *Abies balsamea* in hydroponic culture at pH 3.8 in aluminium concentrations of 0 to 740 μM. There was no effect of aluminium concentration on shoot development, but root growth declined from 185 μM aluminium upwards, giving rise to stunted roots and reduced uptake of Mn, Mg and Zn. Similarly, Alexander & Miller (1985) showed that there was little effect of aluminium up to 148 μM on plant growth, but some root damage occurred at higher levels. Entry et al. (1987) also showed that mycorrhizal formation and plant growth was decreased at reduced pH and elevated aluminium concentrations in *Abies balsamea* inoculated with *Cenococcum geophilum, Hebeloma crustuliniforme, Laccaria laccata* and an unnamed mycorrhizal fungus. They pointed out, however, that the interaction between pH, aluminium concentration and plant growth are complex.

Cumming et al. (1986) demonstrated an imbalance in phosphorus uptake by *Picea rubens* seedlings in the presence of aluminium at 37 μM using radiotracer techniques. The ^{32}P content of roots was increased by two orders of magnitude in aluminium treated plants compared with controls and translocation to the apical bud and primary needles was significantly

reduced. This immobilization of phosphorus in the roots may be associated with adsorption-precipitation phenomena of phosphorus-aluminium complexes in the extracellular and intercellular material of the root cortex found by McCormick & Borden (1974) and Kottke & Oberwinkler (1990).

Simulated acid rain has been applied to seedling trees with differing mycorrhizal associations in a number of studies. Shafer et al. (1985) applied an acid rain adjusted with 70 meq SO_4 and 30 meq NO_3 to give pHs of 4.0, 3.2 and 2.4 to *Pinus taeda* seedlings grown in a mixture of steamed loamy piedmont soil and sand. After 16 weeks it was shown that increased acidity reduced the formation of mycorrhizas at the two intermediate pH levels, but at pH 2.4 the acid addition stimulated the formation of mycorrhizas. The nature of the mycorrhizal associations were, however, not established. In a similarly designed experiment on *Pinus strobus* inoculated with *Pisolithus tinctorius*, Stroo & Alexander (1985) showed that mycorrhizal infection, but not seedling tree growth was reduced by addition of pH 3.5 rain at three times ambient rates in steamed soil. In unsterilized soil, however, there was no effect of acid rain on the mycorrhizal infection. In addition, in a range of soils, the effect of acid rain was only significant in 3 out of the 9 soils tested, where mycorrhizal infection was stimulated by addition of rain containing sulphate only and in only one soil where the rain contained additional nitrogen.

In pot experiments and open-top chambers, Reich et al. (1985, 1986) examined the effects of acid rain on the mycorrhizal development of *Pinus strobus* and *Quercus rubra*. In all cases mycorrhizal infection declined with decrease in rain pH. In pot experiments with juvenile plants, Keane & Manning (1987) found a significant reduction of frequency of mycorrhizas with the application of simulated 'acid rain' on *Betula papyrifera*, but not on *Pinus strobus*. They did not point out which species of mycorrhizal fungi were involved and did not describe any possible change in soil chemistry. As the trees were sprayed with the acidified water, a change in tree photosynthesis may not be unlikely. It is very difficult to distinguish between soil and tree mediated effects in this type of experiment.

The effect of added simulated acid rain at pH 3.0 on the mycorrhizas of *Pinus sylvestris* seedlings in monolith soil lysimeters in a humoferric podzol have been studied by Dighton & Skeffington (1987). Acid was added at 30 times the rate of wet H^+ deposition normally found at the field site. Although there was no difference in the fine root length between acid and control lysimeters, there was a significant reduction in the number of fine root tips in the upper soil horizons of the acid treated lysimeters. The

reduction in root tip numbers was related to the change in dominance of mycorrhizal types, with two coralloid types being dominant in the control lysimeters and inhibited in the acid treated lysimeters. The authors suggested that development of these coralloid forms characterized by the production of large amounts of extramatrical hyphae was suppressed by the toxic effects of elevated aluminium concentration on the growth of the fungus (Firestone et al. 1983, Thompson & Medve 1984). Similar changes in the mycorrhizal flora of roots has been demonstrated by Kumpfer & Heyser (1986) in *Fagus sylvatica*. Here, under the influence of acidic stemflow, "type 1" mycorrhizas characterized by limited extramatrical hyphal development, flourished close to the tree base at low pH and low Ca:Al. At a distance from the tree base, where pH was higher and the Ca:Al ratio was higher, mycorrhizal forms ("type 4") with extensive extramatrical hyphal development could exist.

The potential damage by increased mobility of other metal ions as a result of reduced soil pH, has been shown by Dixon & Buschena (1988). Ectomycorrhizal development of seedlings of *Pinus banksiana* and *Picea glauca* with *Suillus luteus* was reduced in the presence of Cd, Ni, Zn, Cu and Pb in a sandy loam rooting medium. Growth of mycorrhizal trees was greater than non-mycorrhizal trees in the presence of these toxic elements and the foliar concentration of metals reduced in the mycorrhizal plants. This data supports the hypothesis of mycorrhizal protection against accumulation of toxic levels of heavy metals in roots (Bradley et al. 1982; Denny & Wilkins 1987a and b; J. Colpaert, pers. comm.). Similarly, *Paxillus involutus* mycorrhizas have been shown to protect tree roots from high concentrations of calcium on calcareous soils (Lapeyrie 1987). In such situations, the mycorrhizal fungus enables the tree to grow on soils where it could not grow without this fungal partner.

In a hydroponic culture experiment, McQuattie & Schier (1987) investigated the combined effects of O_3 fumigation and aluminium concentration in solution. They showed that the effect of each factor was synergistic in suppressing the development of *Pisolithus tinctorius* mycorrhizas on *Pinus rigida* seedlings (Table 3). The greatest effect was with ozone at high levels of aluminium.

The variable response of both plants and mycorrhizal fungi to direct effects of pollution or through soil chemical changes mediated by pollution make it difficult to give generalized conclusions on the effect of pollutants on mycorrhizas. Many workers have used much higher concentrations of pollutants than are found in nature, presenting worst case scenarios.

Table 3. Proportion of mycorrhizal infection of *Pinus rigida* seedlings when treated with ozone and aluminium in different concentrations. (After McQuattie & Schier 1987).

Concentration of aluminium (g m^{-3})	concentration of ozone (g m^{-3}) 0	0.1	0.2
0	25	11	12
25	37	15	18
50	13	5	5

When considered with some of the highest recorded concentrations of these elements in soil solution at the Solling site in FRG, aluminium at 270 μM and manganese at 39 μM (Ulrich et al. 1979), it is uncertain if there is any significant direct effect of toxicity on the fungal component of the mycorrhizal association. In addition, in experiments where the pollutant was applied to both the plant and soil, it became impossible to separate the effects of soil chemical change and possible reduction of photosynthesis and altered carbohydrate partitioning on the development or functioning of mycorrhizas.

4.2.3 Nitrogen

As already mentioned in section 3, deposition of NO_3 and NH_3 may result in exceeding the critical load of nitrate and ammonia in the soil. Apart from causing a lowered pH, due to nitrification of ammonia and uptake of ammonia by plants, input of nitrogen gives mainly a fertilization effect especially on nitrogen poor soils. Number and frequency of mycorrhizas is greatest in soils with low levels of available nutrients, of which nitrogen is probably the most important, and their functioning depends strongly on the availability of soluble nitrogen (Alexander 1983). Nitrogen is usually limiting in the organic layers where most mycorrhizas occur, so its acquisition is of high importance for mycorrhizal fungi (Dighton 1990). Input of nitrogen will have direct consequences on growth of mycorrhizal fungi. Fungi show N optima for growth, so a slight input of N in N poor soils is expected to increase growth for many species. At large inputs,

lower growth is expected except for nitrophilous species with optima at high nitrogen levels.

Field observations

Observations in areas with large nitrogen input (locally in The Netherlands) showed a very low fruitbody production in medium old and mature stands of *Pinus sylvestris* (Termorshuizen & Schaffers 1987a) and both very low fruitbody production and mycorrhizal status in medium old and mature stands of *Pseudotsuga menziesii* (Jansen & De Vries 1988, Jansen 1990). In young stands, effects of nitrogen input could not be proven. In a nitrogen fertilizer field experiment, Termorshuizen et al. (1988) found a significant negative effect on the numbers of fruitbodies of mycorrhizal (and also of saprophytic) fungi. The effect depended very much on the form of nitrogen and the amount applied, with nitrate being more detrimental than ammonia.

Jansen & De Vries (1988) derived a kind of optimum curve for production of fruitbodies and frequency of mycorrhizas in relation to estimated nitrogen input in plantations of *Pseudotsuga menziesii*: moderate nitrogen input resulted in more fruitbodies and higher mycorrhizal frequency than low or high input.

Ohenoja (1988) also found a reduction in fruitbody production with nitrogen fertilization, although sometimes a short living stimulus was seen in some species. Effects depended on soil type, age of the stand, type of fertilizer used and fungal species. Appearance of new species, i.e. species not observed in the forest stands before fertilization, has never been reported (Kuyper 1989).

The greater the amount of nitrogen used, the lower, in general, the number of fruitbodies of mycorrhizal fungi. Fructification seems to be more inhibited by nitrogen fertilization than occurrence of mycorrhizas, and the decrease appears to be stronger in medium old and mature stands than in juvenile stands before canopy closure (Ohenoja 1988, Kuyper 1989). The effects are strongest on dry and on oligotrophic soils (Ohenoja 1988). Also Meyer (1962) found that nitrogen addition had more negative effects on mor than on mull soils. Pathways of these effects are not clearly known. One of the effects is a higher concentration of nitrogen in the fungal hyphae, an effect also observed after calcium fertilization, which may, therefore, be a secondary effect.

Where a balanced NPK fertilizer (Growmore) was applied to a *Fagus* wood, Hall (1978) found that mycorrhizal fruitbody production increased from 50% of the total dry weight of all fruitbodies to 75% in the year following

fertilization. *Hygrophorus chrysaspis, Lactarius blennius, Russula fellea* and other *Russula* species were most affected. Similar increases in *Lactarius rufus* and *Paxillus involutus*, as a result of addition of balanced fertilizer, are reported by Hora (1959) and Laiho (1970).

Pot experiments

Several authors studied the effects of nitrogen addition in pot experiments. Richards (1965) found a reduction in proportion of mycorrhizas on *Pinus taeda* seedlings after adding $NaNO_3$. Such a reduction was not found with the application of Na_2CO_3, so he concluded that the reduction was an effect of the nitrate ion. As a result of the nitrate application the pH increased. This did not cause the reduction in mycorrhizas, as increased pH due to lime application in combination with a Fe application to prevent chlorosis, did not reduce the mycorrhiza development. As already mentioned in section 2.2, Richards (1965) attributed a good mycorrhizal status to the balance between carbohydrates and nitrogen in the root tissues.

Treatment of 3-5 years old Douglas-firs with a solution of $(NH_4)_2SO_4$ at different levels caused a significant reduction in the frequency of mycorrhizas (Gorissen et al. in prep.). This effect must be attributed to the N, and not to the S-part of the chemical used. Although the level of the treatment, increased NH_4 concentration, lowered pH and increased aluminium concentration were closely interrelated, statistical analysis of the data indicated that the mycorrhizas reacted most strongly to the lowered pH, and less to NH_4 or Al concentration, or to Al:Ca ratio. It is, however, likely that high nitrogen concentration together with a low pH affect mycorrhizas in a different way than low nitrogen concentration and low pH, caused by agents other than nitrogen such as H_2SO_4.

Gorissen & Jansen (in prep.) found interactive effects between nitrogen and ozone (see also section 4.3.2) with positive effects of ozone treatment on the frequency of mycorrhiza of juvenile *Pseudotsuga menziesii* plants being nullificated by high additions of ammonium sulphate.

This has again highlighted that the balance of fertilizer composition is important for the growth of the tree. The addition of a single nutrient element, although satisfying a deficiency of that element may lead to enhanced deficiencies of other elements, causing reduced tree growth. This has been demonstrated for a variety of tree genera, e.g. *Picea, Pinus* (Ingestadt 1979), *Betula* (Ingestadt & Lund 1979), *Pseudotsuga* (Van der Burg 1975), *Eucalyptus* (Dighton et al. 1990). The difference between the results of these fertilizer experiments may well lie in the balance of available

nutrients in soil solution following the addition. In the case of addition of nitrogen alone, this may satisfy a single deficient nutrient and lead to reduced dependency of the trees on mycorrhizas, lowered fungal biomass production or cause nutritional imbalance which is detrimental to fungal and tree growth. Additions of nitrate may be more detrimental to mycorrhizal fungi than additions of ammonium, because not all fungi are able to utilize nitrate. Nitrogen fertilization generally reduces the amount of the fructification of most species of mycorrhizal fungi.

Kuyper (1989) suggested two possible pathways to explain the negative effects of N addition on mycorrhizas and fruitbodies: either nitrogen inhibits production of phytohormones by mycorrhizal fungi, or nitrogen induces changes in shoot:root ratio, where a higher shoot:root ration could be the result of less carbohydrates transported to the roots. As more detailed pathways these fit the general hypotheses explaining mycorrhizal decline: soil mediated versus tree mediated effects (see section 2.3). Dixon et al. (1981) found an increase in mycorrhizal development when nitrogen was applied to the foliage and a decrease when applied to the soil. This strongly indicated that if there are negative effects of nitrogen on mycorrhizas, these are soil mediated.

4.3 Indirect effects: changes in carbon availability

In addition to the potential direct effects of SO_2 and O_3, increased soil acidification and mobilization of toxic metal ions, the physiological status of the host plant could significantly affect the degree of mycorrhizal infection of its root system. Since the mycorrhizal association is mutualistic, the ability of the host plant to supply carbon to the fungal partner must also be taken into consideration. After an estimation of the carbon demand by the population of mycorrhizal fungi, we will discuss the effects of reduced photosynthesis on this population.

4.3.1 Carbon demands by mycorrhizal fungi

Little is known about the energy balance of a mutualistic tree-ectomycorrhizal fungus association. In their review on ecological aspects of mycorrhizal symbiosis, Harley & Smith (1983) stated that "no actual measurements of the quantities of carbon compounds used by the fungus as a

Table 4. Production of (mycorrhizal) fungi in various forests. Figures in kg dry weight ha^{-1} yr^{-1}.

	Source of data				
	A	B	C	D	E
fruitbodies					
above ground	180	41	30	3.1	0.3-44
below ground	nd	24	380	--	--
sclerotia	nd	2158	2700	nd	nd
fungal part of mycorrhizas	nd	6104	630	± 500	0-3400
hyphae	nd	6991	nd	nd	nd
total	>180	15318	>3740	>500	>0.55-3410

A: Romell(1939, cited by Harley & Smith 1983), *Pinus sylvestris*, fruitbodies of *Suillus bovinus* only.
B: Fogel & Hunt (1979), 35-50 year old stands of *Pseudotsuga menziesii*, not distinguished between mycorrhizal and saprophytical fungi.
C: Vogt et al. 1982, 180 year old stand of *Abies amabilis*.
D: Ellenberg et al. 1986, *Fagus sylvatica*.
E: Jansen (own observations) *Pseudotsuga menziesii*, stands of 7-55 year old.
For D and E: no data of below ground fruitbodies; in these ecosystems the amounts of below ground fruitbodies are negligibly small.
nd = not determined.

proportion of total photosynthesis have been made, but some estimates are available for some ectomycorrhizal plants and some ecosystems". However, some estimates can be made.

Calculations of carbon use by mycorrhizal fungi start with estimates of their annual production (Table 4). The annual production of mycorrhizal fungi is the sum of the production of fruitbodies (above and below ground), sclerotia, fungal part of mycorrhizas, and mycelium and strands growing from the mycorrhizas into the soil.

Production of above ground fruitbodies is easiest to assess. Values found range from 3 to 180 kg (dry weight) ha^{-1} yr^{-1} (Fogel & Hunt 1979, 1983) in natural stands of *Pseudotsuga menziesii* in the USA, and 0.3 to 44 kg (dw) ha^{-1} yr^{-1} (Jansen own observations) in *Pseudotsuga menziesii* plantations in The Netherlands. For below ground fruitbodies and sclerotia values of 24 to 2700 kg ha^{-1} yr^{-1} are reported (Fogel & Hunt 1979; Vogt et al. 1982).

Estimates of belowground fungal biomass associated with mycorrhizas have also been made. The mass of mycorrhizal roots (standing crop) is estimated by Vogt et al. (1983) at 400 and 650 kg ha^{-1}, and between 0.02 and 9730 kg (dw) ha^{-1} by Jansen (own obervations). Assuming that ca. 35% of a mycorrhiza is fungal tissue (34%, Alexander 1981; 39.1, 37.0, 34, and 20-30%, several authors cited by Harley 1989), these values equal between 0.006 and 3400 kg fungal tissue (dw) ha^{-1}.

In the calculation of carbon use by mycorrhizal fungi, annual fungal biomass production is more important than biomass or standing crop. Fogel & Hunt (1979) estimated that in a 35 to 50 years old *Pseudotsuga menziesii* stand 15314 kg ha^{-1} yr^{-1} (= 50.5% of the total production) was produced by the fungal component (sheaths of mycorrhizas 20%, sclerotia and fruitbodies 7.6% and hyphae 23%). The contribution of fungi in the total stand annual production is much higher than their contribution in standing crop, as "fungal turnover is five times faster than that of the forest floor and the throughput of mycorrhizae (mantle, plus host tissue) is three times larger than the combined throughput of foliage, branches, and boles". They did not verify, however, whether all sclerotia and hyphae were mycorrhizal. Vogt et al. (1982) estimated that of the total net *Abies amabilis* forest primary production, 13.9 to 15% was due to mycorrhizal turnover, which equaled 3260 to 3740 kg ha^{-1} yr^{-1} fungal biomass (Table 4). Jansen (own observations) did not exactly measure the turnover rate of mycorrhizas, but found that the annual production was at least equal to the biomass at a certain moment. The annual production of mycorrhizal fungi in the *Pseudotsuga menziesii* stands she studied varied between 0.55 and 3410 kg (dry weight) ha^{-1} yr^{-1} (Table 4).

A rough estimate of the sugar consumption needed to realize that production can be made. According to Harley & Smith (1983), "the gross amount of photosynthates needed to form these fungal structures is more than twice their mass". Assuming an efficiency of 40%, the estimated consumption of photosynthates by mycorrhizal fungi is between 1.4 kg ha^{-1} yr^{-1} (lowest value, found by Jansen) and $38.3 \cdot 10^3$ kg ha^{-1} yr^{-1} (highest value, found by Fogel & Hunt).

Measuring photosynthesis and respiration is another method to calculate carbon use by mycorrhizal fungi. Tranquillini (1964, cited by Harley & Smith 1983) compared actual dry weight gain with the expected dry weight gain computed from net photosynthesis. He found that the actual dry weight gain was only 30% of the expected dry weight gain. The missing 70%, or 1.55 g per g dry weight of leaves, was assumed to be used by mycorrhizal fungi. Harley

& Smith (1983) concluded that even with some errors in the measurements of photosynthesis and respiration, ..."the amount of unaccounted photosynthate illustrates that the demands of the mycorrhizal fungus must be considerable". Ellenberg et al. (1986) estimated the respiration of fine roots and mycorrhizas to be 1300 kg C ha^{-1} yr^{-1}, which equaled the respiration by the canopy at night. Together with the respiration of the mycelium in the soil and the fruitbodies of mycorrhizal fungi, they estimated the total respiration of carbon as $4.3 \cdot 10^3$ kg ha^{-1} yr^{-1}. Although the authors stated that this figure is probably too high, they concluded that the respiration of fine roots and symbiotic fungi is remarkably high.

Although data are scarce, one may conclude that mycorrhizal fungi contribute much to the production in a forest and use considerable amounts of carbohydrates to realize this production. In natural stands of *Pseudotsuga menziesii* and *Abies amabilis*, the contribution of fruitbodies and sclerotia is much bigger (ca. 5 times) than of mycorrhizal sheaths and mycelium. However, in planted stands of *Pseudotsuga menziesii* and *Fagus sylvatica* in western Europe the production of the fungal part of mycorrhizas is much higher than of fruitbodies. This is especially so because the quantity of below ground fruitbodies is negligibly small.

4.3.2 Reduced availability of photosynthates

Atmospheric pollutants have the potential to affect the photosynthetic capacity of tree foliage and to influence the distribution of photosynthates through the tree. The resulting reduction in carbohydrates available to root systems would cause a decrease in root growth and mycorrhizal development. Several authors studied the effects of gaseous pollutants on the mycorrhizal development of juvenile plants. In these studies photosynthesis and transport of photosynthates, and reduction in these processes, were generally not measured. Effects on the mycorrhizas, positive or negative, are therefore not easy to explain.

Amundson et al. (1986) reported significant reductions in net photosynthesis rates of one year old needles of *Pinus contorta* and *Pinus banksiana* when exposed to 25 mg l^{-1} and above of SO_2 for 30 min. This, combined with shorter needle retention time and chlorosis could account for considerable loss of carbon allocation to the roots. Similarly, Van Hove (1989) reported reduced photosynthesis in *Pseudotsuga menziesii* with 112 μg m^{-3} SO_2.

In contrast to sulphur dioxide, ozone did not significantly reduce net photosynthesis, as was showed by Skärby et al. (1987) when exposing a 20 year old stand of *Pinus sylvestris* to 120 to 400 $\mu g\ m^{-3}$ O_3. However, increase in transpiration, stomatal conductance and dark respiration at levels of O_3 greater than 250 $\mu g\ m^{-3}$ could account for a 60% higher accumulated respiration from the shoots. This induced loss of carbon in respiration could result in reduced allocation to the root component for maintenance of mycorrhizas.

In an ozone gassing experiment on juvenile *Pseudotsuga menziesii*, Gorissen found an accumulation of carbon in the needles, so indications of a blocked transport to other plant organs (Gorissen pers. comm., Gorissen & Jansen in prep.). Net CO_2 fixation was not affected by the ozone gassing. The ozone gassed plants, however, had higher proportions of mycorrhizas, 33.3% than the not treated plants, 21.4%. The effect of ozone was only stimulatory at low or moderate doses of nitrogen (5 or 50 kg N $ha^{-1}\ yr^{-1}$); at high nitrogen dose (200 kg N $ha^{-1}\ yr^{-1}$) there was no difference in mycorrhizal development between the ozone treated and not treated plants.

Stimulatory effects of ozone on mycorrhiza development were earlier observed by Reich et al. (1985, 1986). Ozone gassing of *Pinus strobus* and *Quercus rubra* in open top chambers had a stimulatory effect at intermediate levels of exposure (70 $\mu g\ m^{-3}$) and in some case at high levels of O_3 (120 $\mu g\ m^{-3}$). Reich et al. hypothesized that "effects of ozone on growth or 'chemical communication' between shoots and roots may enhance mycorrhizal infection, and (or) ozone may act as a general stress upon the whole plant and thereby allow increased infection of a less resistant plant". Also Gorissen & Jansen (in prep.) think it possible that the effect of ozone on enzymes, phenolic compounds or free amino acids influences the establishment of mycorrhizal infections. However, pathways of this effect, and of the interactions with nitrogen in the soil, are completely unknown.

In some experiments, however, ozone treatment reduced the mycorrhizal frequency. Keane & Manning (1987) reported a reduction in mycorrhizal infection in *Betula papyrifera* in five of the eight treatments. Such contradictory results, and especially the reported stimulation of mycorrhizal infection, warrant further studies.

Keane & Manning (1987) showed that gassing with SO_2 had no effect on the frequency of mycorrhizas of *Pinus strobus* and a slight, but not significant effect on *Betula papyrifera*. Exposure to O_3 and SO_2 combined resulted in a positive effect on the frequency of mycorrhizas in case of *P. strobus*, but interactions between O_3 and simulated acid rain did not occur. From the

details of the methods given, it is not clear if the effect on mycorrhizas is a direct effect of the pollutant on the roots and mycorrhizas or, more likely, an indirect effect due to reduced photosynthesis or carbohydrate transport.

In their study on the effects of ozone and sulphur dioxide on *Pinus taeda*, Mahoney et al. (1985) showed that exposure to either pollutant alone did not affect the mycorrhizal formation of the tree seedlings. However the presence of mycorrhizas on the roots of the trees promoted shoot and root growth such that there was no effect of fumigation treatment on the mycorrhizal seedlings. Effects of nitrogen in form of NH_3 gas or NH_4OH dissolved in rain is reported to be very detrimental to mycorrhizas. Van der Eerden et al. (1988) found a very significant reduction in frequency of mycorrhizas of 3 years old Douglas firs (*Pseudotsuga menziesii*) after 13 weeks of fumigation with NH_3. Both Mahoney et al. and Van der Eerden et al. did not measure photosynthesis and transport. If fumigation alone affects the mycorrhizal development, a tree mediated effect is, however, likely.

Schulze et al. (1987) showed an indirect effect of atmospheric pollution on photosynthesis. In a site receiving acid rain, fine root and mycorrhizal development was reduced, causing magnesium deficiency in needles. The yellow needles photosynthesized at a lower rate than green needles from the same site and from a similar site unaffected by acid rain. There were no differences in photosynthetic rate between green needles from both sites.

The dependence of mycorrhizas on carbon supply from the host plant is well documented. Reduction in that supply either by reduction in photosynthetic capacity or changes in carbon partitioning within the plant will reduce the development and efficiency of mycorrhizas. The degree to which pollutants reduce photosynthesis and alter internal translocation pathways in trees is not clearly understood. Also the interrelationships between carbon allocation to roots and mycorrhizas and nutrient uptake need to be evaluated further to fully understand the potential effects of pollution and tree nutrition.

4.4 Effects on ecosystem level

4.4.1 Fungal community development in forest plantations

Succession, or changes in composition of the population of mycorrhizal fungi with time, is observed by many authors. In pre-canopy closure stages of very young stands (plantations), the number of species and of fruitbodies is rapidly increasing each year (Mason et al. 1982, 1984, 1987; Dighton et al. 1986; Fleming et al. 1984; Last et al. 1987). These numbers are highest at the stage of canopy closure, because both the fungi from the pre-canopy closure stage as those of the mature forest stage are present. In the pre-canopy stage the stand is colonized by 'early stage fungi' (Deacon et al. 1983), which also could be called pioneer fungi and classed as r-strategists (Dighton & Mason 1985). Species of the pioneer phase of the stand disappear around canopy closure when species of the more mature phase of the stand appear. Species of mature phase of a stand can be classed as K-strategists (Dighton & Mason 1985).

Older stands were amongst others studied by Ricek (1980)(Table 5). Following stands for 24 years, he found a correlation between increasing stand age and increasing number of species up to ca. 10 years after the first canopy closure. The highest species diversity was found in stands of 20-30 year old. In later stages, the pioneer fungi started to disappear, resulting in a lower number of species in stands of about 40 year. Bendiksen (1981) compared the occurrence of species of mycorrhizal fungi in 8 stands of different age without giving detailed information about the canopy development. He found both the highest number of species and the highest number of fruitbodies in stands of about 20 year old. The number of fruitbodies was lower in the mature stands; the number of species remains the same. Although his data comprised of only 4 genera of mycorrhizal fungi, it is likely that they are representative of the whole spectrum of mycorrhizal fungi in these vegetation types. Higher numbers of species, and fruitbodies, in young stands compared to older stands are also reported by Termorshuizen & Schaffers (1987a), Hintikka (1988), Jansen & De Nie (1988) and Jansen (1990).

There is evidence that this succession seen in fruitbodies has a below ground basis. Deacon et al. (1983) and Jansen & De Nie (1988) found good correlations between fruitbodies and mycorrhizas. Jansen & De Nie (1988) found higher numbers of mycorrhizas per volume of soil in young stands, compared to older stands.

Table 5. Number of species of mycorrhizal fungi, seen as fruitbodies, as a function of stand age.

	Source of data									
	A		B		C		D		E	
	stand age	no. of fungi	stand age	no. of fungi	stand age	no. of fungi	stand age	no. of fungi	stand age	no. of fungi
pre-canopy closure	0-10	15	3-5	1	5-15	26	4-13	41	--	--
early canopy closure	10-20	38	ca.10	15	20-30	39	--	--	8-19	30
late canopy closure and thinning stage	20-30	47	ca.20	28	30-50	34	--	--	20-38	22
mature forest	ca.40	36	40-70	29	>70	29	50-80	18	41-55	22

A: Ricek (1980): 19 stands of *Picea abies*, FRG, followed from 1966-1979;
B: Bendiksen (1981): 8 stands of *Picea abies*, Norway, followed from 1978-1980;
C: Hintikka (1988): 25 stands of *Pinus sylvestris*, Finland, 1975-1977;
D: Termorshuizen & Schaffers (1987a): 35 stands of *Pinus sylvestris*, The Netherlands, 1986-1987;
E: Jansen (own observations): 25 stands of *Pseudotsuga menziesii*, The Netherlands, 1986-1989.

In general, in a newly planted stand the number of species and of fruitbodies of mycorrhizal fungi gradually increase, reaching the highest number around canopy closure. These numbers fluctuate due to management practices (fertilization, thinning etc.). Eventually, there is a slight decrease, so in old, mature stands there are slightly lower numbers of species and fruitbodies. There is a considerable shift in species composition.

If we can consider the fungi occurring mainly with younger forest stands as being 'r-selected' and the fungi occurring late in the forest rotation as 'K-selected' (sensu MacArthur & Wilson 1967), then from theoretical considerations we can assign characteristics to these fungi in terms of growth, reproduction and competitive stability (Heal & Ineson 1984). K-strategists are relatively more complex and have the potential for closer associations with other organisms in the environment, whereas the r-strategists are opportunistic. Although K-strategists have a greater capacity to withstand perturbating conditions, if the external pressure leads to extinction, than the community will revert to the opportunistic r-strategists.

4.4.2 Disturbed succession in polluted areas

The effect of pollutants, if sufficient to upset the stable equilibrium of K-selected organisms, would tend to favour the development of opportunistic individuals (r-strategists) in a less stable environment. In addition, with sustained pressure of the pollution, resistant indivíduals may subsequently emerge and survive under the stressed environment. These organisms are adversity (A-) selected (Southwood 1977) or stress tolerant (S-) species of the R-C-S (Ruderals, Competitors, Stress-tolerators) model of Grime (1979). If we look at the data on the fungal decline in the Netherlands (Arnolds 1985, 1988, 1989) it can be seen that many of the fungi lost from forests as a result of pollution are those which can be classed as K-selected organisms. Thus we are approaching a system of mature forests becoming increasingly associated with fungi that actually belong to an earlier stage of stand development and which are not necessarily appropriate to these older trees.

The effect of atmospheric pollution, causing a decline of mycorrhizal fungal species of older stands (K-strategists), relative to more opportunistic species (r-strategists) of young stands has been observed by

Termorshuizen (pers. comm.) and Jansen (1990). Both studied young and old stands in relatively more and less polluted areas in the Netherlands. Young stands appeared to have the same number of species irrespective of region, so irrespective of amount of atmospheric pollution, and also the number of fruitbodies did not differ according to region.

In mature stands, both the number of species and of fruitbodies were considerably lower in the more polluted areas. Schlechte (1986) found the same when comparing 2 mature stands, one influenced, one not influenced by air pollutants. Mycorrhizas are influenced in the same way. No differences in number and frequency of mycorrhizas could be observed in young stands according to region, according to air pollution levels. In mature stands of Douglas fir (*Pseudotsuga menziesii*) significant lower numbers and frequencies of mycorrhizas were observed in the more polluted areas (Jansen 1990).

The nutritional implications for trees of the changes in fungus flora from K-strategists to r-strategists are not clearly understood, particularly in relation to the acquisition of nutrients from different pools in the soil (Dighton & Mason 1985, Dighton 1990).

5 GENERAL DISCUSSION AND CONCLUSIONS

The mechanism of pollution effects on the development and functioning of mycorrhizas is not yet known in detail. We have considerable knowledge of the morphology and structure of mycorrhizas and some understanding of their physiology in relation to nutrient uptake, carbon demand and regulation of formation. However, this knowledge is far from complete, especially with respect to the physiology of extramatrical hyphae. It is evident that mycorrhizas need carbohydrates for their growth which they get, at least partially, from the tree host. Björkman's 'carbohydrate theory' stated that "Mycorrhizae develop characteristically if the roots of the host plant contain a surplus of soluble carbohydrates" (Björkman 1942, 1944, 1949, cited by Nylund 1988). This theory could, however, not explain the influences of soil chemistry and its changes on the occurrence of mycorrhizas.

When the influence of several 'plant hormones' produced by the mycorrhizal fungi on the host was recognised, Slankis formulated his 'hormone theory'. This theory stated that the symbiosis was brought about by plant hormones produced by the mycorrhizal fungus and that "the fungus through its release of growth regulators strongly influences the host carbon balance" (Slankis 1951, 1967, 1973, cited by Nylund 1988). This theory was a good step forward, but again, it could not explain everything.

That is why Nylund (1988) found it time for a new, 'unifying theory', which brings together probably not all, but many of the "ideas concerning regulation of mycorrhiza synthesis brought up during the last half century". Such a 'unifying theory' is necessary to interpret the effects of air pollutants on mycorrhizas, and probably even to describe the symptoms. Richards (1965) already stated that mycorrhizal formation was dependent on the balance between carbohydrates and nitrogen. The new 'unifying theory' as Nylund proposed will be even more complex than Richards' balance theory and will deal with many variables concerning the physiology of fungi and tree hosts, their inter- and intra-specific variation, changing tree physiology with age, variation in soil nutrient and water supply, hormone production, growth strategies of trees and fungi, whole plant physiology, etc. Effects and phenomena observed in air pollution studies can contribute much to such a theory, as these will give indications on working mechanisms that could not be observed in normal, undisturbed situations.

There appear to be two possible main indirect effects of pollutants on mycorrhizas. Firstly, reduced carbon allocation to the fine roots may result

in less energy for the growth and the maintenance of roots and mycorrhizas, and secondly, induced soil chemical changes may cause nutritional imbalance in the soil and/or increased solubility of toxic elements.

It seems likely that the amount of photosynthate available to fine roots and mycorrhizas determines maximum growth, regrowth and functioning of the system of fine roots, mycorrhizas and fruitbodies of mycorrhizal fungi, thus the upper limit of the annual production of fine roots, mycorrhizas and fruitbodies. The flux of photosynthates to the roots may depends on the age of the tree, season, the carbohydrate reserves in fine roots, the rate of transformation into fungal sugars (e.g mannitol and trehalose) or other compounds (e.g. ergosterol) by mycorrhizal fungi and their storage in the mycorrhizas. One can assume that the flux of photosynthates to the root system is a purely mechanical process driven by roots and mycorrhizas acting as a sink. But it seems more likely that this transport is regulated by the action of 'plant hormones'.

Evidence has been cited above to suggest that SO_2 and O_3 may reduce the photosynthetic capacity of trees and/or alter the carbon partitioning within the trees. A reduced allocation of carbohydrates to the roots may inhibit the development of mycorrhizal fungal structures and, because of the relationship between carbon supply and nutrient uptake, have severe consequences for tree nutrition. The very few available quantitative data on reduced carbon transport to the fine roots do, however, not suggest that this transport is completely blocked. It is not known whether the plant is able to prevent the carbohydrates to be transported into the mycorrhizas, and if so, by which mechanism.

A shortage of photosynthates in the fine roots is probably not the only cause of decreased mycorrhizal infection. If it were, one should expect that all mycorrhizal species would be affected in the same way, and this is definitely not the case. Arnolds (1988) has shown that the occurrence of fruitbodies of some mycorrhizal species are reduced and others appeared to be tolerant to pollution. Apart from carbohydrate shortage, there are apparently other factors restricting mycorrhiza formation. This is in line with Persson's idea (1988) that growth of mycorrhizal fungi is not limited by carbon but by nitrogen, phosphate and magnesium.

In forests stands there are probably no or only very small direct effects of gaseous SO_2 and O_3 on roots and mycorrhizas, due to the low degree of penetrability of these gases in soil and rapid change from the gaseous to liquid phases in soil solution. Study of the effects of pollutant gases on

carbon allocation and changes in soil chemistry are probably a more fruitful area of research in the future.

Despite the soil chemical changes induced by acid rain or deposition of other pollutants, much may be learned about the distribution and functioning of mycorrhizal fungi from different soil types. In a survey of the distribution of fungal fruitbodies in *Fagus* forests of southern Sweden, Tyler (1985) showed that mycorrhizal fungal fruitbody abundance was greater on acidic soils with more or less well defined mor properties. Decomposer fungi, in contrast, were more frequent on less acid mull soils. The consequences, not only of the shift in dominance of fungal type, but in the physiology of both saprotrophic and mycorrhizal fungi under different soil conditions is of importance in understanding the effects of soil chemical changes as a result of pollution.

However, changes in soil chemistry occur as a result of atmospheric deposition. Acidification *per se* can reduce fungal growth and increase solubility of toxic metal ions. These ions reduce growth and physiological activity of mycorrhizal hyphae as well as causing structural damage to the mycorrhizal and non-mycorrhizal roots. Variations in tolerance of such acidifying conditions by different mycorrhizal fungal species has been shown to lead to changes in the mycorrhizal fungal flora of tree root systems. Similarly, the effect of nitrogen addition, either from atmospheric deposition or applied as fertilizer, can change the population of mycorrhizas in forest stands. In areas with extreme high nitrogen deposition, e.g. locally in The Netherlands, this is considered the main detrimental factor for populations of mycorrhizal fungi. When nitrogen is not longer limiting, due to deposition, phosphorus may become the limiting element. Consequences are probably a decreased ability to detoxify aluminium and an increased competition for phosphorus with endomycorrhizas and endomycorrhizal plants (grasses in forests stands).

We have very little information on the comparative physiology of different mycorrhizal fungal species, although there is documented evidence to suggest that changes in mycorrhizal fungal species associated with trees depends on successional age. This may be related to the relative availability of mineral nutrients in soil (Dighton & Mason 1985, Dighton & Harrison 1990). The consequences of changes in development, number of mycorrhizas and mycorrhizal fungal species composition on tree roots on the physiology and nutrition of the tree can only be answered by resorting to the unanswered fundamental questions of mycorrhizal physiology and ecophysiology.

If changes in soil chemistry affect the rate of extension of mycorrhizal hyphae as well as the rate of development of recognizable ectomycorrhizal structures, what is the consequence for tree nutrition? From the work of Frankland & Harrison (1988) it is suggested that a significant contribution of a mycorrhizal fungus to tree nutrition may occur before recognizable mycorrhizal structures (Hartig net and sheath) are visible. How much importance then, should we attach to the correlation between nutrient content and number of recognizable ectomycorrhizas?

Nutrient uptake has been determined by comparisons of nutrient concentrations in mycorrhizal or non-mycorrhizal plants. Harley (1969) and Harley & McCready (1981) have also determined nutrient influx into excised roots on a unit weight basis. Others suggest that surface area of mycorrhizal roots is an adequate unit of measure for influx studies. Very few studies have actually used intact mycorrhizal systems to determine influx via extramatrical hyphae. The degree of development of these hyphae may be restricted by pollution and thus the volume of exploited soil reduced. Unless we are able to evaluate nutrient uptake on a per unit extramatrical hyphal surface area basis, the effect of reduced growth of these hyphae can not be determined. Different mycorrhizal fungal species produce different amounts of extramatrical hyphae. The inherent difference in physiology between fungi producing diffuse hyphae and those producing complex hyphal structures (strands) has also to be evaluated. Are strand formers more efficient translocators of nutrients? Do they exploit a larger soil volume than diffuse hyphal networks?

The interaction between the requirement of photosynthates and nutrient uptake has been elegantly shown for VA-mycorrhizas by Wang et al. (1987). By shading plants they showed that clover plants (*Trifolium* spp) contained higher shoot concentrations of phosphorus under shade conditions, especially at high levels of applied phosphorus, whereas nitrogen content was unaffected by light, but increased with added phosphorus. In onion (*Allium* spp), mycorrhizal infection was reduced at low light intensity and its effects were more marked with plants fed with NH_4^+ than those fed with NO_3^-. Clover shoot dry weight increased to a plateau with increasing phosphorus supply at high light intensity but at low light, growth peaked and then declined at high phosphorus levels. Although this data refers to VAM plants, it is highly likely that similar effects of light influence the development of ectomycorrhizas and their role in nutrient uptake. Much more work needs

to be done to evaluate the magnitude of effects of pollutants on these processes and the underlying physiology.

Schulze et al. (1987) postulated a pathway for interactive effects of atmospheric pollutants. Proton input results in changes in the soil chemistry, of which the change in Al:Ca ratio is one of the most important changes. The increased Al:Ca ratio reduces the vitality of the mycorrhizas and reduces the number of root tips and mycorrhizas. This causes a decreased uptake of Mg. Trees could handle a lower magnesium uptake, if the growth is reduced. However, due to a high nitrogen input, growth is stimulated. The resulting magnesium deficiency causes a lowered photosynthesis. It is clear that the balance between uptake, photosynthesis and growth of the tree is disturbed.

Evidence suggest that the effect of atmospheric pollution is greater on older forest stands than on younger stands (Sobotka 1964, Termorshuizen & Schaffers 1987 a and b, Jansen 1988). If the underlying hypotheses explaining mycorrhizal successions are correct, than the consequences of altering the mycorrhizal population from K-strategists to r-strategists could be important for tree nutrition. It has been suggested that succession occurs in response to an increasing immobilization of mineral nutrients into organic matter on the forest floor. At this time, r-strategist early stage fungi become less efficient at exploiting low concentrations of inorganic nutrients, whereas K-strategist late stage fungi may have the enzymatic capabilities to degrade organic matter to release nutrients for host tree growth. If the population of K-strategists is reduced in older stands by the effects of pollution, than an inappropriate suite of fungi will exist.

Again, the comparative physiology of these fungi has not been investigated fully and generalizations are difficult to make as a number of the late stage K-strategist fungi are, at present, unculturable, making study of their physiology difficult.

Is amelioration possible?

Mycorrhizas always are an integral part of a forest community. Although their relation to the trees is not yet understood in all detail, their functioning in terms of nutrient, water and hormone supply and protection against root pathogens is thought to be indispensable for the trees. This raised the question whether it would be possible to ameliorate the mycorrhizal status of an existing tree stand. Wüstenhöfer (1989) found that in-between-planting of well mycorrhized young trees increased the frequency

of mycorrhizas on the roots of the mature trees, and that this planting is slightly more successful than spraying of spores. Application of Ca, Mg, K or P fertilizers at the same time inhibited, however, the increase of mycorrhizal frequency. Effect of other fertilizers is not known, nor is the effect on tree vitality and growth.

Mycorrhizal species for such an amelioration should be carefully selected. *Pisolithus tinctorius*, often used in the U.S.A., is a native species from relatively warm climates, and prefers soils with a relatively high pH. It is doubtful if its use in western Europe will be successful. *Scleroderma citrinum*, used by Wüstenhöfer (1989) is a species with a preference for broad leafed trees, especially oaks (*Quercus* species), and it is doubtful if it will be of use in stands of conifer trees.

Such an amelioration is in fact always done with early stage r-strategist fungi. As mentioned earlier, a population of r-strategist mycorrhizal fungi will be inappropriate for older trees as they may have less capacity to release nutrients from organic soil layers. Thus, even if techniques to increase the population of r-strategist mycorrhizal fungi in existing stands are successful, one can not consider this an amelioration. The techniques of in-between-planting of young trees and spraying of spores are likely to be not successful in case of K-strategist mycorrhizal fungi.

Fertilization may have positive effects on tree photosynthesis and growth, but will most likely have negative effects on the populations of mycorrhizal fungi. In forest stands, Ca fertilization is often applied to ameliorate lowered pH and lowered Ca:Al ratio due to atmospheric deposition. Wüstenhöfer (1989) studied the effects of $CaCO_3$ application, and $MgCO_3$ plus potassium and phosphate fertilizer, on fruitbody production and number of mycorrhizas in 45 or 65 year old stands of *Picea abies*. The year after the treatments a higher number of fruitbodies of mycorrhizal fungi was observed (not significant). One year later the treated plots had significantly less fruitbodies than the control plots. Such a reduction in fruitbodies is more often reported (Kuyper 1989). Wüstenhöfer (1989) found a slight, not significant increase in number of living and dead mycorrhizas, but observed a shift in the composition of mycorrhizal types: less *Cenococcum* and more light brown and orange mycorrhizas. Also Semjonova (1989) did not found correlations between the amount of lime and the intensity of mycorrhiza formation.

Richards (1965) found in a pot experiment with *Pinus taeda* seedlings a reduction of the mycorrhiza development with liming. But the proportion of mycorrhiza was 'normal' if the chlorosis, which was caused by the calcium

application, was prevented or cured by a Fe application. Some other authors (Wästerlund 1982, Agerer 1989) also found effects of calcium fertilization on soil pH, and on the Ca:Al ratio. Such a fertilization could, however, not reverse the acidification by atmospheric deposition. According to Kuyper (1989) are the quantities of calcium used often much larger than the requirement for trees and mycorrhizal fungi and are the observed calcium fertilization effects usually secondary effects by changes in soil chemistry. The effects of calcium fertilization are often comparable with those of nitrogen fertilization, as calcium stimulates the mineralization of nitrogen and nitrification of NH_4^+, and changes the availability of phosphorus.

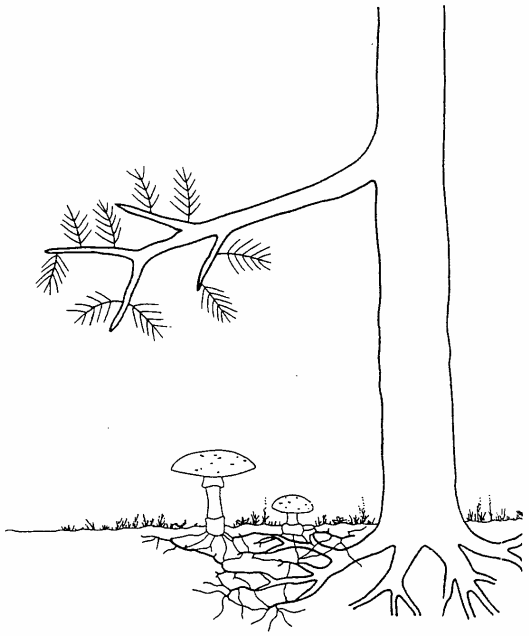
Magnesium fertilization seems to be weakly positive on mycorrhizas. Addition of magnesium, and of potassium, will not have effects as these are usually not limiting (Kuyper 1989). Shortage of magnesium in needles is reported, causing needle yellowing, especially on soils poor in Mg. Whether magnesium shortage is only caused by low soil Mg content or by hampered uptake is unclear, as is the role of mycorrhizal fungi in magnesium uptake. Occurrence of needle yellowing caused by magnesium shortage in air polluted areas and decrease of mycorrhizal status in those areas could be related.

Following their observations on the general decline of the soil's ability to promote mycorrhizal infection as a result of forest decline, Estivalet et al. (1988) showed that the addition of fertilizer resulted in greater growth of *Picea abies* seedlings than in unamended soil from declining forest stands. More controlled experiments allowed them to determine that phosphorus was the main limiting nutrient and that an application of mixed fertilizer with lime gave the best enhancement in growth. Addition of *Laccaria laccata* mycorrhizas partly benefitted the trees. In addition there was an unknown microbial factor which suppressed tree growth in soils from damaged stands. This was alleviated by the application of fungicides or soil pasteurization.

Phosphate fertilization is reported to have (weak) negative or positive effects on fructification (Hora 1959, Garbaye et al. 1979, cited by Kuyper 1989) and *Cenococcum geophilum* is reported to increase (Menge et al. 1977). Phosphate is often applied in combination with nitrogen, in which form it may be weakly compensatorial for the negative effect of the nitrogen (Menge & Grand 1978, cited by Kuyper 1989).

Although effects of fertilizer applications are depending on amount and type used, and on soil type, stand age and probably other factors, the general impression is that they are not very powerful in reversing effects

of atmospheric deposition and the amelioration of the mycorrhizal flora. Amelioration of the mycorrhizal flora by application of spores or mycelium of mycorrhizal fungi is possible, but the technics are not yet enough developed for a successful application of fungi that are appropriate for mature stands.



ACKNOWLEDGEMENTS

This review is as project 201 part of the Dutch Priority Programme on Acidification. The authors wish to thank the Institute of Terrestrial Ecology, Merlewood Research Station (Cumbria, U.K.) and the Wageningen Agricultural University, dept. of Phytopathology (Wageningen, The Netherlands) for providing facilities. Dr. Eef J.M. Arnolds, Helen E. Jones, Dr. Thomas W. Kuyper and Dr. Henrik W. de Nie are thanked for their comments on an earlier version of this review. Frits W. de Vries and Corrie F. Geerds are thanked for their various help and support.

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