Acta Bot. Neerl. 39(4), December 1990, p. 349–358

Effects of ammonium and pH on growth of some ectomycorrhizal fungi *in vitro*

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SUMMARY

In The Netherlands, together with the vitality of trees, most of the associated ectomycorrhizal fungi are declining. This decline is strongly correlated with the deposition of ammonium sulphate. The effect of ammonium and pH on pure culture growth of ectomycorrhizal fungi originating from Douglas fir (*Pseudotsuga menziesii*) plantations was examined at a range of ammonium and pH levels. One isolate each of three ectomycorrhizal fungi, *Laccaria bicolor*, *Lactarius rufus* and *L. hepaticus* were selected and responded to an increase in the ammonium concentration from 1 to 10 mM, with a considerable increase in biomass production and a decrease in radial growth. During growth, acidification of the medium occurred. Addition of ammonium up to concentrations of 10 mM caused this acidification to increase considerably. Buffering the medium slightly affected the effect of ammonium upon biomass production; however, the radial growth production found in unbuffered medium was not apparent in *L. bicolor* and *L. rufus*, and was diminished in *L. hepaticus*. The sensitivity to medium acidification increased in the order *L. rufus*, *L. hepaticus*, *L. bicolor*. Both *Lactarius* isolates showed optimal radial growth at pH 4·0, whereas radial growth of *L. bicolor* increased with increasing pH values up to 6·6. Optimal biomass growth occurred at lower pH values of 3·0, 4·0 and 4·8 for *L. rufus*, *L. hepaticus* and *L. bicolor*, respectively. In contrast with elevated ammonium concentrations, low pH had strong inhibitory effects on two of the fungi examined. That soil pH *per se* is a key factor in the decline of *L. rufus* and the increase of *L. hepaticus* is not supported by the pH response in pure culture.

Key-words: acidification, ammonium, ectomycorrhizal fungi, growth, *Laccaria bicolor*, *Lactarius hepaticus*, *Lactarius rufus*, pH.

INTRODUCTION

Ectomycorrhizal fungi have been reported to increase growth and nutrient uptake by trees, especially when nutrients are at lower concentrations (Harley & Smith 1983). Laboratory experiments have shown that inoculation of *Pseudotsuga menziesii* with an ectomycorrhizal fungus enhanced ammonium uptake (Rygiewicz *et al.* 1984; Kamminga-van Wijk & Prins 1989). In The Netherlands, on the other hand, inorganic nitrogen is

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accumulating in forest soils due to high deposition rates of ammonium sulphate. Ammonium sulphate also causes soil acidification and is seen as a major environmental problem (Van Breemen *et al.* 1982).

In recent decades a decline in ectomycorrhizal fungi in the Dutch forests has occurred, especially among species associated with coniferous trees on acidic, nutrient and humus-poor sandy soils. The decrease has been mainly attributed to acid precipitation and its effect on soil chemistry and the vitality of trees (Arnolds 1988). According to Termorshuizen & Schaffers (1987) a negative correlation exists between the number of carpophores and the number of ectomycorrhizal fungi in 'old' (50–80 years) stands of *Pinus sylvestris* and the amount of NH_3 emission. Jansen & de Vries (1988) have found a comparable relation in Douglas fir stands, although ammonium deposition may also stimulate fruit body formation of ectomycorrhizal fungi to a certain extent. Among the ectomycorrhizal fungi recorded, *Lactarius hepaticus* is one of the most abundant species and *Laccaria bicolor* and *Lactarius rufus* are also common species. Indications exist that the appearance of *L. rufus* has decreased during recent decades, while the frequency of *L. bicolor* is unchanged and that of *L. hepaticus* has markedly increased. An increase of *L. hepaticus* in mature coniferous plantations in recent decades has been reported by De Vries *et al.* (1985). In this context it should be noticed that the species composition of ectomycorrhizal fungi changes with ageing of stands (Mason *et al.* 1982, 1983).

In-vitro studies of ectomycorrhizal fungi have shown that raising the ammonium concentration in a nutrient medium stimulates biomass growth (Littke *et al.* 1984; Boxman *et al.* 1986), whereas radial growth is stimulated or reduced by an increase in the ammonium concentration, depending on the species involved. Ammonium uptake by ectomycorrhizal fungi and ectomycorrhizas is accompanied by medium acidification, inducing indirect effects on growth (Littke *et al.* 1984; Rygiewicz *et al.* 1984). Considerable variations between the response of ectomycorrhizal fungi upon changes in pH have been reported, both interspecific and intraspecific (Laiho 1970; Hung & Trappe 1983)

High ammonium concentrations and low medium-pH belong to the group of so called 'acid rain' factors. In the present study both radial and biomass growth responses of some ectomycorrhizal fungi to these factors are investigated. This study is part of a project on the effects of changing soil chemical factors caused by acid rain on the growth and nutrient uptake of ectomycorrhizal fungi of the Douglas fir. Three isolates have been selected of each of the commonly occurring species, *L. bicolor, L. rufus* and *L. hepaticus.*

MATERIALS AND METHODS

Cultures of *Laccaria bicolor* (Maire) P. D. Orton, *Lactarius rufus* (Scop.) Fr. and *Lactarius hepaticus* Plowr. ap. Boud. were obtained from Dr A. E. Jansen (Agricultural University of Wageningen, The Netherlands). These fungi were isolated from fruit bodies from *Pseudotsuga menziesii* (Mirb.) Franco stands in The Netherlands. Data on the soil type and soil moisture composition of the collection sites of the fungi were obtained from Kleijn *et al.* (1989). *L. bicolor* and *L. rufus* were collected from Kootwijk with a humic podzol with an average pH of 3·52 and an ammonium concentration of $0{\cdot}83 \pm 0{\cdot}54$ mM. *L. hepaticus* originated from Amerongen with a leptic podzol with an average pH of 3·36 and an ammonium concentration of $1{\cdot}39 \pm 0{\cdot}90$ mM. These determinations were done on soil solution samples taken from the upper 15 cm soil layer.

Fungal mycelium, used as inoculum for the growth experiments, was grown on solid MMN (modified Melin–Norkrans) medium (Marx 1969), except that the phosphate concentration was reduced from approximately 5·6 to 3·7 mM by replacing $(NH_4)_2HPO_4$ by an

equivalent amount of NH_4Cl. In the ammonium and pH growth experiments described below, solid nutrient medium (Bacto-Agar 15 g l^{-1}) was used. For inoculation, mycelial plugs 6 mm in diameter were cut from the edge of fungal colonies on solid medium. All experiments took place at 22°C in the dark and were carried out in triplicate. Radial growth was determined by measuring the colony diameter at regular intervals. The growth period varied from 38 to 68 days depending on both the type of experiment and the fungal species, after which fungal biomass was determined by dissolving the agar in hot water, collecting the mycelium by filtration, drying at 105°C for 24 h and weighing. According to Oort (1981) the boiling method will cause a loss of water-soluble compounds amounting to approximately 35% of total biomass with little variation between isolates and no effect of the age of cultures on the amount of loss. We determined that the dry weight loss in *L. bicolor* was approximately 18·5%. The pH was determined at both the beginning and the end of the experiments using a standard glass electrode.

Ammonium growth experiments

For the ammonium growth experiments, Petri dishes were filled with 15 ml solid nutrient medium at one-quarter strength. The effect of ammonium in unbuffered medium was examined by addition of ammonium at concentrations of 1–40 mM at an initial pH of 4·7. In a separate experiment, the ectomycorrhizal fungi were grown on a high (10 mM) and low (1 mM) ammonium medium, both in the presence and the absence of 25 mM citric acid/Tris, at pH 4·0. It is unlikely that citric acid is utilized in the presence of glucose, as it is known as a poor or even in some cases as an unsuitable carbon source for growth of ectomycorrhizal fungi (Jayko *et al.* 1962; Palmer & Hacskaylo 1970). Unless otherwise stated ammonium was added as NH_4Cl.

pH growth experiment

To study the effect of pH on fungal growth on solid medium the agar and nutrient solutions, both at double strength, were sterilized separately. This was done to prevent poor setting of the agar at low pH. The nutrient solutions were adjusted to different pH values with 20 mM citric acid/Tris buffer. After autoclaving and cooling in a waterbath to 50°C, tubes with nutrient solution (10 ml) and an agar solution (10 ml) were combined in a Petri dish and swirled. This resulted in full strength MMN medium with 10 mM citric acid/Tris and pH values ranging from 2·7 to 6·6.

RESULTS

Ammonium growth experiments

At first we examined the effect of varying ammonium concentrations on both radial growth and biomass production of the three fungi in an unbuffered medium with a starting pH of 4·7. This relatively high pH was chosen because from preliminary experiments it appeared that at high ammonium concentrations the pH of the unbuffered medium dropped greatly. Increasing the ammonium concentration from 1 to 5 mM had pronounced effects on both biomass and radial growth of all three fungi (Fig. 1) The biomass production increased 3–4 times. Biomass production by *L. bicolor* and *L. hepaticus* was maximal at 10 mM ammonium and decreased slightly at higher ammonium concentrations up to 40 mM. Biomass formation by *L. rufus* showed a slight but significant ($P < 0{\cdot}01$) increase at ammonium concentrations increasing from 5 to 40 mM.

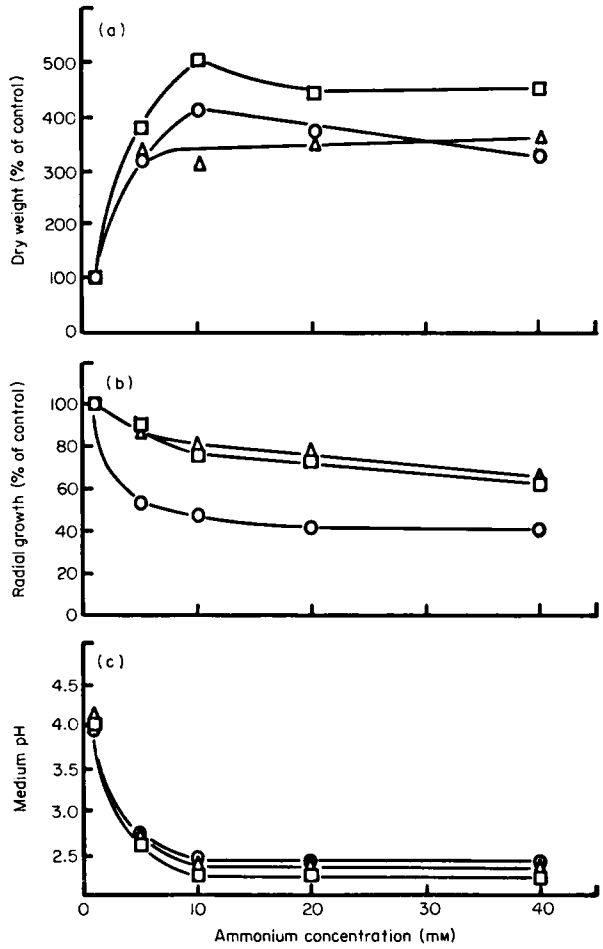

Fig. 1. Biomass production (a), radial growth (b) and medium pH (c) of *Laccaria bicolor* (○), *Lactarius rufus* (△) and *Lactarius hepaticus* (□), after 68 days of growth at various ammonium concentrations in unbuffered solid one-quarter strength MMN medium. Initial pH was 4·7. Results are means of an experiment in triplicate. Standard errors commonly lay within the symbols and are therefore not shown.

The increase in biomass production was accompanied by a reduction in radial growth. This effect was most pronounced in *L. bicolor*. Radial growth of this fungus was reduced 47% by increasing the ammonium concentration from 1 to 5 mM, and was reduced up to 60% at higher ammonium concentrations. The effects of ammonium upon radial growth of both *Lactarius* spp. were similar to those found with *L. bicolor*, but the reduction in radial growth was less severe, amounting to 40% at the highest ammonium concentration.

In the unbuffered medium the pH decreased during growth. Acidification increased with increasing ammonium concentrations and was about the same for the three species examined (Fig. 1c). At 1 mM ammonium the pH decreased from the initial value of 4·7 to 4·0 and at 40 mM ammonium the pH dropped to approximately 2·2.

For a comparison of growth in buffered and unbuffered medium, a starting pH of 4·0, being nearer the pH of natural habitats, was chosen. Two ammonium concentrations were applied, namely 1 mM (low ammonium) and 10 mM (high ammonium). The lowest one was

Table 1. Dry weight production (mg) of the ectomycorrhizal fungi grown for 50 days (*Laccaria bicolor*) and 64 days (*Lactarius rufus* and *Lactarius hepaticus*) on buffered (25 mM citric acid/Tris) or unbuffered solid one-quarter strength MMN medium with high N (10 mM NH_4) or low N (1 mM NH_4)

Treatment	Species		
	L. bicolor	*L. rufus*	*L. hepaticus*
Unbuffered medium			
Low N	$16{\cdot}0 \pm 0{\cdot}3^a$	$13{\cdot}1 \pm 0{\cdot}3^a$	$10{\cdot}2 \pm 0{\cdot}3^a$
High N	$58{\cdot}6 \pm 1{\cdot}2^c$	$49{\cdot}6 \pm 0{\cdot}5^c$	$33{\cdot}5 \pm 4{\cdot}0^c$
Buffered medium			
Low N	$16{\cdot}4 \pm 0{\cdot}6^a$	$11{\cdot}8 \pm 0{\cdot}2^a$	$8{\cdot}4 \pm 0{\cdot}1^b$
High N	$84{\cdot}9 \pm 2{\cdot}3^d$	$44{\cdot}0 \pm 2{\cdot}7^c$	$35{\cdot}8 \pm 7{\cdot}0^c$

Initial medium pH was 4·0. Final medium pH of the three species together was on average: $3{\cdot}8 \pm 0{\cdot}1$ (unbuffered, low N), $2{\cdot}4 \pm 0{\cdot}1$ (unbuffered, high N), $4{\cdot}0 \pm 0{\cdot}0$ (buffered, low N) and $3{\cdot}8 \pm 0{\cdot}1$ (buffered, high N).
Means and standard errors are shown for an experiment in triplicate. Data in the same column followed by a different letter are significantly different $P<0{\cdot}01$; Student's *t*-test.

similar to the ammonium concentration prevailing in the soil, whereas at 10 mM almost maximal growth stimulation occurs, as shown in Fig. 1. When buffered medium was used, an increase in the ammonium concentration had a significantly greater effect on the biomass production of *L. bicolor* than when unbuffered medium was used (Table 1). With *L. rufus* and *L. hepaticus* no significant effect of the buffering on the relative biomass increase by increasing ammonium concentrations was found. The addition of the buffer at 1 mM ammonium caused a slight but significant decrease in biomass production with *L. hepaticus*. Possibly this effect is due to the accompanying increase in ionic strength of the medium (data not shown).

Figure 2 shows that in the presence of the citric acid/Tris buffer the initial rates of radial growth of the fungi were reduced. This effect was most pronounced in both *Lactarius* spp., and only slight in *L. bicolor*. In the absence of buffer the rate of radial growth decreased more rapidly after an initial increase than in the presence of buffer. This effect was more pronounced at 10 mM ammonium than at 1 mM. With *L. bicolor* and *L. rufus* a reduction in the radial growth of 51% and 29%, respectively, was caused by raising the ammonium concentration from 1 to 10 mM in unbuffered medium, however, no appreciable decrease was found in buffered medium. This indicates that the reduction in radial growth by 10 mM ammonium in the unbuffered medium was an indirect effect. With *L. hepaticus*, however, a decrease in radial growth at 10 mM ammonium was observed in buffered as well as in unbuffered medium, but also here the reduction in radial growth was most pronounced in unbuffered medium. The final reductions in the colony growth were 7 and 21% in buffered and unbuffered medium, respectively.

pH growth experiment

Because in the absence of added buffer increasing the ammonium concentration led to an increased acidification of the medium, the effects of ammonium on growth may partly be indirect, being due to a more pronounced decrease in medium pH. Species-specific effects

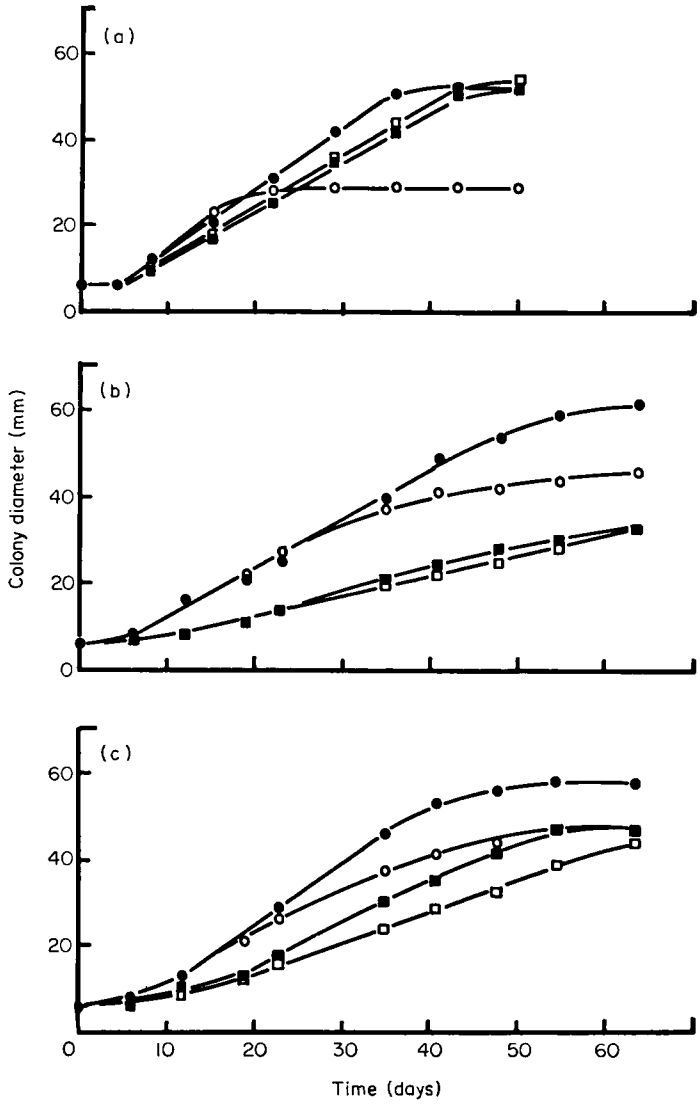

Fig. 2. Growth curves of *Laccaria bicolor* (a), *Lactarius rufus* (b) and *Lactarius hepaticus* (c) on solid one-quarter strength MMN medium. Treatments were: unbuffered medium with 1 mM ammonium (●) or 10 mM ammonium (○), buffered medium with 1 mM ammonium (■) or 10 mM ammonium (□). Initial pH was 4·0. Results are means of an experiment in triplicate. Standard errors commonly lay within the symbols.

of ammonium on both radial growth and dry weight production may be a reflection of differences in the pH sensitivity of the species examined. For this purpose we examined the effect of the medium pH under buffered conditions on the growth of the fungi. We chose a full strength medium to lower the chance that the medium would become exhausted, which would mask the effect of suboptimal pH values on growth. In this medium the ammonium concentration amounted to 4 mM. *L. bicolor* had its optimum for biomass production at a higher pH compared to both the *Lactarius* spp. (Fig. 3). The dry weight production of *L. bicolor* was maximal at pH 4·8 and was 'good' (differing no more than

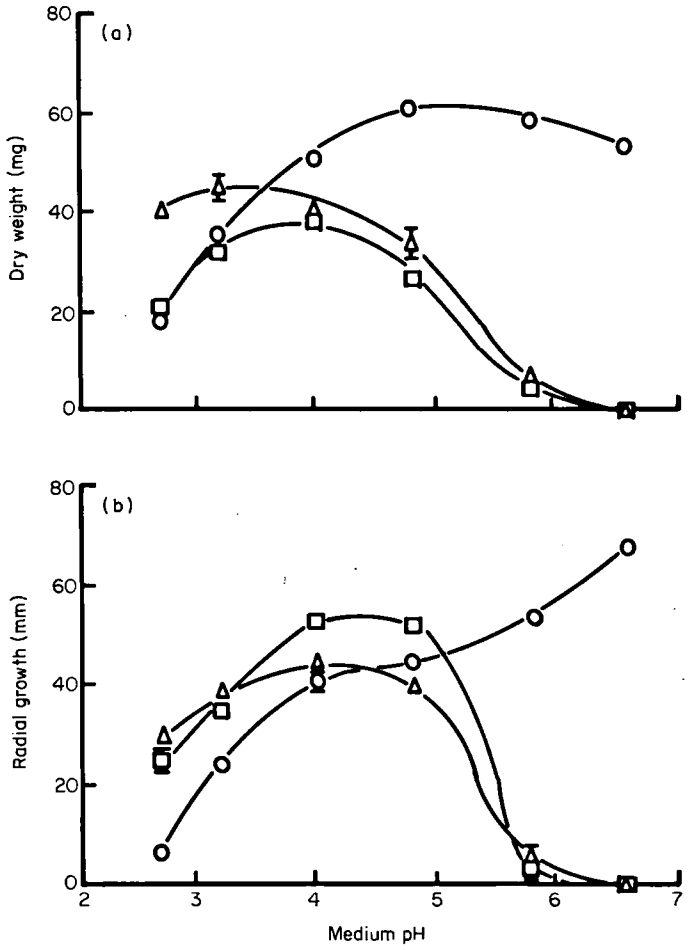

Fig. 3. The pH dependence of biomass production (a) and radial growth (b) of *Laccaria bicolor* (○), *Lactarius rufus* (△) and *Lactarius hepaticus* (□) on buffered solid MMN medium. The growth period was 38 days for *Laccaria bicolor* and 52 days for both *Lactarius* spp. Results are means of an experiment in triplicate. Only when the SEM exceeds 2 mg and 2 mm is it included.

20% with the growth at optimum pH (Hung & Trappe 1983)) at pH 3·9–6·6. The pH dependence of radial growth differed from that of biomass production and increased with pH over the entire range tested, suggesting that at least two pH-dependent processes are involved. Both dry weight and radial growth decreased rapidly when the pH was lowered from 4·0 to 2·7. The growth patterns of *L. rufus* and *L. hepaticus* showed some similarities but *L. rufus* was somewhat more acidophilic than *L. hepaticus*. Dry weight production and radial growth of *L. rufus* were good in the pH range 2·7–4·5 and 3·1–4·9, respectively. For *L. hepaticus* the pH range for good growth was quite narrow, namely 3·1–4·5 and 3·5–5·0 for dry weight production and radial growth, respectively. Growth of both *Lactarius* spp. was poor at pH 5·8 and virtually zero at pH 6·6. In another experiment with liquid instead of solid medium, growth response (dry weight production) of the fungi was comparable (data not shown). Furthermore, it appeared that growth was completely stopped at pH 2.

The pigmentation of *L. bicolor* also depended on the medium pH. The violet colour of its mycelium was most intense at pH 3·2 and 4·0 and was absent at pH 6·6. In spite of the presence of a buffer in the medium some acidification still occurred. This acidification was on average 0·3 pH units. The pH values denoted in Fig. 3 are the mean of the initial and final pH.

DISCUSSION

The nutritional effect of ammonium is evident from the experiment where the pH was not kept constant. Under those conditions a pronounced stimulation of biomass growth was found by raising the ammonium concentration from 1 to 5 mM. This 'fertilizing' effect of ammonium is in agreement with the results of other studies on ectomycorrhizal fungi (Littke *et al.* 1984; Boxman *et al.* 1986).

In buffered medium the relative increase in biomass production caused by increasing the ammonium concentration from 1 to 10 mM is higher than in the unbuffered medium, namely 42%, 31% and 6% with *L. bicolor*, *L. hepaticus* and *L. rufus*, respectively. This phenomenon can be ascribed to the accompanying acidification of the medium which occurs in unbuffered medium (Morton & MacMillan 1954). This acidification increases with increasing ammonium concentrations, because ammonium uptake is accompanied by proton efflux (St John *et al.* 1985). In unbuffered medium this pH can drop to far below the optimal pH for all three species. From the pH dependence of biomass production one would expect that *L. bicolor* would show the largest indirect effect of increasing ammonium concentrations in poorly buffered media and *L. rufus* the lowest effect. Apparently the pH dependence of the fungi also influences the dependence of these fungi on the ammonium concentration besides the direct effect of ammonium as the main nitrogen source for growth.

Whereas biomass production is increased by ammonium, radial growth is either decreased or unaffected. Littke *et al.* (1984) also found for some ectomycorrhizal fungi equal or slightly reduced radial growth on increasing the ammonium concentration. However, some other species only exhibited an increase in radial growth. As seen from the time dependence of the radial growth of the three fungi in unbuffered medium, an initial increase in the ammonium concentration from 1 to 10 mM has little effect. However, after 15–23 days, depending on the species examined, radial growth in the presence of 10 mM ammonium increases less rapidly than at 1 mM ammonium. In buffered medium neither *L. bicolor* or *L. rufus* shows an appreciable decrease in radial growth at 10 mM ammonium. This indicates that the impairment of radial growth found in unbuffered medium is due to the acidification of the medium accompanying uptake of ammonium into the cells. In *L. hepaticus* in buffered medium, a transient decrease in radial growth is found indicating that with this isolate, besides acidification of the medium, a direct effect of ammonium on radial growth is involved. From the pH dependence of radial growth it is seen that *L. bicolor* is the most sensitive of the three fungi. The differences between *L. rufus* and *L. hepaticus* are not very clear and are complicated further by the fact that in *L. hepaticus* ammonium also has a direct effect on radial growth. Boxman *et al.* (1986) reported an inhibitory effect of increased ammonium concentrations on the radial growth of various ectomycorrhizal fungi, but because unbuffered medium was used they did not account for the possibility that it was due to strongly decreasing pH.

Both biomass increment and radial growth were determined because of the different significance of these parameters for the functioning of the mycorrhizas. Mycorrhizal fungi

increase the nutrient and water uptake of trees both by increasing the absorbing area and by providing efficient cellular transport mechanisms (Harley & Smith 1983). Radial growth especially will contribute to the expansion of the absorbing area in the soil, making water and nutrients with a low mobility more easily attainable, while increase in biomass may increase the capacity for accumulation of nutrients. An increased level of ammonium in the soil may, especially when the buffer capacity of the rhizosphere is low, lead to a more compact mycelial growth by an increase in biomass formation together with a reduction in radial growth. This may result in a reduction in nutrient uptake. Boxman *et al.* (1986) proposed that ectomycorrhizal fungi might protect themselves against unfavourable soil constituents, such as high ammonium concentrations, by growing more compactly.

Kamminga-van Wijk & Prins (1989) used the same *L. bicolor* isolate as in the present study for inoculation of Douglas fir seedlings in solution culture. They observed mycorrhizal formation at pH 4·0, but not at a pH below 3·5. For this fungus the potential to form mycorrhizas at low pH may be related to its response to low pH. However, extrapolation of pure culture growth of ectomycorrhizal fungi to the ability to form mycorrhizas in natural conditions is not always justified. Hung & Trappe (1983) found that the pH optima for *in-vitro* growth of ectomycorrhizal fungi are quite different from that of the habitat source. In that study, however, unbuffered nutrient medium was used; therefore their pH optima for *in-vitro* growth may have been overestimated due to acidification of the medium during fungal growth.

Several studies have shown that both raising the pH and the ammonium concentration drastically reduces the formation and fructification of ectomycorrhizas. Wästerlund (1982) demonstrated that addition of acid to the soil of a *Pinus sylvestris* stand raised the amount of fruit bodies and number of ectomycorrhizal species, while treatment with limestone gave the opposite effect. A decrease in pH effect can also act indirectly by changing the availability of nutrients. In this context it is interesting that the fruit body dry weight production of ectomycorrhizal fungi including *L. rufus* was reduced by liming (Fiedler & Hunger 1963). The liming induced a pronounced raise in the pH of the humus layers suggesting that the pH plays a major role in the decline. However, the nutrient uptake of the fungi was unaffected.

Fertilization experiments with ammonium strongly reduced fruit body production and infection of ectomycorrhizal fungi including *L. rufus* (Wästerlund 1982; Alexander & Fairley 1983; Meyer 1985; Jentschke *et al.* 1989). In apparent contradiction with this, the present study has shown that an increase in ammonium availability sharply stimulates biomass production of isolated ectomycorrhizal fungi in nutrient medium, indicating that the interaction of the tree and the mycorrhizal fungus must be quite complicated.

The soil pH is a determinant factor in the manifestation of ectomycorrhizal fungi. In poorly buffered forest soils an increase in the ammonium concentration may lead to a considerable acidification of the rhizosphere. This could alter the species composition of ectomycorrhizal fungi, favouring the acidophilic ones. However, evidence for this view is not provided by the pH-dependent *in-vitro* studies of ectomycorrhizal fungi presented here.

ACKNOWLEDGEMENTS

We wish to thank Dr A.E. Jansen for kindly providing the fungal isolates. We are grateful to Dr H.B.A. Prins for critical reading of this manuscript and to Dr R.A. Gage for correcting the English text. This study was financed by the Dutch Ministry of Housing,

Physical Planning and Environment and is part of the Dutch Priority Programme on Acidification.

REFERENCES

Alexander, I.J. & Fairley, R.I. (1983): Effects of N fertilisation on populations of fine roots and mycorrhizas in spruce humus. *Plant Soil* **71:** 49–53.

Arnolds, E. (1988): The changing macromycete flora in the Netherlands. *Trans. Br. Mycol. Soc.* **90:** 391–406.

Boxman, A.W., Sinke, R.J. & Roelofs, J.G.M. (1986): Effects of NH_4^+ on the growth and K^+ (^{86}Rb) uptake of various ectomycorrhizal fungi in pure culture. *Water, Air Soil Pollut.* **31:** 517–522.

De Vries, B., Jansen, A.E. & Barkman, J.J. (1985): Verschuivingen in het soortenbestand van fungi in naaldbossen in Drenthe, 1958–1983. *Wet. Meded. Kon. Ned. natuurhist. Ver.* **167:** 74–83.

Fiedler, H.-J. & Hunger, W. (1963): Über den Einfluß einer Kalkdüngung auf Vorkommen, Wachstum und Nährelementgehalt höherer Pilze im Fichtenbestand. *Arch. Forstw.* **12:** 936–962.

Harley, J.L. & Smith, S.E. (1983): *Mycorrhizal Symbiosis.* Academic Press, London.

Hung, L.L. & Trappe, J.M. (1983): Growth variation between and within species of ectomycorrhizal fungi in response to pH *in vitro*. *Mycologia* **75:** 234–241.

Jansen, A.E. & de Vries, F.W. (1988): *Qualitative and quantitative research on the relation between ectomycorrhiza of* Pseudotsuga menziesii, *vitality of the host and acid rain.* Report 25–02. Dutch Priority Programme on Acidification (RIVM, Bilthoven). Agricultural University, Wageningen.

Jayko, L.G., Baker, T.I., Stubblefield, R.D. & Anderson, R.F. (1962): Nutrition and metabolic products of *Lactarius* species. *Can. J. Bot.* **8:** 361–371.

Jentschke, G., Godbold, D.L. & Hüttermann, A. (1989): Effects of ammonium and nitrate on mycorrhizal infection of Norway spruce seedlings under controlled conditions. *Agric. Ecosyst. Environ.* **28:** 201–206.

Kamminga-van Wijk, C. & Prins, H.B.A. (1989): The influence of pH on ectomycorrhizal development of *Pseudotsuga menziesii* inoculated with *Laccaria bicolor* on hydroculture. *Agric. Ecosyst. Environ.* **28:** 213–217.

Kleijn, C.E., Zuidema, G. & de Vries, W. (1989): *De indirecte effecten van atmosferische depositie op de vitaliteit van Nederlandse bossen. 2. Depositie, bodemeigenschappen en bodemvochtsamenstelling van acht Douglasopstanden.* Rapport nr. 2050, Stichting voor Bodemkartering. Wageningen.

Laiho, O. (1970): *Paxillus involutus* as a mycorrhizal symbiont of forest trees. *Acta For. Fenn.* **106:** 1–72.

Littke, W.R., Bledsoe, C.S. & Edmonds, R.L. (1984): Nitrogen uptake and growth *in vitro* by *Hebeloma crustuliniforme* and other Pacific Northwest mycorrrhizal fungi. *Can. J. Bot.* **62:** 647–652.

Marx, D.H. (1969): The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. I. Antagonism of mycorrhizal fungi to root pathogenic fungi and soil bacteria. *Phytopathology,* **59,** 153–163.

Mason, P.A., Last, F.T., Pelham, J. & Ingleby, K. (1982): Ecology of some fungi associated with an ageing stand of birches *(Betula pendula* and *B. pubescens). Forest Ecol. Manage.* **4:** 19–39.

—, Wilson, J. & Last, F.T. (1983): The concept of succession in relation to the spread of sheating mycorrhizal fungi on inoculated tree seedlings growing in unsterile soils. *Plant Soil* **71:** 247–256.

Meyer, F.H. (1985): Einfluß des Stickstoff-Faktors auf den Mykorrhizabesatz von Fichtensämlingen im Humus einer Waldschadensfläche. *Allg. Forstztg.* **40:** 208–219.

Morton, A.G. & MacMillan, A. (1954): The assimilation of nitrogen from ammonium salts and nitrate by fungi. *J. Exp. Bot.* **5:** 232–252.

Oort, A.J.P. (1981): Nutritional requirements of *Lactarius* species, and cultural characters in relation to taxonomy. *Verh. Kon. Ned. Akad. Wet., Afd. Nat., 2e,* **76:** 1–87.

Palmer, J.G. & Hacskaylo, E. (1970): Ectomycorrhizal fungi in pure culture. I. Growth on single carbon sources. *Physiol. Plant.* **23:** 1187–1197.

Rygiewicz, P.T., Bledsoe, C.S. & Zasoski, R.J. (1984): Effects of ectomycorrhizae and solution pH on [^{15}N]ammonium uptake by coniferous seedlings. *Can. J. For. Res.* **14:** 885–892.

St John, B.J., Smith, S.E., Nicholas, D.J.D. & Smith, F.A. (1985): Enzymes of ammonium assimilation in the mycorrhizal fungus *Pezizella ericae* Read. *New Phytol.* **100:** 579–584.

Termorshuizen, A.J. & Schaffers, A.P. (1987): Occurrence of carpophores of ectomycorrhizal fungi in selected stands of *Pinus sylvestris* in the Netherlands in relation to stand vitality and air pollution. *Plant Soil* **104:** 209–217.

Van Breemen, N., Burrough, P.A., Velthorst, E.J., van Dobben, H.F., de Wit, T., Ridder, T.B. & Reijnders, H.F.R. (1982): Soil acidification from atmospheric ammonium sulphate in forest canopy throughfall. *Nature* **299:** 548–550.

Wästerlund, I. (1982): Försvinner tallens mykorrhizasvampar vid gödsling? *Svensk. Bot. Tidskr.* **76:** 411–417.