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Nutrient uptake by intact mycorrhizal *Pinus sylvestris* seedlings: a diagnostic tool to detect copper toxicity

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Summary We developed a nondestructive method for detecting early toxic effects of sublethal copper (Cu) concentrations on ectomycorrhizal and non-mycorrhizal (NM) Scots pine (*Pinus sylvestris* L.) seedlings. The fungal symbionts examined were *Paxillus involutus* (Fr.) Fr., *Suillus luteus* (Fr.) S.F. Gray and *Thelephora terrestris* (Ehrh.) Fr. The accumulation of Cu in needles and fungal development (ergosterol) in roots and substrate were assessed. Inorganic phosphate (P_i) and ammonium (NH_4^+) uptake capacities were determined in a semi-hydroponic cultivation system on intact P-limited plants that were exposed for 3 weeks to 0.32 (control), 8 or 16 μM Cu^{2+}. Short-term effects of a 1-hour exposure to 32 μM Cu^{2+} on nutrient uptake rates were also determined.

None of the Cu^{2+} treatments affected plant growth or root ergosterol concentrations. The active fungal biomass in substrate invaded by *S. luteus* was reduced by 50% in the 16 μM Cu^{2+} treatment compared with the control treatment; however, colonization by *S. luteus* prevented an increased accumulation of Cu in the needles. In contrast, the 16 μM Cu^{2+} treatment caused a 2.2-fold increase in needle Cu concentration in NM plants. Ergosterol concentrations in the substrate colonized by *P. involutus* and *T. terrestris* were not affected by 16 μM Cu^{2+}. Although *P. involutus* and *T. terrestris* were less sensitive to Cu^{2+} than *S. luteus*, *T. terrestris* did not prevent the accumulation of Cu in needles of its host plant in the 16 μM Cu^{2+} treatment.

Mycorrhizal plants consistently had higher P_i and NH_4^+ uptake capacities than NM plants. In the control treatment, specific P_i uptake rates were almost 10, 4 and 3 times higher in plants associated with *P. involutus*, *S. luteus* and *T. terrestris*, respectively, than in NM plants, and specific NH_4^+ uptake rates were about 2, 2 and 5 times higher, respectively, than those of NM seedlings. Compared with the corresponding control plants, a 3-week exposure to 8 μM Cu^{2+} had no effect on the nutrient uptake potential of plants. In contrast, the 16 μM Cu^{2+} treatment significantly reduced P_i uptake capacity of all plants and decreased NH_4^+ uptake capacity of seedlings colonized by *S. luteus* or *T. terrestris*. The 32 μM Cu^{2+} 1-h shock treatment reduced specific NH_4^+ and P_i uptake rates of roots colonized by *S. luteus* to 39 and 77%, respectively, of the original rates. The Cu^{2+} 1-h shock treatment reduced the NH_4^+ uptake rate of NM plants by 51%.

Keywords: ectomycorrhiza, heavy metals, nutrient uptake rate, Paxillus involutus, Suillus luteus, Thelephora terrestris.

Introduction

Copper (Cu) is an essential element for plant and fungal metabolism; however, at elevated concentrations, Cu becomes toxic. For decades, Cu has been used as a fungicide in agriculture, and soil Cu toxicity is found in old abandoned vineyards as a result of accumulation from fungicide applications. Other sites with elevated Cu concentrations are copper mines, natural copper outcrops and contaminated areas around Cu refineries and smelters. Severe Cu toxicity affects plant associations and can eliminate arbuscular mycorrhizal colonization of metal-tolerant grasses (Griffioen et al. 1994). Rühling et al. (1984) observed a strong negative correlation between carpophore production of some ectomycorrhizal fungal genera and the Cu concentration in the soil organic layer around a zinc-copper smelter in south-eastern Sweden.

Several laboratory studies have demonstrated that ectomycorrhizal (ECM) fungi can protect tree seedlings against heavy metal toxicity (Jones and Hutchinson 1986, 1988, Denny and Wilkins 1987, Colpaert and Van Assche 1992, Bücking and Heyser 1994, Marschner et al. 1996). The ameliorating effect of ECM fungi has been attributed to reduced translocation of heavy metals to the host plant (Leyval et al. 1997), although mycorrhizal-induced increases in nutrient uptake can also alleviate heavy metal stress in highly mycotrophic tree species. The extent of protection varies with both the ECM fungal species and the heavy metal (Jones and Hutchinson 1986, Colpaert and Van Assche 1992, Marschner et al. 1996). Protection of the host plant by the fungus implies the existence of mechanisms that protect the mycobiont from heavy metal toxicity. However, few studies have attempted to identify the physiological processes underlying the differences in heavy metal sensitivity of ECM fungi (Hartley et al. 1997).

In polluted soils, the external mycelia are the first components of the ECM root system that have to cope with high external concentrations of heavy metals. Copper can have

negative effects on membrane integrity, and on nutrient uptake systems, such as the plasma membrane ATPase activity of plants and fungi (Sandmann and Böger 1983, Kennedy and Gonsalves 1989, Gadd 1993). If Cu ions disturb nutrient uptake mechanisms of ECM fungi, the extent of this response could account for inter- and intraspecific differences in Cu sensitivity of ECM fungi and their respective host plants. Therefore, analyses of nutrient uptake rates may provide a diagnostic method for detecting differences in sensitivity to sublethal heavy metal concentrations.

We studied the effects of elevated copper concentrations on mycorrhizal and NM Scots pine (*Pinus sylvestris* L.) seedlings. A new nondestructive technique was used to determine nutrient uptake rates of intact plant–fungus associations. We investigated the effects of a 3-week exposure to elevated Cu concentrations on mycorrhizal colonization and specific uptake rates for ammonium and inorganic phosphate (P_i). We also determined the effects of a 1-h exposure to 32 μM Cu^{2+} on specific uptake rates.

Materials and methods

Plant and fungus material

Pinus sylvestris seeds from a single tree were surface-sterilized for 15 min in 35% H_2O_2, sown in a 2:1 (v/v) mixture of perlite and vermiculite and watered weekly with Ingestad's balanced nutrient solution for *P. sylvestris* (Ingestad and Kähr 1985). Exact formulae of stock solutions are given in Nylund and Wallander (1989). Nitrogen was supplied as 0.7 mM NH_4NO_3. The weight proportions of the other macro-nutrients in the solution were 100 N:9 P:54 K:18 S:6 Ca:6 Mg, with P as the growth-limiting nutrient. After 7 weeks, seedlings were selected for uniformity and inoculated with a mycorrhizal fungus or left non-mycorrhizal. Three ectomycorrhizal species were used for inoculation: *Suillus luteus* (Fr.) S.F. Gray, *Paxillus involutus* (Fr.) Fr. and *Thelephora terrestris* (Ehrh.) Fr. The fungi were isolated from fruiting bodies collected from Scots pine forests in the autumn of 1995. The *S. luteus* and *T. terrestris* isolates originated from a highly metal-polluted acid sandy soil in Lommel, Belgium. The site is described in detail by Vangronsveld et al. (1996). The *P. involutus* fungus was collected from an uncontaminated area. The seedlings were inoculated by a sandwich technique (Colpaert et al. 1996). Ten seedlings were harvested immediately after inoculation to determine their P content.

Growth conditions

Three to four days after inoculation, the plants were transplanted to transparent 70-ml syringes (27-mm diameter) containing 5 g dry weight of acid-washed perlite, with a total water retention capacity of 17 ± 0.5 ml. (Perlite was a suitable substrate for this experiment because it has a low adsorption capacity for ions.) The perlite in each container was covered with small quartz stones and dark plastic foil to prevent algal growth. All of the plant containers were inserted in holes in a PVC lid that was placed over a large dark plastic box. To obtain similar nutrient concentrations in mycorrhizal and NM plants, an exponential nutrient addition regime according to the Ingestad concept was used (Ingestad and Ågren 1995). The nutrient addition rate aimed to maintain a low constant relative growth rate of 3.0% day^{-1} and was calculated from the P contents of the seedlings harvested at the time of inoculation. The daily nutrient requirements to maintain this relative growth rate were supplied in the mean volume of the daily water requirement of the plants. The NH_4^+ concentration in the added nutrient solution was about 0.78 mM, and the concentration of P was close to 65 μM. Plants were maintained in non-sterile conditions in a growth chamber in an 18-h photoperiod at 400 μmol m^{-2} s^{-1} PAR (photosynthetically active radiation) with a day/night temperature of 22/15 °C and at least 70% relative air humidity. Six weeks after inoculation, mycorrhizal colonization was checked through the transparent walls of the plant containers. Successfully inoculated plants were randomly assigned to one of three copper treatments: 0.32 (control; $n = 3$), 8 ($n = 3$) and 16 μM Cu^{2+} ($n = 4$). At the start of the 3-week Cu treatment, all plant containers were slowly rinsed with 50 ml of the respective nutrient solutions in order to obtain the desired Cu concentration in the growth substrate. Copper concentrations in the eluted solution were monitored. After the start of the treatment, a constant relative nutrient addition rate of 3.0% day^{-1} was maintained until harvest.

Nutrient uptake measurements

After a 3-week exposure to the copper treatments, net uptake of NH_4^+ and P_i by the plant–fungus combinations were determined by measuring the depletion of these nutrients from a solution that continuously circulated through the plant containers (Figure 1). The uptake measurements were performed under the growth conditions.

An excess of 100 ml of Ingestad's nutrient solution was percolated through each plant container by means of a peristaltic pump at a flow rate of 5 ml min^{-1} in order to establish similar nutrient solution concentrations for all plants. This Ingestad test solution contained 65 μM P, 780 μM NH_4^+ and the Cu^{2+} concentration of the respective treatment. After equilibra-

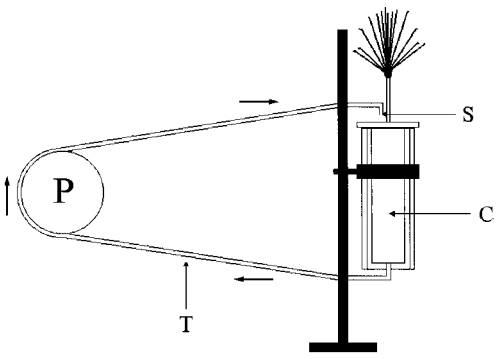

Figure 1. Experimental set-up for measuring nutrient uptake in *Pinus sylvestris*. Abbreviations: P = peristaltic pump; S = sampling point; C = plant container; and T = tubing that connects the plant container with the peristaltic pump. At point S, the nutrient solution drips back into the plant container. Arrows indicate the direction of flow of the nutrient solution in the tubing.

tion, each container was plugged into a closed loop with a 1.3-m length of silicone tube connected to a peristaltic pump (Figure 1). Immediately thereafter, each plant received 15 ml of Ingestad test solution that was continuously circulated through the plant container at a flow rate of 5 ml min^{-1}. The total volume of solution circulating in each container was 32 ± 0.5 ml. Over a period of 2 h, five 1-ml samples of nutrient solution were collected and analyzed for NH_4^+, P_i and pH. The circulation of nutrient solution was then stopped, and the excess solution collected in test tubes and analyzed for Cu. As a control, parallel measurements were performed in containers filled with perlite but without a plant. With this system, successive assessments of plant nutrient uptake could be made on single intact plant–fungus associations. Consecutive uptake measurements on the same plant did not affect P_i or NH_4^+ uptake rates. Preliminary tests with this experimental system confirmed that depletion of NH_4^+ and P_i in the circulating nutrient solution is attributable solely to active transport. The addition of the membrane decoupler carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP) immediately and completely inhibited uptake of these nutrients (unpublished results).

The effect of an acute copper shock on the nutrient uptake rates of plants that had been exposed to elevated Cu concentrations for 3 weeks was also determined. The Cu concentration in the test solution was raised to 32 μM and the solution was percolated through each plant container for 1 h. Immediately thereafter, we analyzed temporal changes in ammonium, inorganic phosphate, and copper concentrations in the circulating test solution.

Inorganic phosphate was determined colorimetrically by the phosphomolybdate method (Murphy and Riley 1962), ammonium was determined by the Berthelot reaction according to Botton and Chalot (1991) and copper was measured by atomic absorption spectrophotometry.

During the nutrient uptake measurements the P_i and NH_4^+ concentrations decreased from 65 μM to less than 1 μM, and from 780 to 30 μM respectively. Nutrient depletion over these concentration ranges was almost exponential. Inorganic phosphate uptake rates were calculated based on an external inorganic phosphate concentration of 40 μM, and NH_4^+ uptake rates were calculated based on an external ammonium concentration of 500 μM.

Harvest and analyses

After the nutrient uptake measurements, the 16-week-old plants were harvested. Fresh weights of shoots and roots were determined before they were oven-dried for at least 3 days at 75 °C. Dried samples were ashed for 6 h at 600 °C. Ashes were taken up in 20% HCl and copper was determined by atomic absorption spectrophotometry. The P concentration in the needles was determined in ashed (2 h at 500 °C) 100-mg subsamples that were each dissolved in 3 ml 7% HCl and partially neutralized with NaOH. Phosphate was determined colorimetrically according to Murphy and Riley (1962).

Two 0.5-g root samples, and two 5-g perlite samples were frozen in liquid nitrogen for ergosterol analyses. Ergosterol is a fungus-specific membrane component, and is considered to be a reliable indicator of metabolically active fungal biomass (Nylund and Wallander 1992). The concentration of ergosterol was determined by a modification of the method of Nylund and Wallander (1992). Perlite samples were saponified (100 °C for 30 min) in 10 ml of 1M KOH in MeOH, and root samples were saponified in 5 ml of this solution. After cooling and addition of 1 ml of H_2O, the samples were extracted with 10 ml of *n*-hexane (5 ml of *n*-hexane for root samples). The hexane fraction was evaporated to dryness below 40 °C. The extract was redissolved in 1 ml of HPLC-grade methanol. Ergosterol was then quantified by high-performance liquid chromatography on a reverse-phase C18 column, with methanol as the mobile phase, an injection volume of 100 μl and a flow rate of 2 ml min^{-1}. Peaks were detected with a UV detector at 282 nm.

Statistical analysis

Data from each plant–fungus combination in the Cu^{2+} treatments were subjected to analysis of variance (ANOVA) to detect treatment effects on specific nutrient uptake rates, and needle P, needle Cu, and ergosterol concentrations. Nutrient uptake rates obtained before and after the 32 μM Cu^{2+} shock treatment were compared by a paired *t*-test.

Results

Plant and fungal growth

There were no significant copper treatment effects on shoot and root biomass. The seedlings had a mean shoot dry weight of 1.00 ± 0.12 g, and a mean root dry weight of 1.29 ± 0.16 g. Calculated mean relative growth rate of the plants was 3.05% day^{-1}.

After the 3-week exposure to 8 or 16 μM Cu^{2+}, mycorrhizas and long-root meristems appeared healthy. All mycorrhizal plants were well colonized, with 80–100% of all fine roots infected. At the end of the experiment, the perlite substrates of the mycorrhizal plants were completely invaded by external mycelium. Root ergosterol concentrations (Figure 2) and visible assessment of the root systems confirmed that the 8 and 16 μM Cu^{2+} treatments had no detectable effect on ectomycorrhizal colonization of the roots. New short roots became rapidly colonized with a mantle mycelium and had, at least macroscopically, the same appearance in all treatments. The concentration and total content of ergosterol in the pine roots were similar for the three fungi used. However, the ECM fungi differed in the extent to which they colonized the perlite substrate. In the control treatment, *P. involutus* and *S. luteus* developed a denser external mycelium than the *T. terrestris* isolate (Figure 3). The 16 μM copper treatment reduced the ergosterol concentration of perlite colonized by *S. luteus*, but it did not affect the ergosterol concentration of perlite colonized by *T. terrestris*, and increased the ergosterol concentration of perlite colonized by *P. involutus* (Figure 3).

Plant analyses

The P concentrations of the needles (Figure 4) corresponded to concentrations previously found for P-limited pine seedlings (Cumming 1996, Colpaert et al. 1997). Needle P concentrations were similar in the three plant–fungus combinations.

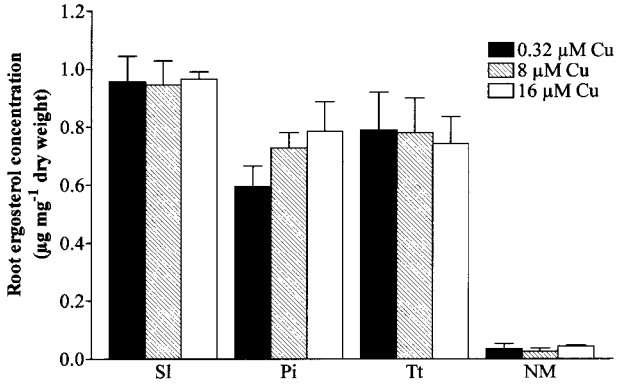

Figure 2. Root ergosterol concentrations of ectomycorrhizal and non-mycorrhizal *Pinus sylvestris* seedlings after a 3-week treatment with 0.32, 8 or 16 µM Cu^{2+}. Error bars represent the standard error of the mean. Abbreviations: Sl = *Suillus luteus*; Pi = *Paxillus involutus*; Tt = *Thelephora terrestris*; and NM = non-mycorrhizal.

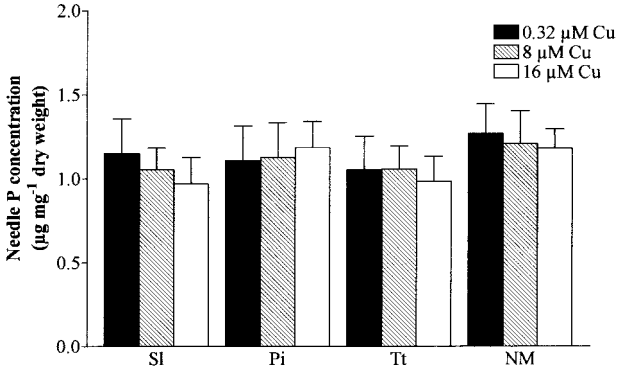

Figure 4. Needle phosphorus concentrations of ectomycorrhizal and non-mycorrhizal *Pinus sylvestris* seedlings after a 3-week treatment with 0.32, 8 or 16 µM Cu^{2+}. Error bars represent the standard error of the mean. Abbreviations: Sl = *Suillus luteus*; Pi = *Paxillus involutus*; Tt = *Thelephora terrestris*; and NM = non-mycorrhizal.

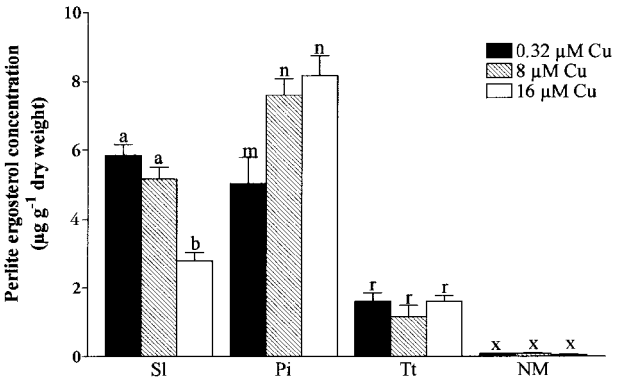

Figure 3. Perlite ergosterol concentrations of ectomycorrhizal and non-mycorrhizal *Pinus sylvestris* seedlings after a 3-week treatment with 0.32, 8 or 16 µM Cu^{2+}. Adjoining bars marked with different letters are significantly different at $P < 0.05$. Error bars represent the standard error of the mean. Abbreviations: Sl = *Suillus luteus*; Pi = *Paxillus involutus*; Tt = *Thelephora terrestris*; and NM = non-mycorrhizal.

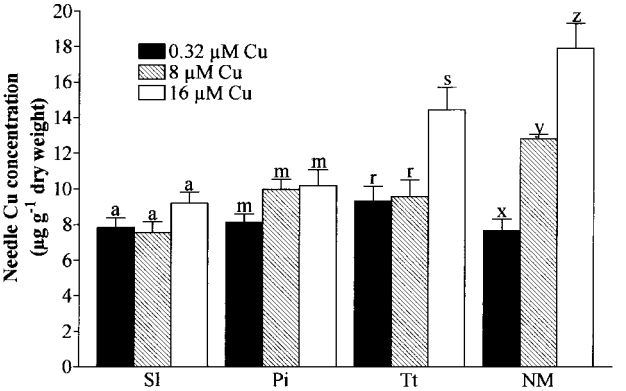

Figure 5. Needle copper concentrations of ectomycorrhizal and non-mycorrhizal *Pinus sylvestris* seedlings after a 3-week treatment with 0.32, 8 or 16 µM Cu^{2+}. Adjoining bars marked with different letters are significantly different at $P < 0.05$. Error bars represent the standard error of the mean. Abbreviations: Sl = *Suillus luteus*; Pi = *Paxillus involutus*; Tt = *Thelephora terrestris*; and NM = non-mycorrhizal.

Needle copper concentrations differed considerably between mycorrhizal and NM plants and among host plants of different mycobionts (Figure 5). In both elevated Cu^{2+} treatments, NM plants had higher needle copper concentrations than mycorrhizal plants. Seedlings mycorrhizal with *T. terrestris* had an elevated needle copper concentration in the 16 µM Cu^{2+} treatment, whereas plants colonized with *S. luteus* or *P. involutus* did not have increased needle copper concentrations when grown at elevated Cu^{2+} concentrations.

Copper concentrations in roots were not affected by either the copper treatments or the ectomycorrhizal fungi (data not shown). Application of the 32 µM Cu^{2+} 1-h shock treatment led to high extracellular adsorption of Cu on plant and fungal cells.

Nutrient uptake capacities

A time course of nutrient depletion in the circulating test solution is illustrated in Figure 6. Within a few hours the nutrient solution was almost completely depleted. In the control treatment (0.32 µM Cu^{2+}), specific P_i and NH_4^+ uptake rates were considerably higher in ectomycorrhizal plants than in NM plants (Figures 7A and 8A). Among the plant–fungus associations studied, plants infected with *P. involutus* had the highest P_i uptake rate. At an external concentration of 40 µM P_i, plants in the control treatment colonized with *P. involutus* had a mean specific P_i uptake rate of 1.83 nmol $g_{root,dw}^{-1}\ s^{-1}$ compared with a P_i uptake rate of 0.19 nmol $g_{root,dw}^{-1}\ s^{-1}$ for NM plants. High P_i uptake rates were not always linked to high NH_4^+ uptake rates. Among the plant–fungus associations studied, host plants of *T. terrestris* had the highest specific NH_4^+ uptake rates with a mean of 7.31 nmol $g_{root,dw}^{-1}\ s^{-1}$ in the control treatment. Non-mycorrhizal plants had a specific NH_4^+ uptake rate of only 1.43 nmol $g_{root,dw}^{-1}\ s^{-1}$. The NH_4^+ uptake rates of the other ectomycorrhizal plants fell between

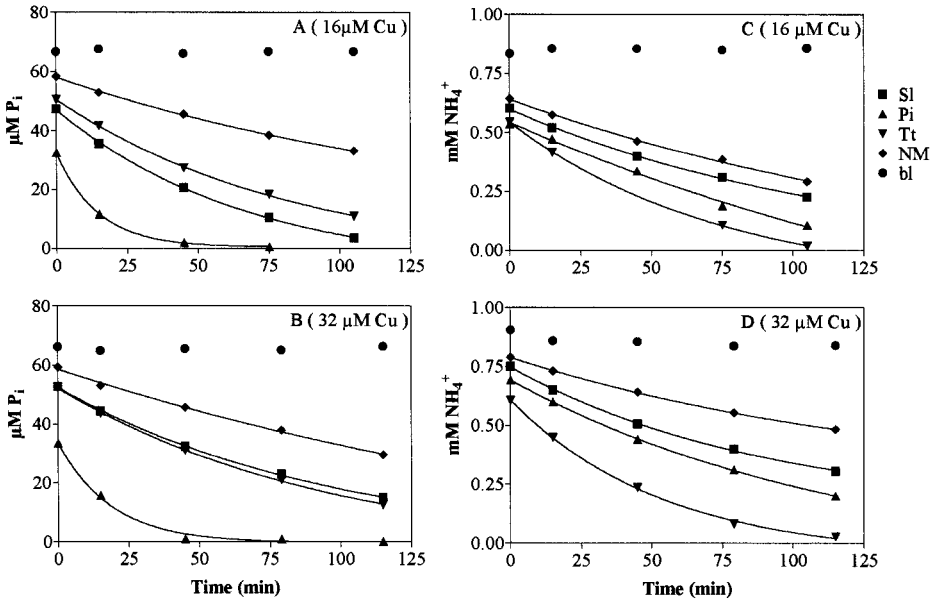

Figure 6. Example of nutrient depletion curves of plants in the 16 μM Cu^{2+} treatment. Depletion of P_i at an external Cu^{2+} concentration of 16 μM (A) and 32 μM (B). Depletion of NH_4^+ at an external Cu^{2+} concentration of 16 μM (C) and 32 μM (D). The lines are exponential curves fitted through the measurement points. Abbreviations: Sl = *Suillus luteus*; Pi = *Paxillus involutus*; Tt = *Thelephora terrestris*; NM = non-mycorrhizal; and bl = blank.

these two values.

A comparison of the NH_4^+ and P_i uptake rates after a 3-week exposure to elevated Cu^{2+} revealed changes in the nutrient uptake capacity of the three plant–fungus combinations. A copper treatment effect on NH_4^+ uptake rate did not necessarily result in a similar response in P_i uptake. All seedlings showed a significantly decreased specific P_i uptake rate in response to the 16 μM copper treatment (Figure 7), whereas a 3-week exposure to elevated Cu^{2+} reduced NH_4^+ uptake rates only in plants colonized with *T. terrestris* or *S. luteus* (Figure 8).

The 32 μM 1-h shock treatment resulted in a significant decrease in specific P_i uptake rate only in plants mycorrhizal with *S. luteus*. Ammonium uptake was more sensitive to the application of an acute copper dose than P_i uptake. Non-mycorrhizal plants and host plants of *S. luteus* showed decreased specific NH_4^+ uptake rates in response to the 32 μM 1-h shock treatment, irrespective of the previous copper treatment (Figure 8). Plants colonized with *P. involutus* showed decreased specific NH_4^+ uptake rates only when previously grown at 16 μM Cu^{2+}. There was no effect of the 32 μM 1-h shock treatment on NH_4^+ uptake rates of plants colonized with *T. terrestris*.

During the nutrient uptake measurements, the pH of the nutrient solution decreased from 4.0 to 3.4, but after a few hours it slowly increased to 4.0. These changes in pH were observed in all Cu^{2+} treatments for both mycorrhizal and non-mycorrhizal plants. The fluctuation is attributable to a preferred uptake of NH_4^+ over NO_3^-, when both ions are present in solution (Marschner 1995). The Cu^{2+} concentration in the circulated solution stayed within 10% of the supplied concentration.

Discussion

Because we grew the mycorrhizal and NM seedlings in a semi-hydroponic system with a controlled nutrient addition rate, all plants were a similar size and had a similar nutrient status. These "steady state" growing conditions allowed direct comparisons of the physiological properties of the three intact plant–fungus associations.

During the 3-week exposure to elevated copper concentrations, root and shoot growth remained at the control value of about 3.0% day^{-1}. Similarly, we conclude that the copper treatments did not affect fungal colonization of the root systems because neither the ergosterol concentration nor total ergosterol in the roots was reduced by high Cu^{2+} concentrations (Figure 2). The absence of any effects of copper on root biomass, the relatively low accumulation of copper in needles in most plant–fungus associations (Figure 5), and the continued net influx of nutrients in plants (Figures 7 and 8) indicate that the Cu^{2+} concentrations used were not lethal in our semi-hydroponic system. In contrast, in culture solutions, taproot elongation of non-mycorrhizal *Pinus pinea* L. and *P. pinaster* Ait. seedlings was reduced in the presence of 1 μM Cu^{2+}, and complete inhibition of root growth occurred in the presence of 5 μM Cu^{2+} (Arduini et al. 1995). In our semi-hydroponic system, we recently found a decrease in root growth in NM Scots pine seedlings exposed for 3 weeks to 32 μM Cu^{2+} concentration and complete inhibition of root growth occured in the presence of 47 μM Cu^{2+} (authors' unpublished observations). Jones and Hutchinson (1986) found a 40 and 70% reduction in root biomass in NM birch seedlings grown for 18 weeks in silica sand irrigated with Ingestad's nutrient solution containing 32 and 63 μM Cu^{2+}, respectively. Differences in Cu sensitivity among studies are probably related to differences in seedling age, test species, uptake conditions or degree of exposure in the different culture systems.

The finding of greater copper sensitivity for *S. luteus* than for *P. involutus* and *T. terrestris* (Figure 3) contrasts with results from *in vitro* metal-tolerance studies showing high Cu^{2+} tolerance indices for *S. luteus* and low tolerance indices for *P. involutus* and *T. terrestris* (Colpaert and Van Assche 1987,

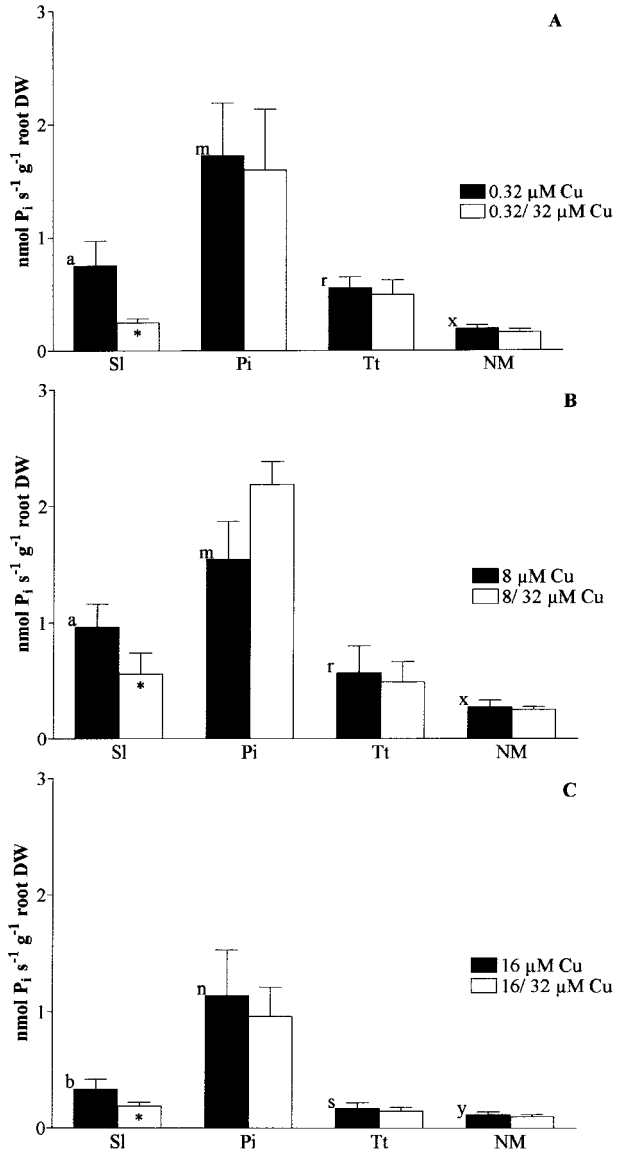

Figure 7. Specific inorganic phosphate uptake rates of ectomycorrhizal and non-mycorrhizal *Pinus sylvestris* seedlings after a 3-week treatment with 0.32 µM Cu^{2+} (A), 8 µM Cu^{2+} (B) and 16 µM Cu^{2+} (C); and after a subsequent 1-h shock treatment with 32 µM Cu^{2+}. The uptake rates are means of calculated values for a P_i concentration in the nutrient solution of 40 µM. Bars for the same plant–fungus association marked with different letters are significantly different at $P < 0.1$. Paired bars marked with an asterisk are significantly different at $P < 0.05$. Error bars represent the standard error of the mean. Abbreviations: Sl = *Suillus luteus*; Pi = *Paxillus involutus*; Tt = *Thelephora terrestris*; and NM = non-mycorrhizal.

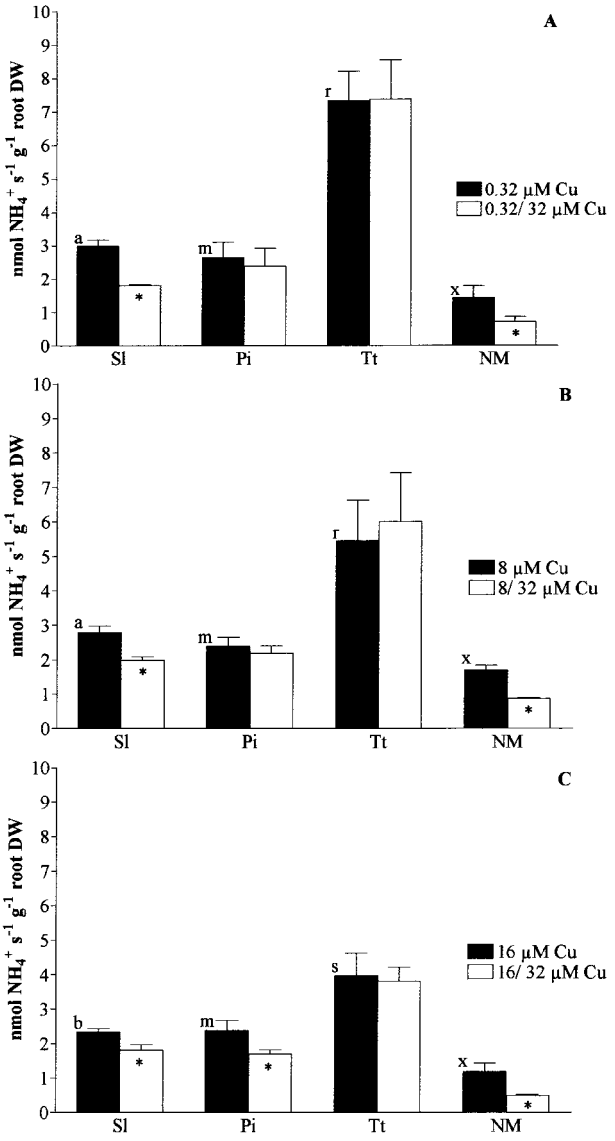

Figure 8. Specific ammonium uptake rates of ectomycorrhizal and non-mycorrhizal *Pinus sylvestris* seedlings after a 3-week treatment with 0.32 µM Cu^{2+} (A), 8 µM Cu^{2+} (B) and 16 µM Cu^{2+} (C); and after a subsequent 1-h shock treatment with 32 µM Cu^{2+}. The uptake rates are means of calculated values for an NH_4^+ concentration in the nutrient solution of 500 µM. Bars for the same plant–fungus association marked with different letters are significantly different at $P < 0.1$. Paired bars marked with an asterisk are significantly different at $P < 0.05$. Error bars represent the standard error of the mean. Abbreviations: Sl = *Suillus luteus*; Pi = *Paxillus involutus*; Tt = *Thelephora terrestris*; and NM = non-mycorrhizal.

Tam 1995). This discrepancy supports the contention that *in vitro* metal-tolerance studies are of low diagnostic value, although intraspecific differences among isolates may explain such anomalies.

The copper concentrations in Scots pine needles increased with increasing concentrations of Cu^{2+}. The increase was lower in mycorrhizal plants than in NM plants, indicating that the mycorrhizas reduced translocation of Cu^{2+} to the host plant. Copper rapidly becomes toxic when it is transported to leaves, because photosynthesis (photosystem I) is very sensitive to Cu ions (Balsberg Påhlsson 1989). Copper concentrations of 15–25 µg g_{dw}^{-1} in shoots of higher plants are generally toxic (Balsberg Påhlsson 1989); however, in our study, these concentrations were only reached in NM plants and in plants inoculated with *T. terrestris* (Figure 5). Reduced translocation

of Zn, Cd, Ni and Pb to shoots of ectomycorrhizal tree seedlings has been reported in many experiments (Jones and Hutchinson 1986, Denny and Wilkins 1987, Dixon 1988, Colpaert and Van Assche 1992, 1993, Bücking and Heyser 1994). Of the three mycobionts we studied, *T. terrestris* was the least effective in reducing translocation of Cu^{2+} to the shoots, even though the growth of the fungus itself was not affected by exposure to the copper treatments and both P_i and NH_4^+ uptake potential remained higher than for NM plants in the corresponding copper treatments. Both *P. involutus* and *S. luteus* prevented translocation of Cu^{2+} to the shoots, even though the active biomass of the external mycelium of *S. luteus* was reduced in the 16 μM Cu^{2+} treatment. Damage to the external mycelium of *S. luteus* was also observed in an experiment with elevated Cd additions (Colpaert and Van Assche 1993). Despite the copper-induced reduction in active external biomass, the nutrient uptake capacity of the plants associated with *S. luteus* remained higher than that of the NM plants, even at the highest external Cu^{2+} concentrations.

The nutrient uptake measurements revealed large differences in nutrient uptake capacity between mycorrhizal and NM root systems. Root systems colonized with *P. involutus* had P_i uptake rates almost 10 times higher than those of NM roots in the control (0.32 μM) copper treatment. Ammonium uptake was three times higher for plants associated with *T. terrestris* than for NM seedlings. Although comparable specific NH_4^+ and P_i uptake rates have been reported based on values calculated from nutrient contents in plants that were sequentially harvested (Jones et al. 1991) or from plants that were transferred to nutrient solutions containing isotopes (Rygiewicz et al. 1984), large differences in P_i and NH_4^+ uptake potential between ECM and NM plants were not identified in these studies. The higher nutrient uptake capacity of mycorrhizal plants compared with NM plants can probably be ascribed to an increase in the nutrient absorbing surface area of the root system as a result of the development of an extensive mycelium (Rousseau et al. 1994), although differences in the kinetics of the nutrient influx mechanisms in the different fungi and NM plant roots might also affect the efficiency of nutrient uptake. The ECM fungi may differ in the number of carriers in the plasmalemma, and exhibit different V_{max} and K_m values for uptake of particular nutrients.

In general, Cu toxicity is believed to act through a decrease in membrane integrity, root transmembrane potential or H^+-ATPase activity (Gadd 1993, Marschner 1995). We observed a reduction in nutrient uptake potential, both in plants exposed to high Cu concentrations for 3 weeks and in plants subjected to a 1-h exposure to 32 μM Cu^{2+}. These responses to elevated Cu concentrations were observed before significant effects on growth, nutrition or Cu accumulation became apparent.

Inhibition of specific P_i uptake potential was not always associated with a reduction in specific NH_4^+ uptake capacity, suggesting that external elevated Cu^{2+} concentrations differentially affect the nutrient uptake mechanisms of these two ions. A 3-week exposure to 8 μM Cu^{2+} had no effect on the uptake of P_i or NH_4^+ by the plants; however, in all plant–fungus associations, there was a significant reduction in P_i uptake potential after a 3-week exposure to 16 μM Cu^{2+} (Figure 7). The acute Cu^{2+} shock treatment had no additional effect on P_i uptake capacity of the Cu-treated plants, except in plants associated with *S. luteus*. All plants colonized by *S. luteus* showed a general inhibition of P_i net influx immediately after the external Cu^{2+} concentration was increased to 32 μM Cu^{2+} for 1 h. This inhibitory effect of copper must be the result of a direct failure of the P_i uptake mechanism, because it cannot be ascribed to a change in the nutrient absorbing surface area of the mycorrhizal roots.

The copper treatments had a smaller effect on NH_4^+ uptake than on P_i uptake (Figure 8). Of the ectomyorrhizal species examined, roots colonized with *S. luteus* were the most sensitive to high concentrations of Cu ions in the external medium, and they also exhibited a decreased NH_4^+ uptake capacity when exposed to the 32 μM Cu^{2+} 1-h shock treatment. A 3-week exposure to 16 μM Cu^{2+} caused a small decrease in NH_4^+ uptake capacity of plants colonized with *T. terrestris*; however, the acute 32 μM Cu^{2+} dose had no effect on NH_4^+ uptake capacity of these plants. The 3-week exposure to 8 or 16 μM Cu^{2+} had no effect on NH_4^+ uptake capacity of NM plants; however, when the external Cu^{2+} concentration was suddenly raised to 32 μM, NH_4^+ uptake of NM roots was reduced to 50% of that observed in the previous Cu^{2+} treatment (Figure 8).

In summary, ECM fungi can reduce the translocation of Cu to the foliage of their host plants. Protection of the photosynthetic apparatus against Cu damage is an advantage for both partners because their carbon nutrition relies on carbon fixation by the plant. Of the mycobionts examined, *Thelephora terrestris* was the least efficient mycobiont in reducing Cu transport to the shoots. An analysis of the P_i and NH_4^+ uptake potential of mycorrhizal and NM root systems in the presence of elevated Cu^{2+} revealed a complex pattern of responses. In all of the copper treatments, P_i and NH_4^+ uptake potential of mycorrhizal plants was higher than that of NM plants. In both mycorrhizal and NM plants, P_i and NH_4^+ uptake rates were sometimes reduced by the copper treatments. Based on the nutrient uptake and ergosterol data, we conclude that *S. luteus* was more sensitive to high external Cu^{2+} concentrations than *Paxillus involutus* and *T. terrestris*. We also conclude that the semi-hydroponic system can be used to identify the nutrient uptake potential of intact mycorrhizal and NM seedlings. Furthermore, the method was sufficiently sensitive to detect early toxic effects of copper on membrane functioning.

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