THE INFLUENCE OF MYCORRHIZAL ASSOCIATIONS ON PAPER BIRCH AND JACK PINE SEEDLINGS WHEN EXPOSED TO ELEVATED COPPER, NICKEL OR ALUMINUM

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ABSTRACT. Acid deposition may adversely affect northern forest ecosystems by increasing the concentration of metals in the soil solution. This study investigates the effects of ectomycorrhizal fungi on paper birch and jack pine seedlings exposed to elevated Cu, Ni, or Al in sand culture. One of four mycorrhizal fungi, Scleroderma flavidum, was able to reduce Ni toxicity to the birch seedlings. It did this by reducing transport of Ni to the stems. None of the fungi affected Cu toxicity in birch. In separate experiments, jack pine seedlings were exposed to combinations of Al and Ca. Infection with Rhizopogon rubescens increased seedling susceptibility to Al. Seedlings inoculated with Suillus tomentosus showed a greater growth stimulation by Ca than uninoculated jack pines. Thus, for both tree species, the mycorrhizal association could alter the response of seedlings to high concentrations of certain metals, although this varied with fungal species.

1. INTRODUCTION

Jack pine and paper birch are widespread and important components of the Canadian boreal forest, a region which is subject to acid rain. Aluminum is a major constituent of boreal forest soils, while Cu and Ni are serious contaminants in soils around the Sudbury smelters in Ontario. Under acid soil conditions the solubility of such metals increases, enhancing their potential availability for plant uptake.

The presence of ectomycorrhizae reduces the uptake of Zn into Betula seedlings grown at potentially toxic concentrations of Zn (Brown and Wilkins, 1985). Mycorrhizae are also essential for the survival of several ericoid species in soils contaminated with Cu and Zn (Bradley et al., 1982).

The present study was initiated to determine: a) whether infection of either paper birch or jack pine by mycorrhizal fungi affects their growth in, and uptake of, Ni, Cu, or Al; and b) whether elevated Al concentratons affect the infectivity of two ectomycorrhizal fungi on jack pine, and whether Ca influences this effect.

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2. MATERIALS AND METHODS

2.1 Birch: Ni and Cu exposure

Sterile Betula papyrifera Marsh. seedlings, were grown in silica sand in 50 mL culture tubes from seeds collected from the Sudbury area. Seedlings were inoculated with agar plugs from colonies of Laccaria proxima, Lactariusm hibbardae, Lactarius rufus, or Scleroderma flavidum, or with sterile agar plugs. Following mycorrhizae formation, the seedlings were transplanted to sterile 5 cm pots which contained acid-washed silica sand, and which were topped with Al caps. Seedlings were fertilized every two weeks with 1/10 modified Ingestad's solution (Mason, 1980), at pH 3.5 with either 5 ppm Ni, as $NiCl_2$, 4 ppm Cu, as $CuCl_2$, or no metal added. After 18 weeks of treatment the seedlings were harvested, oven-dried, and digested in concentrated HNO_3. The digests were analyzed for Ni or Cu on a Perkin Elmer Model 460 atomic absorption spectrophotomer. At harvest, the degree of infection of each root system was scored as being between 0 and 1/4, 1/4 and 1/2, 1/2 and 3/4 or 3/4 and 1.

2.2 Jack pine: Al, Ca exposure

Isolates of Rhizopogon rubescens and Suillus tomentosus were cultured from basidiocarps collected in a 12 yr-old and 64 yr-old stand, respectively, of Pinus banksiana Lamb. Cultures were fragmented (Molina and Palmer, 1982), and 10 mL of inoculum applied to each seedling of P. banksiana growing in sterile chambers consisting of two nested 7.5 cm plastic pots. The upper pot contained silica sand, and was topped with an inverted translucent plastic 400 mL beaker. Four 2 cm holes in each beaker were plugged with cotton. Seedlings received one of four treatments: 1 ppm Al + 1 ppm Ca, 1 ppm Al + 20 ppm Ca, 10 ppm Al + 1 ppm Ca, or 10 ppm Al + 20 ppm Ca biweekly for 16 weeks. Al and Ca were added in a 1/4 strength modified Ingestad's solution adjusted to pH 3.8. At harvest the root area was measured using a Li-Cor LI-3100 Area Meter. The number of infected root tips on each seedling was counted under a dissecting scope. This number was divided by the seedling root area to give an indication of the degree of infection. Seedlings were then oven-dried, weighed, and digested in concentrated HNO_3. The digests were analyzed for Al and Ca using an Applied Research Laboratories 3400 Simultaneous Spectrometer.

3. RESULTS

3.1 Paper birch

Ni and Cu reduced the growth of birch by 37 and 60%, respectively, relative to controls (Figure 1a). Under Ni treatment, seedlings infected with Scleroderma flavidum weighed more than other mycorrhizal or non-mycorrhizal seedlings principally due to enhanced root growth (Figure 1b). This enhanced growth correlated with a reduced transfer of Ni to the stems of the S. flavidum-infected seedlings (Figure 2a).

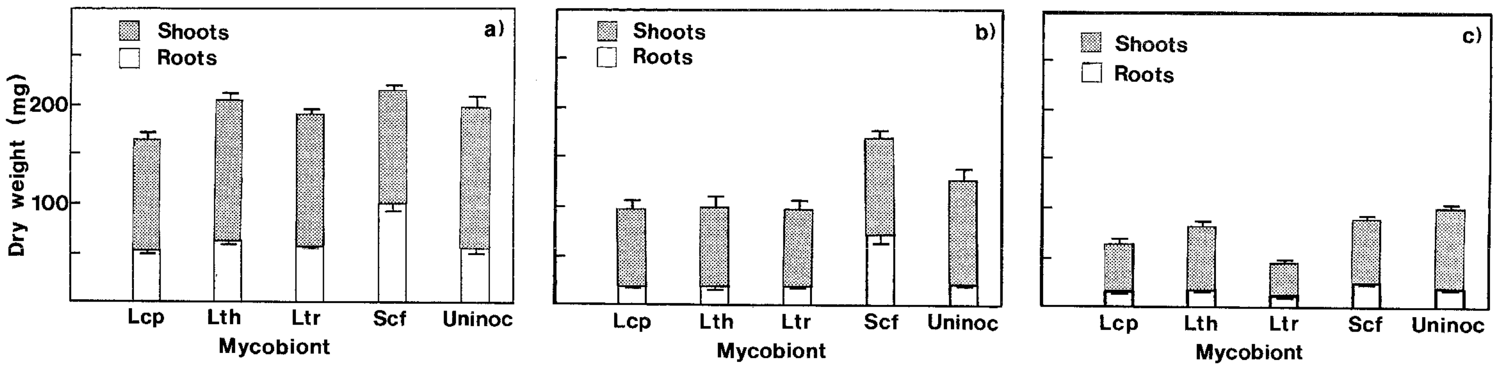

Fig. 1. Dry weights (mg) of birch seedlings grown for 18 wk a) in the absence of additional Ni or Cu, b) in the presence of 5 ppm Ni, or c) in the presence of 4 ppm Cu. Lcp = Laccaria proxima, Lth = Lactarius hibbardae, Ltr = Lactarius rufus, Uninoc = uninoculated. Means + standard errors. The significance levels for inoculation effects as detected by one-way ANOVAs are: a) shoots, $P<0.05$; roots, $P<0.001$; b) shoots, n.s.; roots, $P<0.001$; c) shoots, $P<0.001$, roots, $P<0.001$.

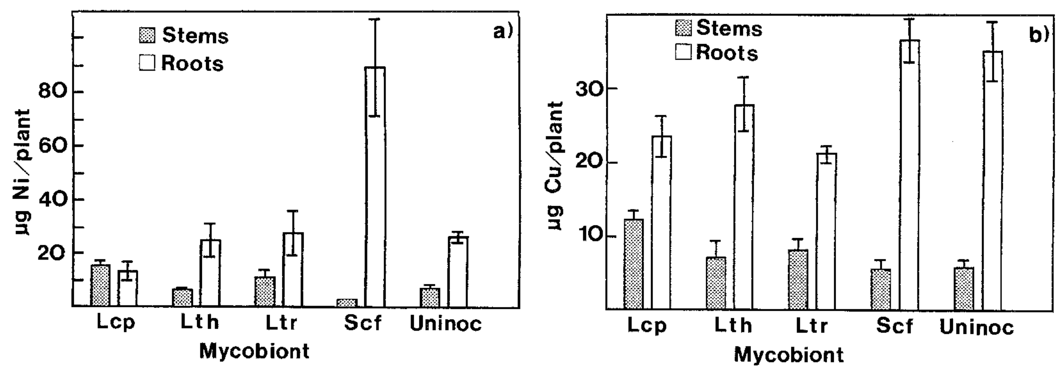

Fig. 2. The Ni or Cu content (µg per plant) of the stems and roots of birch seedlings after 18 wk of growth in the presence of a) 5 ppm Ni or b) 4 ppm Cu. The 4 mycobionts are as listed in Fig. 1. Means + standard errors. The significance levels for inoculation effects as detected by one-way ANOVAs are: a) stems, $P<0.01$; roots, $P<0.001$; b) stems, n.s., roots, $P<0.05$.

Other treatments resulted in at least twice as much Ni in the stems and half the amount of Ni in the roots.

None of the mycorrhizal fungi ameliorated the effect of Cu (Figure 1c). While root weights differed only slightly between inoculation treatments, shoot weights were markedly lower in Lactarius rufus-infected plants. There were no differences in the transfer of Cu to the stems of seedlings infected with different fungi (Figure 2b).

Rates of infection varied between the four fungi. Infection was greater than 1/2 in 82% of the seedlings inoculated with either Laccaria proxima or S. flavidum. Lactarius hibbardae infected only poorly with 66% of seedlings showing less than 1/4 infection. Infection by L. rufus was variable.

Neither Ni or Cu affected infection by any of the fungal species (Kruskal Wallis). Under either metal treatment, S. flavidum was the only fungus to show a significant relationship between the degree of infection and host weight ($p < 0.05$). When all seedlings were analyzed as a group, there was a positive relationship between degree of infection and root weights ($p < 0.05$) under the Ni treatment. Shoot weights were not correlated with infection for either metal.

3.2 Jack pine

Pine seedlings infected with Suillus tomentosus were smaller than uninoculated seedlings, regardless of Al or Ca treatment (Figure 4). Aluminum at 10 ppm reduced both shoot height and root weight in seedlings infected with R. rubescens. There was no corresponding effect of Al on uninoculated seedlings. Calcium at 20 ppm increased root growth in seedlings infected with S. tomentosus, especially when combined with high Al.

As well as inhibiting growth, 10 ppm Al reduced the infectivity of R. rubescens (Figure 3). There was no ameliorative effect of Ca. Aluminum did not affect the proportion of root infected by S. tomentosus.

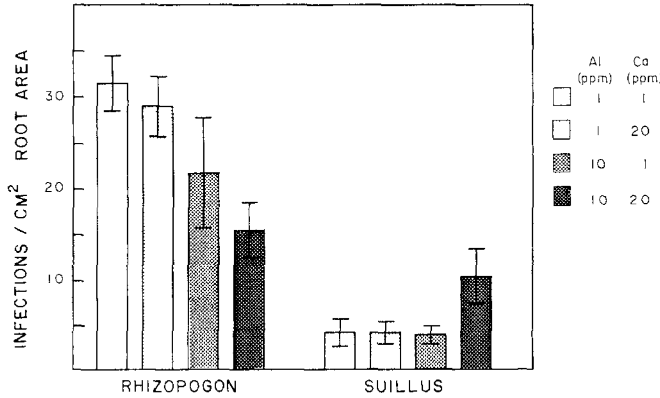

Fig. 3. The mean number of ectomycorrhizal root tips per cm2 of root area, with standard errors, for jack pine seedlings inoculated with one of two mycorrhizal fungi and treated with one of four Al and Ca combinations. The significance level for Al treatment effect on Rhizopogon rubescens infection is p + 0.005 (two-way ANOVA).

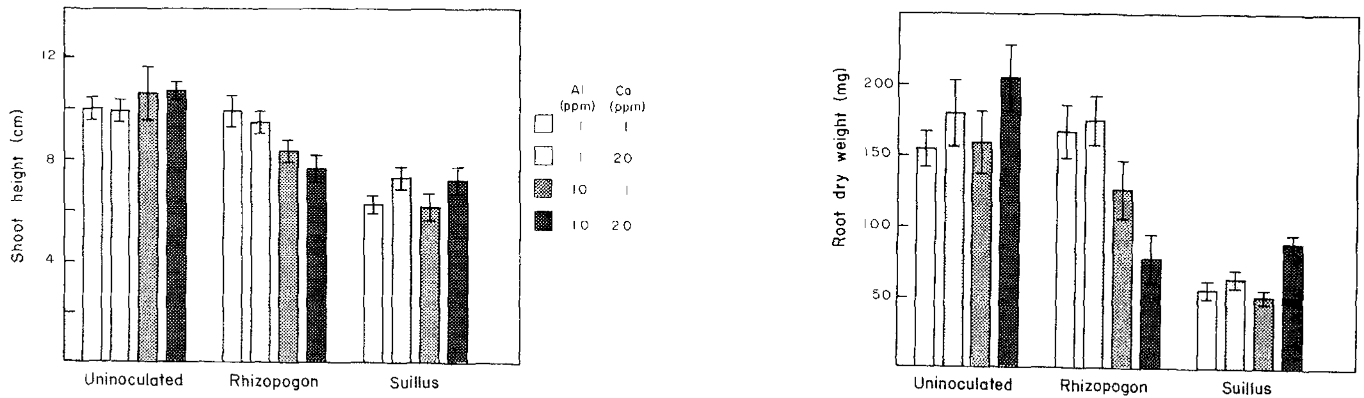

Fig. 4. The mean shoot height (cm) and root dry weight (mg) for jack pine seedlings when inoculated with one of two mycorrhizal fungi, or when not inoculated, and grown in the presence of two Al and Ca concentrations in combination. The bars indicate standard errors. The significance levels for Al treatment effects on seedlings inoculated with Rhizopogan rubescens are p=0.005 for shoot height and p=0.001 for root weight (two-way ANOVAs). Significance level for Ca treatment effect on root weight of seedlings inoculated with Suillus tomentosus is p=0.002.

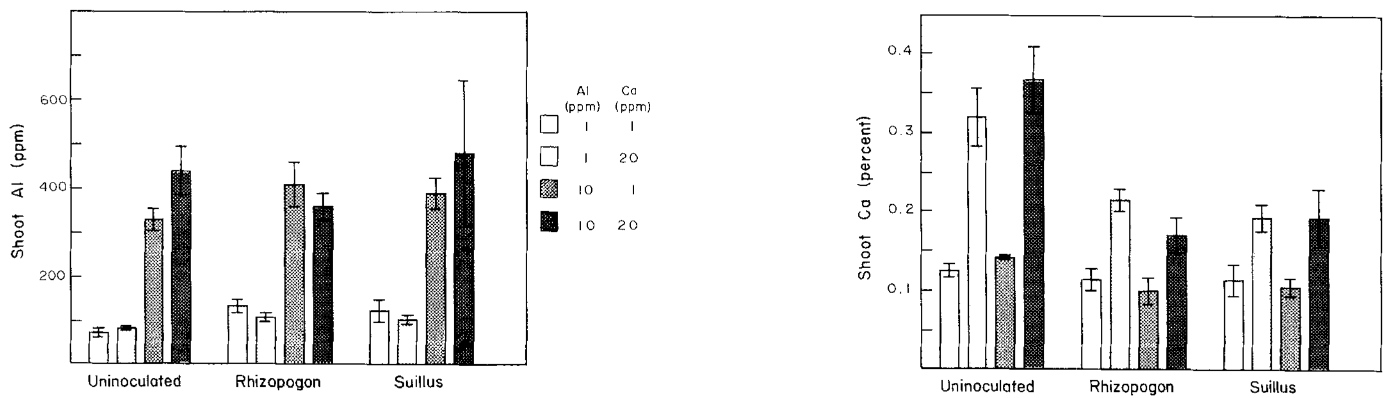

Fig. 5. The Al and Ca concentrations (ppm dry weight) in the shoots of jack pine seedlings grown in the presence of combinations of two concentrations of Al and Ca. Bars indicate standard errors. The seedlings were either uninoculated or inoculated with one of two mycorrhizal fungi. The significance level for Al treatment effect on Al concentration is $p<0.001$ (three-way ANOVA). Significance levels for inoculum treatment and Ca treatment effects on Ca concentrations are $p<0.001$ and $p=0.001$, respectively.

The concentration of Al in shoots (Figure 5a) or roots of the seedlings was not influenced by fungal treatment, and thus cannot explain differences in growth between the various inoculum treatments. Calcium treatment had no effect on Al concentration in the shoots.

Shoot Ca concentration was reduced by both fungal species, especially at the high Ca level (Figure 5b).

4. DISCUSSION

Mycorrhizal and non-mycorrhizal seedlings responded differently to Al, Ca, Cu, and Ni but no generalization about the influence of ectomycorrhizal infections can be made. The differences in response of R. rubescens and S. tomentosus to Al and Ca may relate to the characteristics of the sites from which these isolates originated. The R. rubescens isolate came from a young jack pine stand. Soil water extracts (top 10 cm) from this site had low levels of both Al and Ca (Al 0.8 ppm, Ca 1.8 ppm, Browning, 1986). The S. tomentosus isolate was obtained from a mature jack pine stand where soil water extracts had higher levels of both elements (Al 40.9 ppm, Ca 70.8 ppm). It is not surprising then that R. rubescens showed a greater sensitivity to Al. In contrast, the isolates of Laccaria proxima, Lactarius hibbardae, Lactarius rufus, and Scleroderma flavidum used to infect the birch seedlings were all obtained from the same site near Sudbury, Ontario.

These results do not reflect differences in infectivity between fungi. Although infection of jack pine by S. tomentosus was lower than that of R. rubescens, it had a major effect on seedling characteristics relative to uninoculated plants. If the effect was strictly related to the degree of infection, seedlings infected by Suillus tomentosus would be expected to be more similar to uninoculated seedlings that those infected by R. rubescens. This was not observed. The same results occurred in birch seedlings which were only lightly infected by L. hibbardae. The response of birch seedlings was positively correlated with the degree of infection only for Scleroderma flavidum, the fungus which increased resistance to the metals, relative to the other fungi.

When grown in Ni, the effect of the fungi on the birch was related to their ability to control the distribution of the metal within the plant. Brown and Wilkins (1985) found that infection of Betula by either Amanita muscaria or Paxillus involutus increased Zn tolerance of the host in a similar manner, by reducing Zn translocation to the shoot. Mycorrhizae may retain metals in the polyphosphate bodies of the fungi, or in the interfacial matrix (Bradley et al., 1982; Jensen et al., 1982).

Differences in Cu tolerance were not due to differences in total uptake or translocation of Cu to the shoots. Instead, the fungi may begin to divert increasing amounts of photosynthate from the host.

Aluminum uptake, like that of Cu, was not affected by mycorrhizal formation in this study. It may be the partitioning of internal Al which differs between inoculated and uninoculated seedlings. Jack pine seedlings infected with Pisolithus tinctorius appeared to exclude Al from the nuclei of root cortical cells (Cochrane and McNabb, 1979).

Calcium has often been shown to ameliorate Al toxicity (Foy, 1974).

The reduced translocation of Ca observed in the mycorrhizal seedlings may explain why infection with R. rubescens produced Al-sensitive seedlings. The response of seedlings infected with Suillus tomentosus is complicated by the fact that this fungus is normally found on mature tree root systems. The much smaller size of all seedlings infected with S. tomentosus may indicate that this fungus has a higher carbohydrate demand than R. rubescens.

5. CONCLUSIONS

Depending on the species of mycobiont present, growth of paper birch and jack pine may be inhibited by elevated concentrations of Cu, Ni, or Al in the soil solution. This should be considered in cases where the mycobiont present can be selected for tolerance, for example, in the outplanting of containerized nursery stock to sites contaminated by metals or prone to acid deposition.

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REFERENCES

Bradley, R., Burt, A.J. and Read, D.J.: 1982, New Phytol. 91, 197.

Brown, M.T. and Wilkins, D.A.: 1985, New Phytol 99, 101.

Browning, M.H.R.: 1986, M.Sc. Thesis, University of Toronto, p. 211.

Cochrane, C.E. and McNabb, H.S.: 1979, 'Aluminum and pH effects on growth and mycorrhizal relationships of Pisolithus tinctorius' In: Fourth North American Conference on Mycorrhizae, Colorado State University.

Foy, C.D.: 1974, 'Effect of aluminum on plant growth', In: The Plant Root and its Environment, Ed. W. Carson, University Press, Charlottesville, VA, pp. 601-642.

Jensen, T.E., Baxter, J., Rachlin, J.W. and Jani, V.: 1982, Environ. Poll. (A) 27, 119.

Mason, P.A.: 1980, 'Aseptic synthesis of sheathing (ecto-) mycorrhizas' In: Tissue Culture Methods for Plant Pathologists. Eds. D.S. Ingram and J.P. Hegelson, Blackwell Scientific, Oxford, pp. 173-178.

Molina, R. and Palmer, J.G.: 1982, 'Isolation, maintenance, and pure culture manipulation of ectomycorrhizal fungi'. In: Methods and Principles of Mycorrhizal Research, Ed. N.C. Schenk, American Phytopathological Society, St. Paul, pp. 115-129.