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Growth Response of Ectomycorrhizal Norway Spruce Seedlings Transplanted on Lead - Polluted Soil

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Summary

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Growth response, photosynthetic activity, photosynthetic pigments content and the content of P, Ca and K were followed in 3 years old mycorrhized and noninoculated spruce (*Picea abies* (L.) Karst.) seedlings after transplantation on lead-polluted soil. Measured parameters showed that the advantages of nursery inoculation with the fungi *Pisolithus tinctorius* and *Lactarius piperatus* did not change in the year and a half after transplantation. In contrast, we can already say that spruce seedlings inoculated with *Laccaria laccata* are not suitable for the reforestation of lead polluted areas.

Introduction

Contamination with heavy metals is a most serious ecological problem. The interactions of fungi and heavy metals are comperatively known, but we know less of the role of mycorrhizal symbiosis in heavy metal uptake and transport into the host plant. Both AM and ectomycorrhizal fungi can increase the plant's resistance to heavy metal concentrations in the soil. The immobilization of metal ions by specific components of the fungal cell wall and the intracellular fixation by peptids are the main mechanisms by which a fungus could moderate transport into the symbiont, thereby making tolerance possible (GALLI & al. 1994).

Lead toxicity manifests itself by many physiological effects, such as inhibition of chlorophyll synthesis, decrease of photosynthetic activity, decreased transpiration; and increased activity of hydrolytic enzymes and peroxidases. It is

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known that Pb negatively influences mineral nutrition and normal root development in tree species such as Norway spruce, *Picea abies* (GODBOLD & KETTNER 1991). Some ericoid fungi improve plant tolerance to lead (HASHEM 1990), but there are reports that hyphal sheath does not represent a barrier to the transport of lead in ectomycorrhiza (JENTSCHKE & al. 1991). The key factor in increased tolerance to lead by mycorrhizal plants lies particularly in the retention of metal ions in the extramatrical mycelium of mycorrhizal symbionts.

Material and Methods

Spruce seedlings (*Picea abies* (L.) Karsten) were successful inoculated with the fungi *Laccaria laccata* (Scop. ex Fr.) Bk. et Br. (evid.no. DB 28), *Pisolithus tinctorius* (Mich. ex Pers.) Coker et Couch (DB 41), and *Lactarius piperatus* (L. ex Fr.) S.F. Gray (DB 31) from the fungal bank of the Dept. of Biology, BF as has been described previously (VODNIK & GOGALA 1994). The controls were noninoculated seedlings. In May 1993, 2-yr old seedlings were transplanted to the vicinity of a lead smeltery, Žerjav, in the Mežica Valley. The soil of the test area was rendzina on limestone and dolomite with a neutral pH (6-7). The total lead concentration in the soil was from 9552 ppm at a depth of 0-5 cm to 98 ppm at 20-30 cm.The height, gain in height were studied and the width of the plant crown was measured with the caliper in three periods. In May and September 1994 photosynthetic activity and the content of assimilation pigments were also determined as described by VODNIK & GOGALA 1994. P, Ca and K in the root system and shoots were determined by photometry.

Results

In the beginning, all three groups of mycorrhizal plants grew faster than the controls, particularly those inoculated with *P.tinctorius*. In the second year, growth was more equal except in *L.laccata*-inoculated where growth rate was significantly decreased. All three inoculated groups had significantly greater plant crowns than the controls. Seedlings inoculated with *L.laccata* gradually lost the advantage gained in the tree nursery while the other mycorrhizal seedlings conserved it. *P.tinctorius*-seedlings showed the most uniform growth throughout the seasons (data not shown). Seedlings inoculated with *L.laccata* contained the least chlorophyll and carotenoids in their needles. The differences were especially noticeable in September due to a large decrease in chlorophyll and carotenoid content in *L. laccata*-inoculated and slightly higher autumn pigment content in the other groups of inoculated seedlings. In May and July, CO_2 assimilation was significantly higher in the mycorrhizal seedlings than in the controls. This difference decreased during the summer and at the measurement in September the net photosynthesis was lower in the mycorrhizal seedlings. The largest seasonal variations were seen on seedlings inoculated with *P.tinctorius*. Compared to the control, P uptake in spring increased in seedlings inoculated with *P.tinctorius* and *L.piperatus*. In autumn there were very high P-concentrations in the roots and particularly the needles of seedlings inoculated with *P.tinctorius*. P-content in *L.laccata*-inoculated was lower than in the controls, but during time Ca-uptake was extremely increased in comparison with other seedlings. Root Ca uptake in the autumn increased in *P.tinctorius*-seedlings (Tab. 1).

Tab. 1. P, K and Ca in roots and shoots, pigment content and photosynthetic activity of inoculated and control seedlings.

Spruce seedlings inoculated with:			May 94 (8-10)	Jul.94 (8-10)	Sept.94 (8-10)
Laccaria laccata	roots	P (µg/g)	85.1 ± 38.5		115.8 ± 7.0
		K (mg/g)	8.02 ± 1.18 *		4.75 ± 0.55 ***
		Ca (mg/g)	8.39 ± 0.99		28.90 ± 3.89 *
	shoots	P (µg/g)	62.8 ± 12.9		90.8 ± 9.9
		K (mg/g)	16.15 ± 1.22 *		7.63 ± 0.86 **
		Ca (mg/g)	6.23 ± 0.47		5.17 ± 0.64
	chlorophyl a (mg/g)		0.91 ± 0.12		0.56 ± 0.08 *
	chlorophyl b (mg/g)		0.40 ± 0.07		0.24 ± 0.03 *
	carotenoids (mg/g)		0.41 ± 0.03		0.27 ± 0.02 *
	NF (µmol CO_2 mg^{-1} h^{-1})		69.47 ± 10.99 *	39.38 ± 6.95	17.21 ± 2.80
Pisolithus tinctorius	roots	P (µg/g)	80.8 ± 19.0 *		169.5 ± 8.1
		K (mg/g)	10.20 ± 1.32 **		14.59 ± 1.31 *
		Ca (mg/g)	7.22 ± 1.47		15.01 ± 3.16
	shoots	P (µg/g)	82.9 ± 17.0		252.6 ± 28.7 ***
		K (mg/g)	21.46 ± 2.63		20.56 ± 1.16
		Ca (mg/g)	3.65 ± 0.47		4.90 ± 0.77
	chlorophyl a (mg/g)		1.05 ± 0.25		1.38 ± 0.07
	chlorophyl b (mg/g)		0.47 ± 0.12		0.60 ± 0.04
	carotenoids (mg/g)		0.45 ± 0.08		0.46 ± 0.06
	NF (µmol CO_2 mg^{-1} h^{-1})		69.49 ± 7.48 **	43.06±4.51**	9.38 ± 3.09
Lactarius piperatus	roots	P (µg/g)	67.1 ± 5.3 ***		155.0 ± 9.0
		K (mg/g)	8.97 ± 1.15 **		10.54 ± 1.17
		Ca (mg/g)	5.97 ± 0.63		11.78 ± 1.66
	shoots	P (µg/g)	59.2 ± 7.7		100.7 ± 12.0
		K (mg/g)	22.63 ± 1.14		16.89 ± 0.92
		Ca (mg/g)	4.72 ± 0.52		5.11 ± 0.90
	chlorophyl a (mg/g)		1.00 ± 0.18		1.47 ± 0.29
	chlorophyl b (mg/g)		0.52 ± 0.10		0.60 ± 0.11
	carotenoids (mg/g)		0.39 ± 0.05		0.50 ± 0.08
	NF (µmol CO_2 mg^{-1} h^{-1})		56.01 ± 6.27	35.63 ± 6.37	15.08 ± 4.05
Control - noninoculated	roots	P (µg/g)	33.4 ± 2.3		142.4 ± 21.1
		K (mg/g)	5.67 ± 0.20		10.06 ± 0.95
		Ca (mg/g)	8.26 ± 2.58		14.92 ± 3.40
	shoots	P (µg/g)	55.1 ± 8.6		107.2 ± 17.0
		K (mg/g)	25.09 ± 3.76		14.87 ± 2.44
		Ca (mg/g)	5.44 ± 0.52		5.35 ± 0.49
	chlorophyl a (mg/g)		1.06 ± 0.22		1.09 ± 0.24
	chlorophyl b (mg/g)		0.57 ± 0.11		0.44 ± 0.10
	carotenoids (mg/g)		0.41 ± 0.08		0.39 ± 0.05
	NF (µmol CO_2 mg^{-1} h^{-1})		41.04 ± 6.51	23.55 ± 2.96	27.55 ± 9.52

Measurements represent the mean ± SE, (n in parentheses) *: Statistical significance is based on the Student's *t*-test. Signicantly different from noninoculated-control at (*) $P<0.05$; (**) $P<0.01$; (**) $P<0.001$. Net photosynthesis (NF) is expressed per mg chlorophyl a+b in the measured shoots.

Discussion

A year and a half after transplantation, we can already say that seedlings inoculated with *L.laccata* are not suitable for reforestation of the Žerjav area. This fungus was chosen as the most appropriate symbiont for seedlings in the tree nursery (VODNIK & GOGALA 1994) because of improved growth and development, but these advantages were not maintained on polluted soil. Several physiological parameters showed that the advantages of inoculation with *P.tinctorius* or *L.piperatus* remained in the year and a half after transplantation. The inoculated seedlings' photosynthetic activity was modified and there was better uptake of K and P. Phosphorus supply to the plant could be crucial in rendzina soil where its content is usually limited. P can be involved in Al (KOTTKE & MARTIN 1994) and also Pb (MARSCHNER 1994) detoxification in ectomycorrhiza. Preliminary analyses show toxical levels of Pb in the needles of seedlings, but for a precise evaluation of differences between groups of seedlings further analyses are necessary. Some of our experiments *in vitro* demonstrated that *L.laccata* is much less resistant to higher lead concentrations than *P.tinctorius* and *L.piperatus* (data not shown). Lower Pb-tolerance may be the reason for the bad growth response of *L.laccata* mycorrhizal seedlings in Žerjav. A precise determination of mycorrhizal state would be necessary to check this presumption. In our experiment mycorrhizal state of seedlings at the time of sampling was just estimated as successful. We must also take into account that such extreme growth conditions as are present in Žerjav lead to other forms of stress. Again, seedlings inoculated with more tolerant species and strains of fungi are at an advantage. Inoculation with a resistant symbiont may be an important means towards successful reforestation. But for a precise evaluation of mycorrhizal seedlings, their growth and development will have to be followed through a much longer period of observation.

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