

# Two genotypes of mycorrhizal *Pinus pinaster* respond differently to cadmium contamination

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## Introduction

Fertilization, a worldwide used standard method to enhance crop yield, is one of the main anthropogenic sources of Cd accumulation in agricultural soils. Forest expansion on impracticable agricultural land may help to stabilize the site and prevent the leaching of metals into the soil deeper layers. However, heavy metal contamination may be an obstacle for plant development. The genetics of the planting material is currently recognized to have a strong influence in tree establishment in a given environment. Moreover, ectomycorrhizal (ECM) fungi are known to form symbiotic associations with forest trees and their ability to influence metal uptake as well as their action as plant growth enhancers under several environmental constraints has been previously demonstrated. We have envisaged that the combination of tree genotype with selected ECM fungi could represent an important strategy for the reforestation of Cd contaminated sites.

## Objectives

❖ The present work was performed with the aim to evaluate how plant genotype may affect the mycorrhizal symbiosis and plant establishment in heavy metal contaminated soils.

## Materials and Methods

❖ The experiment consisted of a factorial design with 3 Cd levels (0, 15, and 30 mg Cd kg<sup>-1</sup>soil), three ECM fungal treatments (no fungi, *Suillus bovinus* and *Rhizopogon roseolus*) and two genotypes of *P. pinaster* (A and B);  
 ❖ Fungal inoculants were selected based on their ability to develop under Cd exposure, previously determined by in-vitro experiments;  
 ❖ Growth traits were determined and metal accumulation was assessed.

## Results

Table 1 - Shoot height, shoot dry weight and root dry weight of genotypes A and B of *P. pinaster* seedlings inoculated with *S. bovinus*, *R. roseolus* and non inoculated control growing under three Cd concentrations: 0, 15 and 30 mg Cd kg<sup>-1</sup>.

Cd mg kg <sup>-1</sup> soil	Genotype	ECM fungal isolates	Biometric parameters			
			Shoot height (cm)	Shoot dry weight (g)	Root dry weight (g)	
0	A	No fungi	12.18 ± 0.503 <sup>a</sup>	0.78 ± 0.055 <sup>a</sup>	0.53 ± 0.103 <sup>a</sup>	
		<i>S. bovinus</i>	12.20 ± 0.263 <sup>a*</sup>	0.79 ± 0.043 <sup>a</sup>	0.91 ± 0.067 <sup>b</sup>	
		<i>R. roseolus</i>	12.13 ± 0.442 <sup>a</sup>	0.75 ± 0.056 <sup>a*</sup>	0.94 ± 0.026 <sup>b</sup>	
	B	No fungi	13.23 ± 0.220 <sup>b</sup>	0.71 ± 0.028 <sup>b</sup>	0.75 ± 0.084 <sup>a</sup>	
		<i>S. bovinus</i>	14.66 ± 0.558 <sup>a*</sup>	0.81 ± 0.043 <sup>c</sup>	1.01 ± 0.070 <sup>a</sup>	
		<i>R. roseolus</i>	11.25 ± 0.336 <sup>a*</sup>	0.58 ± 0.025 <sup>a*</sup>	0.77 ± 0.102 <sup>a</sup>	
	Genotype (G)			** F = 7.016	* F = 4.561	(NS) F = 0.339
	ECM fungal isolates (F)			*** F = 9.280	* F = 4.962	* F = 5.295
	G x F			** F = 8.492	(NS) F = 2.260	(NS) F = 2.121
15	A	No fungi	9.63 ± 0.397 <sup>a</sup>	0.42 ± 0.022 <sup>a</sup>	0.34 ± 0.017 <sup>a*</sup>	
		<i>S. bovinus</i>	11.27 ± 0.429 <sup>b</sup>	0.59 ± 0.026 <sup>b</sup>	0.57 ± 0.088 <sup>b</sup>	
		<i>R. roseolus</i>	10.10 ± 0.219 <sup>a</sup>	0.36 ± 0.052 <sup>a</sup>	0.46 ± 0.064 <sup>a*</sup>	
	B	No fungi	9.70 ± 0.171 <sup>a</sup>	0.44 ± 0.020 <sup>a</sup>	0.54 ± 0.044 <sup>a*</sup>	
		<i>S. bovinus</i>	10.70 ± 0.211 <sup>b</sup>	0.56 ± 0.043 <sup>b</sup>	0.63 ± 0.064 <sup>a</sup>	
		<i>R. roseolus</i>	10.12 ± 0.361 <sup>a*</sup>	0.50 ± 0.041 <sup>a*</sup>	0.69 ± 0.063 <sup>a*</sup>	
	Genotype (G)			(NS) F = 0.404	(NS) F = 2.273	** F = 9.852
	ECM fungal isolates (F)			*** F = 9.875	*** F = 11.032	* F = 3.563
	G x F			(NS) F = 0.671	(NS) F = 2.853	(NS) F = 1.036
30	A	No fungi	10.17 ± 0.347 <sup>b</sup>	0.48 ± 0.037 <sup>b</sup>	0.50 ± 0.057 <sup>b</sup>	
		<i>S. bovinus</i>	9.90 ± 0.239 <sup>b</sup>	0.48 ± 0.026 <sup>b</sup>	0.47 ± 0.099 <sup>b*</sup>	
		<i>R. roseolus</i>	8.60 ± 0.879 <sup>a</sup>	0.35 ± 0.034 <sup>a</sup>	0.21 ± 0.040 <sup>a*</sup>	
	B	No fungi	9.42 ± 0.240 <sup>a</sup>	0.39 ± 0.029 <sup>a</sup>	0.46 ± 0.062 <sup>a</sup>	
		<i>S. bovinus</i>	10.56 ± 0.252 <sup>b</sup>	0.51 ± 0.036 <sup>b</sup>	0.71 ± 0.038 <sup>b*</sup>	
		<i>R. roseolus</i>	9.26 ± 0.278 <sup>a</sup>	0.35 ± 0.026 <sup>a</sup>	0.41 ± 0.048 <sup>a*</sup>	
	Genotype (G)			(NS) F = 0.644	(NS) F = 0.527	* F = 5.963
	ECM fungal isolates (F)			*** F = 9.935	*** F = 10.023	** F = 7.896
	G x F			* F = 4.243	(NS) F = 1.717	(NS) F = 2.541

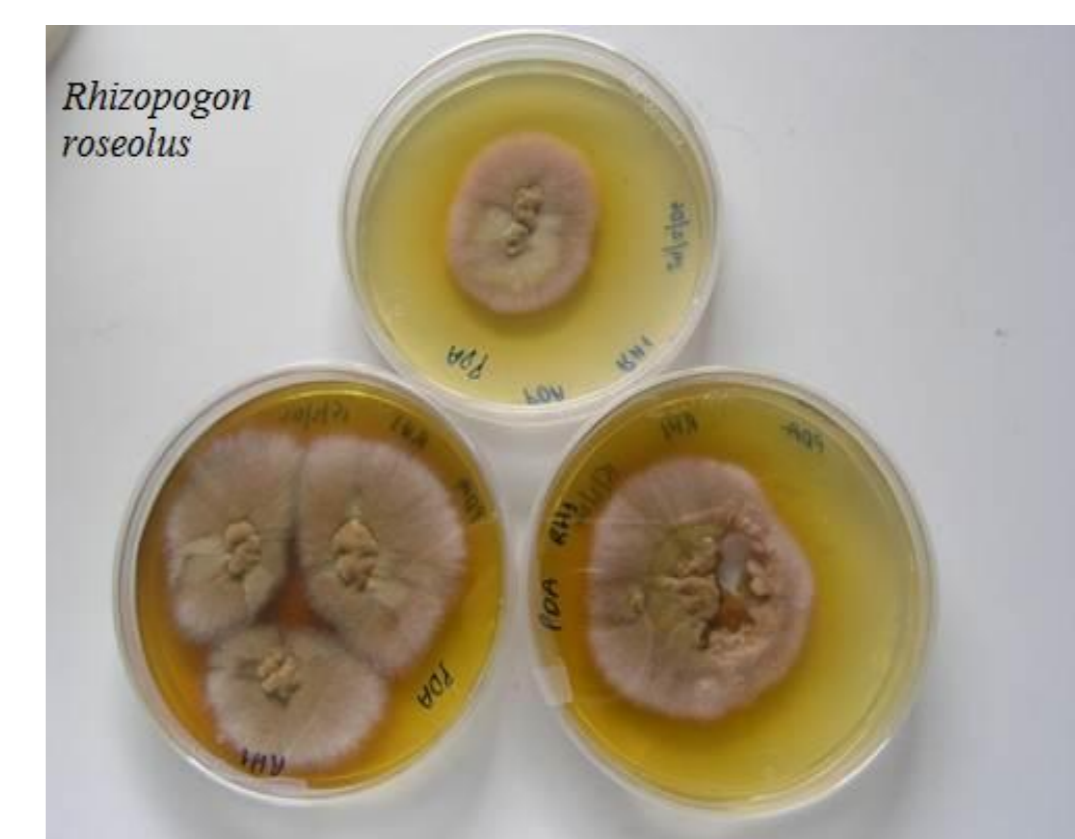


Table 2 - Cadmium concentration of shoot, root and total plant for genotypes A and B of *P. pinaster* seedlings inoculated with *S. bovinus*, *R. roseolus* and non inoculated control, growing under two Cd concentrations: 15 and 30 mg Cd kg<sup>-1</sup>.

Cd mg kg <sup>-1</sup> soil	Genotype	ECM fungal isolates	Cd (mg kg <sup>-1</sup> plant dry weight)			
			shoot	root	total plant	
15	A	No fungi	11.93 ± 1.494 <sup>b</sup>	33.48 ± 0.676 <sup>a</sup>	28.72 ± 3.069 <sup>a</sup>	
		<i>S. bovinus</i>	6.96 ± 0.511 <sup>a</sup>	32.71 ± 2.635 <sup>a</sup>	21.79 ± 1.576 <sup>a</sup>	
		<i>R. roseolus</i>	11.22 ± 1.399 <sup>b</sup>	40.52 ± 3.290 <sup>b</sup>	26.65 ± 2.452 <sup>b</sup>	
	B	No fungi	11.12 ± 0.179 <sup>b</sup>	35.79 ± 2.021 <sup>a</sup>	23.75 ± 1.181 <sup>a</sup>	
		<i>S. bovinus</i>	8.01 ± 0.468 <sup>a</sup>	28.19 ± 2.124 <sup>a</sup>	20.57 ± 0.896 <sup>a</sup>	
		<i>R. roseolus</i>	14.87 ± 0.885 <sup>c</sup>	45.43 ± 3.922 <sup>b</sup>	29.62 ± 2.244 <sup>b</sup>	
	Genotype (G)			(NS) F = 2.550	(NS) F = 0.170	(NS) F = 0.441
	ECM fungal isolates (F)			*** F = 16.643	** F = 11.448	** F = 6.566
	G x F			(NS) F = 2.574	(NS) F = 1.709	(NS) F = 2.002
30	A	No fungi	19.48 ± 2.123 <sup>a*</sup>	79.33 ± 7.078 <sup>a</sup>	43.39 ± 3.046 <sup>a</sup>	
		<i>S. bovinus</i>	17.57 ± 0.410 <sup>a*</sup>	63.81 ± 4.760 <sup>a</sup>	41.52 ± 2.533 <sup>a</sup>	
		<i>R. roseolus</i>	16.70 ± 1.191 <sup>a</sup>	97.45 ± 3.362 <sup>b*</sup>	55.49 ± 3.643 <sup>b*</sup>	
	B	No fungi	11.71 ± 0.494 <sup>a*</sup>	77.88 ± 3.033 <sup>a</sup>	47.65 ± 2.015 <sup>b</sup>	
		<i>S. bovinus</i>	13.14 ± 0.275 <sup>a*</sup>	62.00 ± 1.976 <sup>b</sup>	41.19 ± 1.786 <sup>b</sup>	
		<i>R. roseolus</i>	14.70 ± 0.719 <sup>b</sup>	47.39 ± 2.428 <sup>a*</sup>	33.64 ± 2.668 <sup>a*</sup>	
	Genotype (G)			*** F = 29.083	*** F = 26.287	* F = 7.645
	ECM fungal isolates (F)			(NS) F = 0.054	** F = 7.116	(NS) F = 1.417
	G x F			* F = 3.648	*** F = 21.331	*** F = 13.637

❖ At 30 mg Cd kg<sup>-1</sup> non-inoculated genotype A accumulated more Cd in the shoots (1.7-fold) than genotype B;

❖ At the lowest Cd concentration *S. bovinus* decreased Cd shoot concentration and increased aboveground development in both genotypes;

❖ At the highest Cd dosage inoculation with *R. roseolus* decreased Cd concentration in the roots of genotype B whereas the opposite occurred in genotype A.

## Conclusions

❖ The two genotypes of *P. pinaster* revealed differences in metal accumulation and distribution within the tree organs. Inoculation with ECM fungi affected plant growth, metal uptake and induced different responses in each genotype. The selection of an adequate combination between genotype and associated mycobionts may be an important biotechnological tool to enhance the efficiency of reforestation and phytoremediation processes of degraded land using *P. pinaster*.

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