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Root Exudates of Maize, Pine and Beech Seedlings Influenced by Mycorrhizal and Bacterial Inoculation*

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Abstract

The influence of endo- (*Glomus mosseae*) or ectomycorrhizal fungi (*Laccaria laccata*) and of bacteria (*Enterobacter agglomerans*, *Agrobacterium* sp., *Agrobacterium radiobacter*) on root exudation of maize, pine and beech seedlings, respectively, were studied in a growth chamber or in a greenhouse. Rhizospheric microorganisms modify the total release of carbon in the rhizosphere, the amounts of soluble exudates and their composition in carbohydrates, amino-acids and aliphatic acids. Such modifications influence the carbon balance, the humification processes and the bioweathering of minerals in the rhizosphere.

Introduction

Though the definition of "exudates" has been restricted by some authors such as Rovira et al. (1979) to the soluble carbon compounds released passively by sterile roots, the term includes here the three groups of compounds defined by Lespinat and Berlier (1975), i.e. in this presentation, all the carbon compounds released in the rhizosphere by sterile (without microorganism) and non sterile (with microorganisms) plants. As mycorrhiza are present in 90% of the root systems and as rhizobacteria are always present, experiments have been performed to evaluate the root exudates of maize and pine seedlings and their modifications after inoculation with rhizobacteria and/or mycorrhizal fungi.

Material and Methods

Maize (*Zea mays*) was grown in a phytotron (Laheurte, 1985; Laheurte and

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Berthelin, 1988) and was inoculated or not by an endomycorrhizal fungus (*Glomus mosseae*) and/or a phosphate dissolving bacterium (*Enterobacter agglomerans*). Cultures were performed for nine weeks.

Pine (*Pinus sylvestris L.*) and beech (*Fagus sylvatica* L.) seedlings, inoculated or not with an ectomycorrhizal fungi (*Laccaria laccata*) and/or a phosphate-dissolving bacterium (*Agrobacterium* sp. and *Agrobacterium radiobacter*, respectively) were grown in a greenhouse for 2 years (Leyval, 1988, 1989; Leyval and Berthelin, 1989). All plants were cultivated in cylindric tubes, filled with acid-washed sand, and were fed automatically.

At the end of the experiments, dry weight of plants was determined. Root exudation was evaluated as total organic carbon (total C) released by the roots (CHN Carlo Erba elemental analyser). The soluble fractions were collected from the sand after stirring with distilled water, filtration on paper then on a porous membrane (0.45 μm). Insoluble exudates were determined on sand residue after water extraction of soluble compounds. In the soluble fraction, carbohydrates, amino- and aliphatic acids were analyzed by colorimetry, G.C. and HPLC (Laheurte, 1985; Leyval, 1988).

Results

After two years, *Agrobacterium* inoculation significantly increased pine and beech growth (Table 1) and the total organic carbon released (Table 2). *Enterobacter agglomerans* did not significantly modify maize growth after 9 weeks (Table 1). The ectomycorrhizal fungus, *Laccaria laccata* promoted the release of carbon in the rhizosphere of both tree seedlings and the beech growth (Tables 1 and 2), but had no significant effect on pine growth (Table 1). The endomycorrhizal fungus, *Glomus mosseae*, did not significantly increase maize growth and decreased the total exuded carbon (Tables 1 and 2).

The soluble fraction of the root exudates was substantially greater with maize than with pine and beech (Table 2). Such difference depends on the plants but also on the culture devices which are respectively closed or open (lysimeters) for the maize or the pine and the beech. The open (lysimeters) devices simulated certainly much more the soil plant system than the closed one. The analyses of total carbohydrates, amino-acids

Table 1. Growth of maize (9 weeks), pine and beech (two years) inoculated by bacteria and mycorrhizal fungi. Total dry matter (shoot and roots) in g per plant. Two treatments with the same letter in the same column are not significantly different.

	Maize	Pine	Beech
Non inoculated	4.1a	6.9a	1.6a
+Bacteria	4.3a	7.8b	4.7b
+Mycorrhiza	4.4a	6.2a	4.0b
+Bacteria+Mycorrhiza	4.1a	6.4a	2.1a

Table 2. Influence of bacterial (respectively *Enterobacter agglomerans*, *Agrobacterium* sp. and *Agrobacterium radiobacter*) and/or mycorrhizal (*Glomus mosseae* and *Laccaria laccata*) inoculations on total and soluble organic carbon (mg C per plant) released by maize, pine and beech seedlings. Two treatments with the same letter in the same column are not significantly different.

	Total organic carbon			Total soluble carbon		
	Maize	Pine	Beech	Maize	Pine	Beech
Non inoculated	36a	417a	168a	9.0a	2.4ab	2.1a
+Bacteria	32a	627b	320b	7.0ab	2.7ab	2.5ab
+Mycorrhiza	28b	767c	494c	5.8b	2.7a	2.7b
+Bact+Mycor	43a	713c	189a	6.0b	2.6ab	2.2a

and aliphatic acids in the soluble fraction of exudates were performed on regrouped samples in order to dispose of larger and sufficient amounts of compounds for analysis. So that statistical analyses were not performed between the treatments for theses analyses, but at least 5 samples originated from 5 plants of the same treatment were regrouped. In comparison to sterile maize, the soluble fraction from maize inoculated with *Enterobacter agglomerans* contained a greater amount of carbohydrates (Table 3), with a different composition (fucose, rhamnose, glucose, ribose, arabinose instead of galactose and glucose). Total amount of amino-acids was not modified by *Enterobacter agglomerans* but different amino-acids (arginine, prolin, glutamic and diaminopimelic acids) were found. One of them (diaminopimelic acid) was characteristic of the bacterial inoculation. No more citric and isocitric acids recovered in the axenic maize rhizosphere were collected in presence of the bacterium. For mycorrhizal maize roots (Laheurte and Berthelin, 1986), the soluble fraction contained less carbohydrates (glucose, galactose, mannose), more amino-acids (same acids detected than with bacterial inoculation, except diaminopimelic acid that is produced only by bacteria) than for non mycorrhizal maize. No organic acids were detected. The soluble fraction from maize inoculated with both microorganisms (bacterium and fungus) mainly showed an increased amount of carbohydrates (Table 3) and aliphatic acids (Laheurte, 1985).

After inoculation of pine and beech seedlings with *Agrobacterium* sp. or *Agrobacterium radiobacter* or with *Laccaria laccata*, the total soluble fractions of the carbon released were not significantly modified except for mycorrhizal beech (Table 2). They showed smaller amounts of carbohydrates and amino-acids with pine but larger with beech (Table 3). In the rhizosphere of pine and beech, the analyses of aliphatic acids revealed the presence of citric, malic, fumaric, gluconic, lactic, succinic acids. They are mainly present in the non inoculated rhizosphere of beech (fumaric > citric > malic) and pine (malic > lactic < gluconic). The bacterial inoculation increased the amounts of malic and lactic acids in beech rhizosphere and of malic, citric, fumaric and lactic acids in pine rhizosphere. The mycorrhizal inoculation, except with beech, and the inoculation with

Table 3. Carbohydrates (expressed in μmoles glucose per plant) and amino-acids (expressed in μmoles leucine per plant) present in the soluble organic compounds in the rhizosphere of maize, pine and beech.

	Carbohydrates			Amino-acids		
	Maize	Pine	Beech	Maize	Pine	Beech
Non inoculated	5.3	1.7	0.7	31.3	9.5	2.0
+ Bacteria	6.8	1.2	0.9	29.8	3.5	5.0
+ Mycorrhiza	3.4	0.9	1.5	43.2	4.9	7.9
+ Bact + Mycor	17.5	0.6	3.2	25.0	8.0	5.4

Table 4. Amounts of total aliphatic acids collected in the beech and pine rhizosphere. The known amounts of aliphatic acids determined by HPLC allow to express the results in milliequivalents of carboxylic group found per plant.

	Non inoculated	+ bacteria	+ mycorrhiza	+ bact + mycor
Pine	63.2	103.3	0.2	0.1
Beech	20.4	42.9	21.9	0.1

both microorganisms (the fungus and the bacteria) decreased strongly the amounts of collected aliphatic acids as shown in table 4. Only traces of acids were found.

In these experimental conditions, synergistic effect of the inoculation by the bacterium and the mycorrhizal fungus was not observed neither for the growth nor the root exudation except for total exudation and carbohydrates production by maize roots and carbohydrate production in the beech rhizosphere. Comparatively to increasing effect of bacteria or mycorrhiza alone on the growth or on the production of total exudates, the inoculation by both microorganisms (bacterium + fungus) introduced some competitive effects.

Discussion and Conclusion

As previously reported by different authors, exudation of axenic roots varies according to plant species, but also their age and the nutritional conditions. Quantitative and qualitative differences in composition of root exudates were observed between maize, pine and beech roots, especially, the total amount of organic compounds collected in the rhizosphere in comparison to plant biomass and, the percentage of soluble fraction in comparison to the total carbon release.

Some authors have already observed an increase of the global root exudation in presence of non symbiotic microflora (e.g. Barber and Martin, 1976; Prikryl and Vancura, 1977, 1980; Kraffczyk et al., 1984). In our experiments, mycorrhization and inoculation by rhizobacteria increased the total root exudation in the pine and beech rhizosphere and so the rhizospheric humification. But similar effects were not, in these experimental conditions, observed with the maize except when both microorganisms were present. It seems interesting to underline that, on one hand, bacterial or mycorrhizal inoculation alone have promoted the growth and the total exudation of pine and beech and that, on the other hand, in the same experimental conditions, the double inoculation (bacterium + fungus) have not a synergistic effect. They even eventually have a competitive but not explained effect.

Such presented results must be related to the experimental conditions and do not give information about the rate of root exudation, but about the resulting carbon compounds in the rhizosphere and the possible effect of rhizospheric microorganisms. They show, for instance, the increase of organic acids in pine and beech rhizosphere after inoculation with *Agrobacterium* sp. and *Agrobacterium radiobacter* and the important role that microorganisms can play in the production of compounds involved in the weathering of minerals. Microorganisms can, by such processes, modify the amounts and the nature of the organic compounds that can be involved in the exchange processes between soil and plants.

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