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Rhizodeposition and net release of soluble organic compounds by pine and beech seedlings inoculated with rhizobacteria and ectomycorrhizal fungi

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Summary. A lysimetric experiment was performed in a greenhouse to evalute root deposition and net release of soluble organic compounds after 1 and 2 years from pine and beech seedlings inoculated with an ectomycorrhizal fungus (*Laccaria laccata*) and/or rhizobacteria (*Agrobacterium radiobacter* for beech and *Agrobacterium* sp. for pine). Total C compounds released in the rhizosphere of both plants increased after inoculation with the bacteria or ectomycorrhizal fungus. The rhizobacteria increased root and plant growth and rhizodeposition, but the mycorrhizal fungi appeared to increase only root deposition. Soluble C compounds, collected after 2 years, represented only 0.1–0.3% of the total C compounds released into the rhizosphere, and were modified by inoculation with the microorganisms. After inoculation with the bacteria, levels of sugars and amino acids decreased in pine and beech rhizospheres, whereas organic acids increased, especially in the pine rhizosphere. In the rhizosphere of mycorrhizal beeches, sugar and amino acids increased, and organic acids differed from those released from non-mycorrhizal beeches. In the mycorrhizal pine rhizosphere, however, all compounds decreased. Following dual inoculations, mycorrhizal colonization increased, no effect on plant growth was observed, and virtually no organic acids were detected.

Key words: Ectomycorrhizae – Rhizobacteria – Rhizodeposition – Soluble organic compounds – Pine – Beech – *Laccaria laccata* – *Agrobacterium radiobacter*

Root exudates were initially defined as all organic substances released by living roots (Rovira 1969). More recently, the definition has been restricted to low molecular weight compounds released passively by roots, distinguished from secretions, mucilages, and mucigels, lysates, and microbial or plant "debris" (Martin 1977; Rovira et al. 1979). Although soluble exudates have been more frequently studied, since they are more readily biodegradable and are considered to be responsible for the rhizospheric effect (Kraffczyk et al. 1984), insoluble compounds are also very important in supplying organic matter to soils. Rhizodeposits, including all these organic compounds, have been reported by different authors to represent from 9% to 50% of the root weight, depending upon method (experimental and analytical) or expression of the results (per cent dry matter of plant, of roots, of photosynthetically fixed C, etc.) (Shamoot et al. 1968; Barber and Gunn 1974; Barber and Martin 1976; Prikryl and Vancura 1980; Beck and Gilmour 1983; Haller and Stolp 1985; Laheurte and Berthelin 1986). Root exudation varies according to species, age of the plant, and nutritional conditions (Rovira 1969; Smith 1970; Kraffczyk et al. 1984; Haller and Stolp 1985).

Little information is available on tree root exudates. Reported results have mainly concerned the root necromass (Perrson 1978; Agren et al. 1980) or the release of specific compounds, such as organic acids, lipids, vitamins or hormonal substances (Bowen 1969; Smith 1969, 1970; Krupa and Fries 1971; Fries et al. 1985; Strzelczyk et al. 1986).

Quantitative and qualitative modifications to root exudates have been observed after inoculation of roots with microorganisms that use root exudates as a C source (Barber and Matin 1976; Barber and Lynch 1977; Martin 1977; Prykril and Vancura 1977; Schönwitz and Ziegler 1982; Kraffczyk et al. 1984; Laheurte and Berthelin 1988). Comparisons between mycorrhizal and non-mycorrhizal plants have been reviewed by Ingham and Molina (1991), but have mainly concerned vesicular-arbuscular mycorrhizae colonization (Katznelson et al. 1962; Rambelli 1973; Laheurte and Berthelin 1986).

In a previous paper, we reported results from a greenhouse experiment on the effect of an ectomycorrhizal fungus (*Laccaria laccata* S235) and/or phosphate-solubilizing bacteria (*Agrobacterium radiobacter* or *Agrobacterium* sp.) on the weathering of a mica by pine and beech roots (Leyval and Berthelin 1991). In the pre-

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sent work we studied the effect of these rhizospheric microorganisms on rhizodeposition (total organic compounds) and on the net release of soluble organic compounds possibly involved in the weathering of minerals (amino acids, sugars, organic aliphatic, and phenolic acids) (Berthelin 1983; Robert and Berthelin 1986; Leyval and Berthelin 1991). As the experiment was not carried out under axenic conditions the C compounds studied originated from rhizodeposition and root exudates, eventually transformed by the microorganisms present, and form the microorganisms themselves.

Materials and methods

After germination of disinfected pine (*Pinus sylvestris* L.) and beech (*Fagus silvatica* L.) seeds, young seedlings were repotted in plastic containers on autoclaved sand for inoculation with the ectomycorrhizal fungus *Laccaria laccata*. After 6 months the pine and beech roots were partially disinfected in a gentamicin solution (Kidd et al. 1982). The seedlings were then transferred to plastic cylindric lysimeters. The cylindric tubes (Leyval 1990; Leyval and Berthelin 1991) were filled with 600 g acid-washed sand mixed with 6 g mica (phlogopite) and 1 g rock phosphate, as sole sources of K, Mg, Fe, and P for plant growth (Leyval and Berthelin 1991). This sand–mica–rock phosphate mixture is here termed "soil". The upper part of the cylinders was covered with finer sand coated with silicons (Berthelin and Leyval 1982) to prevent, or at least reduce, growth of mosses and algae. A nutrient solution containing ($mg\,l^{-1}$): $(NH_4)_2SO_4$, 47.1; $CaCl_2 \cdot 2H_2O$, 18.4; H_3BO_3, 2.3; $MnSO_4 \cdot H_2O$, 1.2; $ZnSO_4 \cdot H_2O$, 2.0; $CuCl_2 \cdot 2H_2O$, 0.5; $MoO_3 \cdot H_2O$, 0.6 was added through a silicon tube with an automatic pump ($10\,ml\,day^{-1}$). All lysimeters and minerals were sterilized by autoclaving before the experiment. The lysimeters were placed in a greenhouse for a 2-year period. Average daily greenhouse temperatures ranged from 10 to 20 °C.

The treatments included a control (lysimeter without a plant); non-inoculated plants; plants + bacteria (plants inoculated with bacteria); plants + mycorrhiza (plants inoculated with *Laccaria laccata* S 235, provided by Dr. Le Tacon, CRF-INRA, Champenoux, France); and plants + bacteria + mycorrhiza (plants inoculated with both microorganisms). The pine and beech seedlings were inoculated with two phosphate-dissolving bacterial strains, *Agrobacterium* sp. or *Agrobacterium radiobacter* respectively, isolated from their rhizosphere at a high frequency (Leyval and Berthelin 1988). The bacteria and the ectomycorrhizal fungus were grown in pure cultures on liquid medium (Leyval and Berthelin 1988) to prepare the inoculi. The microbial inoculation procedure has been described previously (Leyval and Berthelin 1991). The plants were inoculated with 1 ml ground fungal mycelium grown for 2 weeks, previously rinsed in sterile distilled water, and/or with 1 ml bacterial suspension (10^8 to 10^9 bacteria ml^{-1}). To maintain the size of the inoculated microbial populations in the experimental devices, where the root environment (pure sand, humidity level) may be different from that in a soil, root inoculations were performed at the beginning of the experiment in October 1984; and repeated in March and May 1985 and March and May 1986. The same inoculum, autoclaved twice, was added to the non-inoculated control plants. There were 15 replicates randomly laid out, except for the controls, which had 6 replicates.

Effluents were collected regularly, the pH determined, and leachates combined over periods of 3 months for analyses. Results have been presented elsewhere (Leyval 1990).

After 1 and 2 years, respectively, 5 and 10 lysimetric cylinders were opened and the plants removed. Non-rhizospheric and rhizospheric fractions of soil were separated, the latter being defined as the soil remaining attached to the root system after it had been shaken by hand (Fig. 1). Rhizospheric soil was separated from the root systems after stirring in 100 ml sterile distilled water for only 30 min, to limit extraction of plant and microbial compounds. The water-soluble and -insoluble compounds were separated by filtration on a tissue membrane (20 µm pore size; UGB, Panissières, France). The water-soluble compounds were filtered using a porous membrane (0.45 µm) (Fig. 1). Insoluble rhizodeposits were determined in the sand remaining after water extraction of soluble compounds. All fractions were kept frozen before analysis. Shoots and root dry matter was recorded. Ectomycorrhizal infection was checked under a binocular microscope and assessed, after acid hydrolysis, using a chitin assay (Plassard et al. 1983). Bacteria in the rhizospheric soil were counted on nutrient agar (Difco, Detroit, Michigan, USA), using the plate-dilution technique.

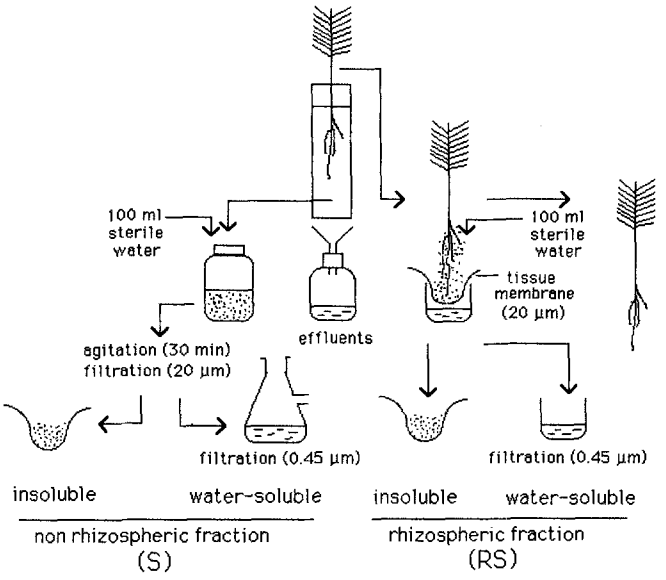

Fig. 1. Experimental procedure for collecting various organic fractions from lysimeters

Total organic C was measured in sand, in the water-soluble fractions (rhizospheric and non-rhizospheric) and in the effluents of the lysimeters with a CHN elemental analyzer and a total C monitor (Carlo Erba). The effluents were not analyzed further, for they contained little organic C.

Well defined organic compounds were identified and measured only in the water-soluble fraction. In analyzing the water-soluble exudates, samples from the same treatment were grouped together. Free and total neutral sugars were analyzed by the anthrone colorimetric method (Brink et al. 1960), and free and total amino acids by the ninhydrin colorimetric method (Moore and Stein 1954), primarily on non-hydrolyzed samples to evaluate free sugars and amino acids. As the amounts detected were small, the samples were then concentrated 10 times by rotative evaporation at 30 °C. After hydrolysis (3 *N* HCl for 16 h at 80 °C), total amino acids were measured. Total sugars were analyzed after a second hydrolysis (1 *N* H_2SO_4 for 16 h). Aliphatic organic acids were separated and identified using high-performance liquid chromatography (Gold System, Beckman) with an Aminex HPX-87 H column (Biorad, Richmond, California, USA; eluant: 0.009 *N* H_2SO_4, 0.7 ml min^{-1} at 65 °C; detection at 210 nm). Phenolic compounds were determined using reverse-phase chromatography (silica column Nova-Pak C 18; eluant: water–methanol (80 : 20); flow rate: 0.7 ml min^{-1}; ultraviolet spectrophotometer detection at 275 nm).

Total C data were statistically analyzed (Student's *t*-test).

Results

Microbial colonization of pine and beech roots and plant growth

The rhizospheric bacterial population was 10 times greater after 2 years and 2000 times greater after 1 year, respectively, in beech and pine rhizospheres inoculated with the agrobacteria than in non-inoculated rhizospheres (Ta-

Table 1. Dry matter, rhizospheric bacterial population, and mycorrhizal infection of pines and beeches inoculated with *Agrobacterium* sp. or *A. radiobacter* and *Laccaria laccata*

	Beech				Pine			
	NI	+B	+M	+B+M	NI	+B	+M	+B+M
Plant dry matter (g)								
After 1 year	2.23 a	3.08 a	2.32 a	1.92 a	2.68 b	2.59 bc	2.23 c	2.31 c
After 2 years	1.41 a	4.72 b	4.0 b	2.07 a	6.91 cd	7.91 d	5.94 c	6.27 c
Bacterial population (CFU g^{-1} dry weight)								
After 1 year	3×10^7 a	10^7 a	10^8 a	7×10^6 a	10^7 a	2×10^{10} b	2×10^8 a	3×10^7 a
After 2 years	3×10^6 a	3×10^7 b	7×10^6 a	3×10^6 a	3×10^7 a	2×10^6 a	8×10^6 a	10^7 a
Fungal dry weight (% of root dry weight)								
After 1 year	1.6 a	1.1 a	1.6 a	2.9 b	1.7 a	1.4 a	5.5 b	8.6 c
After 2 years	0.2 a	0.2 a	0.9 b	0.6 a	0.8 a	1.1 a	2.6 b	4.2 b

Means with the same letter in a row are not significantly different at $P<0.05$. NI, Not inoculated; +B, inoculated with bacteria; +M, inoculated with mycorrhizal fungus; +B+M, dual inoculation; CFU, colony-forming units

ble 1). However, in the other treatments bacterial populations were not significantly different from the non-inoculated control contaminated with air-borne microorganisms. Mycorrhizal infection was higher for pine and beech seedlings inoculated with *Laccaria laccata* than non-inoculated seedlings (Table 1). Fungal dry matter was underestimated, since part of the external hyphae was probably lost when the rhizospheric sand was separated from the roots (Fig. 1). Bacterial inoculations increased mycorrhizal development (except after 2 years for beech).

Plant dry matter (shoots and roots, Fig. 1) was rather low, due to nutrient deficiencies (Leyval and Berthelin 1991), since K, Mg, Fe, and P were applied as insoluble compounds. Bacterial or mycorrhizal inoculations enhanced beech growth. The growth increase appeared after 1 year but was significant only after 2 years. Dual inoculations, however, did not promote beech growth (Table 1). Bacterial inoculation increased pine growth after 2 years, but *Laccaria laccata* either had no significant effect or had a depressing effect on pine growth. Under the present experimental conditions, the promoting effect on plant growth was mainly due to bacteria.

Net C release (rhizodeposits)

Release of total C in the lysimeters was equivalent to 30%–68% and 36%–99% of root C for beech and pine seedlings, respectively, depending on the different root treatments (Fig. 2). Root inoculation with either *Agrobacterium* sp. or *Agrobacterium radiobacter*, or with *Laccaria laccata*, promoted total C production (C in roots and C released by roots). With pines, the larger increase was observed with *Agrobacterium* sp. Mycorrhizal infection increased the percentage of C released compared to total C production (rhizodeposits+roots) for both plants (34%–50%). *Agrobacterium* sp. also increased the percentage of C released in the pine rhizosphere to a lesser extent (18% in comparison to 13% for the non-inoculated pines). Dual inoculation with both microorganisms did not modify total C in the lysimeters (Fig. 2), but with pine seedlings, the ratio of root C to C released into the rhizosphere and into the soil decreased.

C release in the lysimeters was classified into (1) water-soluble compounds (<0.45 μm); (2) water-insoluble compounds (0.45–20 μm), including microbial cells, root, and microbial debris and lysates; and (3) water-insoluble compounds (>20 μm) corresponding to dead roots, mucilaginous debris, and polysaccharide compounds. The C content of fraction 3 was much greater than those of fractions 1 and 2 (Table 2), which represented no more than 2 mg/plant. Inoculation of beech roots with *Agrobacterium radiobacter* or *Laccaria laccata* led to a significant decrease in soluble and insoluble compounds (fractions 1, 2, and 3; Table 2) but dual inoculation did not (no significant differences). In the pine lysimeters, fractions 1 and 2 were not modified, but insoluble compounds (fraction 3) significantly increased after inoculation with *Agrobacterium* sp. and/or *Laccaria laccata*.

Chemical analyses of soluble C compounds

Quantities of soluble C in the lysimeters (fraction 1) were measured after 1 and 2 years (Table 3). Per gram of soil, they were 20- to 120-fold greater in the beech rhizosphere and 6- to 50-fold greater in the pine rhizosphere than in the non-rhizospheric soils. The amounts of soluble C compounds were smaller after 2 years than after 1. In the rhizosphere of mycorrhizal pines, and of mycorrhizal beeches only after 2 years, lower amounts of soluble C compounds were found than in the rhizosphere of non-mycorrhizal pines. The rhizosphere of beech seedlings inoculated with *Agrobacterium radiobacter* contained lower amounts of soluble C compounds than that of non-inoculated seedlings and the opposite was observed for pines inoculated with *Agrobacterium* sp. (Tables 2 and 3). However, these results were not significant.

Greater quantities of free and total sugars and amino acids were found in the rhizosphere of mycorrhizal beeches (Table 4). Concentrations of free sugars and total amino acids were also increased. However, pine-root water extracts contained smaller amounts of amino acids and

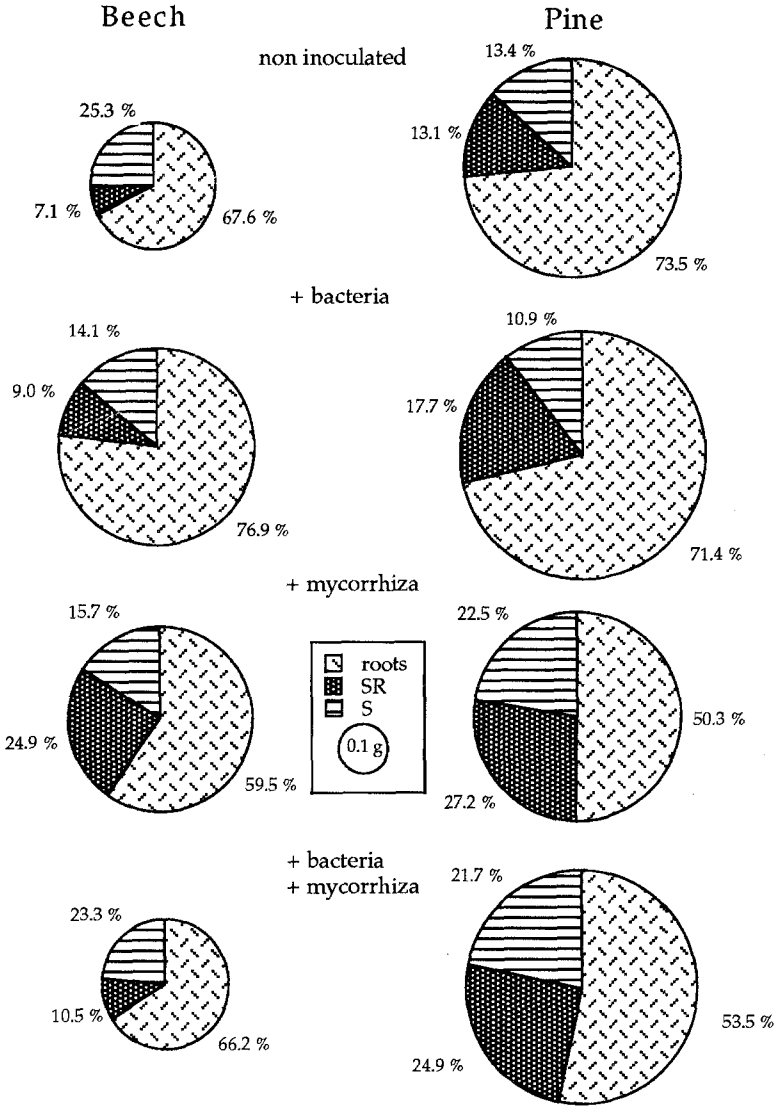

Fig. 2. Effect of rhizobacteria (*Agrobacterium* sp. and *A. radiobacter*) and ectomycorrhizal fungus (*Laccaria laccata*) on total C compounds in lysimeters with pine and beech seedlings after 2 years. Areas of the *circles* are proportional to C quantities; SR, rhizospheric soil; S, non-rhizospheric soil

sugars after inoculation with *Laccaria laccata*. The rhizospheres of pines and beeches inoculated with the bacteria contained smaller amounts of sugars and amino acids than non-inoculated plants (with beech, the quantities per plant increased because the plants were larger). However, the pine rhizospheric extracts then presented higher contents of organic acids (Table 4).

Fumaric, citric, and malic acids were found in beech-root water extracts, whereas in corresponding pine extracts, malic, lactic, and gluconic acids were mainly observed (Table 5). Pure cultures of both *Agrobacterium* spp. also contained gluconic and lactic acids (Table 6). In the rhizospheric extracts of pines inoculated with the bacteria, citric, fumaric, malic, and lactic acids were detected, as they were in non-inoculated plants, but in greater amounts, and gluconic acid was no longer present (Table 5). In the water extracts from beech roots inoculated with *Agrobacterium radiobacter*, the composition of or-

Table 2. Effect of bacteria (+B) and/or ectomycorrhizal fungi (+M) on the amount of C (mg g^{-1} plant dry weight) in pine and beech lysimeters after 2 years

Fraction	Beech				Pine			
	NI	+B	+M	+B+M	NI	+B	+M	+B+M
(1) $<0.45\,\mu m$	0.35	0.04	0.12	0.34	0.11	0.09	0.13	0.13
(2) $0.45\,\mu m \ll 20\,\mu m$	1.13	0.48	0.55	0.72	0.23	0.25	0.32	0.29
t-test on fractions 1+2	a	ab	b	a	a	a	a	a
(3) $>20\,\mu m$	117	67.2	64.75	89.8	59.9	84.1	128.6	113.2
t-test on fraction 3	a	b	b	a	d	f	e	e

See footnotes to Table 1

Table 3. Soluble C compounds ($\mu g\ g^{-1}$ of sand) in lysimeters after 1 and 2 years

	Beech				Pine			
	RS		S		RS		S	
	1 year	2 years	1 year	2 years	1 year	2 years	1 year	2 years
Non-inoculated	160 a	152 a	4.0 e	2.1 ab	220 a	36.2 a	4.0 a	1.8 a
+Bacteria	110 a	73 ab	3.3 e	2.3 ab	230 a	79.6 ab	4.5 a	2.4 ab
+Mycorrhiza	190 a	52 b	3.2 e	2.5 a	60 b	27.3 a	6.2 b	3.1 b
+Bacteria +mycorrhiza	366 a	102 b	3.0 e	1.9 b	40 c	18.3 c	6.6 b	2.6 ab

Means with the same letter in a column are not significantly different at $P<0.05$. RS, rhizospheric soil; S, non-rhizospheric soil

Table 4. Sugar, amino acid, and organic acid content of water-soluble pine and beech rhizospheric extracts

	Beech				Pine			
	NI	+B	+M	+B+M	NI	+B	+M	+B+M
Free sugars								
μg glucose $plant^{-1}$	10	10	230	90	440	430	230	210
μg glucose g^{-1} dry weight	7	2	57	40	60	54	39	33
Total sugars								
μg glucose $plant^{-1}$	120	170	270	570	300	210	160	100
μg glucose g^{-1} dry weight	85	36	67	270	40	26	27	16
Free amino acids								
μM leucine $plant^{-1}$	2.9	4.0	4.8	4.4	0.0	0.0	0.0	5.7
μM leucine g^{-1} dry weight	2.1	0.8	1.2	2.1	0.0	0.0	0.0	0.9
Total amino acids								
μM leucine $plant^{-1}$	2.0	5.0	7.9	5.4	9.5	3.5	4.9	8.0
μM leucine g^{-1} dry weight	1.4	1.1	2.0	2.6	1.4	0.4	0.8	1.3
Organic acids								
mEq $plant^{-1}$	20.4	42.9	21.9	0.1	63.2	103.3	0.2	0.1
mEq g^{-1} dry weight	14.5	9.1	5.5	0.05	9.1	13.0	0.03	0.02

See Table 1 for abbreviations

Table 5. Organic acid composition (μM $plant^{-1}$) of rhizospheric water extracts from pines and beeches inoculated with *Agrobacterium* sp. (+B) and/or *Laccaria laccata* (+M)

	Beech				Pine			
	NI	+B	+M	+B+M	NI	+B	+M	+B+M
Citric acid	3.4	n.d.	6.4	0.0	traces	9.4	0.0	0.0
Malic acid	0.4	14.0	0.0	0.0	17.2	20.3	0.0	0.0
Fumaric acid	4.7	0.1	0.5	0.03	0.04	4.0	0.1	0.03
Gluconic acid	0.0	0.0	0.0	0.0	7.0	0.0	0.0	0.0
Lactic acid	0.0	14.7	1.7	0.0	14.7	26.5	0.0	0.0
Unidentified	0	2	0	1	2	3	1	1

NI, not inoculated; +B+M, dual inoculation

ganic acids was modified, and comprised mainly malic and lactic acids.

In the rhizospheric extracts of mycorrhizal beeches, lower levels of organic acids (expressed per g dry weight) were found than in those of non-mycorrhizal beeches (Table 4); citric acid increased and lactic acid, as produced by the fungus in pure culture (Table 6), appeared. In the case of mycorrhizal pine seedlings, the amount of organic acids detected was very low (Tables 4 and 5).

Following dual inoculation with the agrobacteria and with *Laccaria laccata*, levels of total sugars and amino acids (per g dry weight) were higher in the beech rhizosphere than with single inoculations. Free and total sugars were lower in the pine rhizosphere compared to single inoculations (Table 4). Only very small amounts of fumaric acid were then detected (Table 5) in beech and pine rhizospheres.

Small amounts of aromatic compounds were observed in the soluble water extracts of mycorrhizal beeches, of non-inoculated pines, and of pines inoculated with *Agrobacterium* sp. (Fig. 3). Among these phenolic compounds, benzoic, catechic, protocatechic, vanillic, parahydroxybenzoic, and syringic acids were identified.

Discussion

The observations on the pine and beech rhizospheric environment, mainly on organic matter release, was made after 1 and 2 years of plant growth in the experimental device. The pines and beeches were inoculated several times with phosphate-dissolving bacteria and an ectomycorrhizal fungus in order to maintain the size of the inocu-

Table 6. Production of organic acids (ppm) by pure cultures of *Agrobacterium* sp. and *Laccaria laccata* in liquid medium

	Agrobacterium radiobacter (after 24 h)	*Agrobacterium* sp. (after 24 h)	*Laccaria laccata* (after 10 days)
Gluconic acid	628 ±80	1190±200	
Lactic acid	779 ±12	942±22	+
Fumaric acid	3.5±0.5	0.0	
Tartaric acid			+
Malic acid			+
Succinic acid			+
Acetic acid			+
Cetoglutaric acid			+
Undetermined			4

Means±SE; +, identified but not quantified

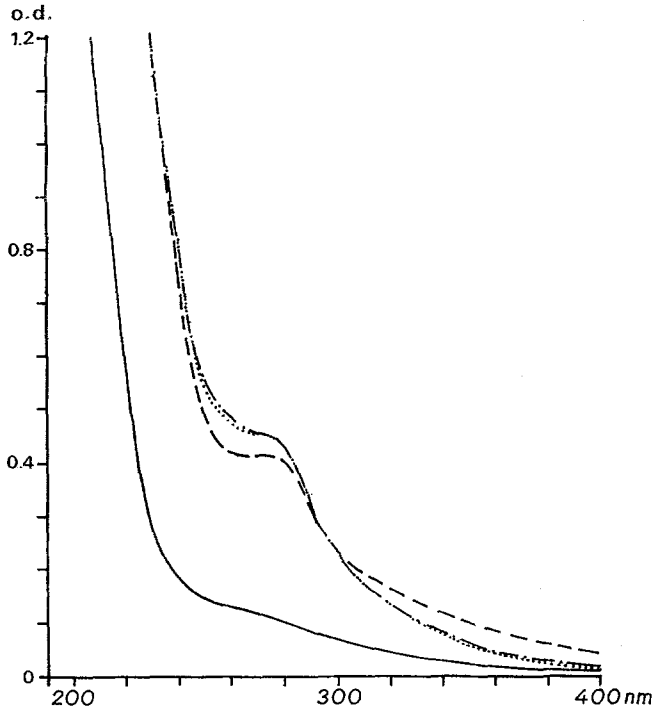

Fig. 3. Aromatic compounds in pine and beech soluble exudates detected by the peak (shoulder) at 280 nm on ultraviolet spectrum. Non-inoculated beech: _____; mycorrhizal beech; – – –; non-inoculated pine: –·–·–; pine inoculated with *Agrobacterium* sp.: . . .; o.d., optical density

lated populations. As a matter of fact, Holl and Chanway (1992) showed that a bacterial population (*Bacillus polymyxa*) inoculated into a pine rhizosphere can decline rapidly, but that re-inoculation after 8 weeks can reverse this effect and re-establish the threshold population size. However, these authors observed the maximum increase in pine dry weight 8 weeks after inoculation with *Bacillus polymyxa*, when its population size had decreased by 100-fold. In the present experiment the dominance of the agrobacteria in pine and beech rhizospheres was not shown. However, the inoculation affected plant growth, rhizodeposition, and the release of soluble organic compounds. Root colonization with *Laccaria laccata* was verified and an effect was observed, as for the agrobacteria.

Rhizodeposits, as defined by Shamoot et al. (1968), were as high as 30%–99% of root C in the lysimeters with pine and beech seedlings after 2 years. Under these experimental conditions it was not possible to evaluate CO_2 production. However, respiration might represent a great part of C losses. Reid et al. (1983) showed that 14.5% of fixed ^{14}C had been transferred to pine roots after 11 months, 0.4% to root exudates, and 11.7% lost by respiration.

More C was released into the rhizosphere when pines and beeches were inoculated with the ectomycorrhizal fungus *Laccaria laccata* or with one of the agrobacteria. With bacterial inoculation, rhizodeposits and root dry matter increased simultaneously. In contrast, with mycorrhizal inoculation, root weights did not change, but the release of C relative to root C increased (Fig. 2). Mycorrhizal external hyphae might account, at least partially, for such an increase.

In this experiment soluble pine and beech water extracts, collected arbitrarily after 2 years, contained 3–10 mg of C per g of root, corresponding to only 0.1%–0.3% of the total C compounds in the rhizosphere after 2 years. Levels of sugars and amino acids, expressed per g of plant dry weight, were lower in pine and beech rhizospheres inoculated with the bacteria. With both plants, the release of organic acids was increased by *Agrobacterium* sp. and *Agrobacterium radiobacter* by 40% and 240%, respectively, which could explain the increased weathering of minerals that was observed in the lysimeters after 2 years (Leyval and Berthelin 1991). However, in relation to plant growth, organic acids increased only in the pine rhizosphere (Table 4). The organic acids changed both quantitatively and qualitatively after inoculation with both *Agrobacteria* spp. Lactic acid, which the bacteria released in pure culture, appeared in plant rhizospheres. Malic and citric acids increased after bacterial inoculation. In an axenic experiment under hydroponic conditions (Leyval 1988) sterile pines also released gluconic and lactic acids. After 8 weeks the sterile pines had released 3.0±0.01 (mean of three replicates and standard error) and 2.4±0.05 ppm of gluconic and lactic acids, respectively. In the tubes where pines were inoculated with *Agrobacterium* sp. the concentration of gluconic and lactic acids was increased (17.0±1.0 and 25.7±3.2 ppm, respectively).

In the rhizosphere of mycorrhizal plants the amounts of soluble C compounds were reduced and modified, as previously observed by several authors (Rambelli 1973; Laheurte and Berthelin 1986). As expressed per gram of soil, they were reduced (Table 3) with mycorrhizal colonization after the 1st and 2nd years for pines and after the 2nd year for beeches. However, this could reflect a dilution effect because the percentage of rhizospheric soil of mycorrhizal pine and beech seedlings increased (Leyval and Berthelin 1989, 1991), thus improving soil exploration. C release in the lysimeters per gram of plant (Table 2) decreased when plant growth (Table 1) was significantly promoted by bacterial of fungal inoculation. This

has been observed previously with maize (Laheurte and Berthelin 1986, 1988). The effect of a phosphate-dissolving bacterium on water-soluble exudates was also dependent on the soluble P content in the medium (Laheurte and Berthelin 1988; Leyval and Berthelin 1989).

For both plant species in the present study, especially mycorrhizal plants, rhizospheric soluble C compounds decreased between the 1st and 2nd years (Table 3), as did mycorrhizal colonization (Table 1). Harris and Paul (1987) suggested that as colonization occurs, the root concentration of P increases, the leakage of exudates through the plant plasmalemma decreases, and mycorrhizal colonization diminishes in proportion (Ingham and Molina 1991). Reid et al. (1983) showed that 32 and 13.4% of ^{14}C fixed below ground was distributed in the roots of mycorrhizal and non-mycorrhizal pines, respectively, after 2 months. But after 10 months, 14.5% had accumulated in the roots of mycorrhizal and non-mycorrhizal pines. However, the percentage of ^{14}C released by respiration from the mycorrhizal roots was twice that from non-mycorrhizal roots after 2 and 10 months.

After inoculation with *Laccaria laccata*, sugar and amino acid contents increased in the beech rhizospheric water extracts, but decreased in the pine extracts, as observed with *Agrobacterium* spp. Laheurte and Berthelin (1986) observed that mycorrhizal roots released less monosaccharides but more amino acids, especially arginine, than non-mycorrhizal roots. The changes in root exudates from easily utilizable sugars to more complex amino acids (Katznelson et al. 1962) could be a strategy to eliminate competitors (Ingham and Molina 1991).

In the rhizosphere of mycorrhizal pines, no organic acids were detected, although *Laccaria laccata* produced organic acids in pure culture (Table 5). However, root exudates and the culture medium used for pure culture of the fungus are completely different and do not necessarily induce production of the same metabolites. Also, other microorganisms were present in the plant rhizosphere and could have used these compounds as sources of C and energy. In the rhizosphere of mycorrhizal beeches, citric and lactic acids appeared, but fumaric acid decreased and the total amount of organic acids was not modified in comparison to non-mycorrhizal plants.

Such compounds can increase the availability of mineral elements to plants and can also modify root membrane permeability, root metabolism, and the concentration of elements in root cells. The pH decreased in the leachates from the lysimeters, from around 6 to 4.5, in all the treatments during the experiment (Leyval 1990). The acidification of the leachates could be related to the NH_4-N source for plant growth. However, the acidification was greater when the beeches were inoculated with rhizospheric microorganisms (Leyval 1990). Certain other bacterial compounds, which were not analyzed in the present experiment, could also be involved in modifying root metabolism and root exudation. For example, phosphate-solubilizing bacteria and bacteria isolated from the rhizosphere of *Pinus sylvestris* (Kampert et al. 1975; Strzelczyk and Pokojska-Burdziej 1984; Vancura and Jandera 1986) have been shown to produce plant growth-promoting substances (Barea et al. 1976). *Agrobacterium radiobacter* produces polysaccharide compounds that promote the growth of sugar-beet roots (Stanek et al. 1983). Microscopic observation of pine roots inoculated with *Agrobacterium* sp. has revealed the presence of polysaccharide coatings (Leyval and Berthelin 1991). Mycorrhizal fungi could also affect root deposition through hormonal effects on plant growth (Stzrelczyk et al. 1986; Frankenberger and Poth 1987; Hanley and Green 1987). In the present experiment, however, they only seemed to increase root deposition (Fig. 2).

Dual inoculation with the ectomycorrhizal fungi and the phosphate-dissolving bacteria increased mycorrhizal colonization of roots (Table 1) and the ratio of released C to root C. However, the bacterial plant growth-promoting effect disappeared. The results for soluble sugars and amino acids in the treatment with bacteria + mycorrhiza were closer to those for the treatment with mycorrhiza alone than for the treatment with bacteria alone (Table 4). This could suggest competition between microorganisms for nutrients, or the production of antimicrobial compounds. However, no inhibition was observed when *Laccaria laccata* and *Agrobacterium* sp. were grown together on agar plates (data not shown). As suggested by Duponnois and Garbaye (1990) the growth stimulation of the mycorrhizal fungus by the bacteria could be a trophic effect induced by the release of organic compounds such as organic acids, which decreased in the rhizosphere of mycorrhizal plants (Table 4).

Table 1 shows that under our experimental conditions the rhizospheric microorganisms increased the growth of beech, which did not grow as well as the pine over the 2 years, more than the growth of pine. In previous experiments (Leyval and Berthelin 1982), the effect of rhizobacteria and endomycorrhizal fungi on maize growth was also dependent on plant growth conditions. When these conditions (nutrients, humidity) were good, only a slight microbial effect on maize growth was observed. In the present experiment the promoting effect on plant growth was mainly caused by bacteria. Some authors have also observed that mycorrhizal fungi occasionally reduce plant growth, especially during early stages of colonization (Ingham and Molina 1991).

The above results only provide data on the rhizospheric environment in lysimeters after 1 and 2 years of seedling growth. Results observed in lysimeters will not necessarily be the same as those obtained under field conditions. The use of sand instead of soil and the lack of a normal root spread and root competition with other plants, which were features of this lysimeter experiment, can greatly influence plant growth, exudation, and microbial effects. Nevertheless, the present experiments have shown clearly that some exudation processes and exudates and rhizodeposits themselves are actually modified by rhizobacteria and mycorrhizas. However, it is not known whether the various organic compounds were excreted by the plants, by living cells of the microorganisms, or were released from dead cells as products of autolysis. Further studies on the release of C compounds by symbiotic fungi and their associated rhizobacteria are necessary for a better understandig of plant-soil-microorganism interactions.

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