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H. A. Azaizeh · H. Marschner · V. Römheld
L. Wittenmayer

Effects of a vesicular-arbuscular mycorrhizal fungus and other soil microorganisms on growth, mineral nutrient acquisition and root exudation of soil-grown maize plants

Abstract Maize (*Zea mays* L. cv. Alize) plants were grown in a calcareous soil in pots divided by 30-μm nylon nets into three compartments, the central one for root growth and the outer ones for hyphal growth. Sterile soil was inoculated with either (1) rhizosphere microorganisms other than vesicular-arbuscular mycorrhizal (VAM) fungi, (2) rhizosphere microorganisms together with a VAM fungus [*Glomus mosseae* (Nicol. and Gerd.) Gerdemann and Trappel], or (3) with a gamma-irradiated inoculum as control. Plants were grown under controlled-climate conditions and harvested after 3 or 6 weeks. VAM plants had higher shoot:root ratios than non-VAM plants. After 6 weeks, the concentrations of P, Zn and Cu in roots and shoots had significantly increased with VAM colonization, whereas Mn concentrations had significantly decreased. Root exudates were collected on agar sheets placed on the interface between root and hyphal compartments. Six-week-old VAM and non-VAM plants had similar root exudate compositions of 72–73% reducing sugars, 17–18% phenolics, 7% organic acids and 3% amino acids. In another experiment in which root exudates were collected on agar sheets with or without antibiotics, the amounts of amino acids and carbohydrates recovered were similar in VAM and non-VAM plants. However, three- to sixfold higher amounts of carbohydrates, amino acids and phenolics were recovered when antibiotics were added to the agar sheets. Thus, the high microbial activity in the rhizosphere and on the rhizoplane limits the exudates recovered from roots.

Key words *Glomus mosseae* · *Zea mays* · Mineral uptake · Root exudation

H. A. Azaizeh (✉) · H. Marschner · V. Römheld
L. Wittenmayer
Institut für Pflanzenernährung (330), Universität Hohenheim, D-70593 Stuttgart, Germany

L. Wittenmayer
Institut für Pflanzenernährung und Düngung, Martin-Luther-Universität, Halle-Wittenberg, D-06108 Halle, Germany

Introduction

The major substrate for microbial activity in the rhizosphere or on the rhizoplane is organic C (rhizodeposition) released by plant roots. This organic C varies from simple organic molecules to mucilage and cells and tissues sloughed off during root growth (Rovira et al. 1983). The simple, low molecular weight compounds consist mainly of sugars, amino acids, organic acids and phenolics. Leakage of organic compounds from roots is an universal phenomenon and can represent a significant loss of photosynthates from the plant. Depending on plant species, age and environmental conditions, these exudates can account for up to 40% of the dry matter produced by plants (Lynch and Whipps 1990). Root exudates of 4-week-old maize plants grown in quartz sand or soil were estimated to be 7% of net photosynthates by measuring the rate of denitrification in a hermetically sealed root system (Haller and Stolp 1985). More root exudates were collected with a flooding method than by percolation, and more total sugars, amino acids and organic acids were collected from P-sufficient than P-deficient plants (Wittenmayer and Gransee 1992).

Vesicular-arbuscular mycorrhizal (VAM) fungi are obligate biotrophs and depend on living plant roots for a supply of organic C. The presence of these fungi in the root may change root exudation by the colonized plants. Mycorrhizal plants often grow better than non-VAM plants, in most instances due to higher P, Zn and Cu uptake (Swaminathan and Verma 1979; Pacovsky 1986; Kothari et al. 1991). The beneficial effects of colonization by VAM fungi may not always outweigh the cost to the host, which in terms of extra carbohydrates translocated to the roots is approximately 6–10% of the net fixed C (Koch and Johnson 1984); in some cases, the C diverted to the fungi is sufficient to decrease plant growth (Bethlenfalvay et al. 1982).

Most studies on root exudation have been carried out on seedlings grown in solution cultures or in quartz sand (Schönwitz and Ziegler 1982; Schwab et al. 1983;

Wittenmayer and Gransee 1992). When plants were grown in soil to examine the effects of VAM fungi on root exudates, exudates were collected after harvest by rinsing the roots in a $CaCl_2$ solution, and then allowing the roots to stand in fresh 0.5 mM $CaCl_2$ for different periods (Graham et al. 1981; Schwab et al. 1983). The composition and the quantities of the exudates collected in such experiments are likely to be different to those produced by plants grown in soil, since factors such as mechanical impedance (Barber and Gunn 1974; Schönwitz and Ziegler 1982), drought stress (Reid and Mexal 1977), anaerobiosis (Whipps and Lynch 1986) and microorganisms (Kraffczyk et al. 1984; Schönwitz and Ziegler 1989) have been shown to affect this process. Also, reabsorption of root exudates can play an important role in solution culture. In maize plants grown in sterile cultures where C was allowed to accumulate over a 10-day growth period, reabsorption was estimated to be about 98% compared with 86% when C was removed daily (Jones and Darrah 1993). Since VAM fungi have significant effects on root growth and morphology of maize plants (Kothari et al. 1990), it is likely that they also affect root exudation.

In our work we concentrate on root exudates such as carbohydrates, sugars, amino acids, phenolics and organic acids. A new technique using agar sheets is described to recover root exudates from soil-grown plants with minimum disturbance to the root system. This paper reports the effects of VAM fungi and other soil microorganisms on growth, mineral acquisition and root exudation of maize plants grown under nonlimiting environmental and nutritional conditions.

Materials and methods

Plant cultivation

Plants were grown in three-compartment pots (compartment size 35 × 25 × 3 cm) and designed to permit spatial separation of plant roots (central compartment) and zones where mycorrhizal hyphae can proliferate in the soil (outer compartments) (Kothari et al. 1990). A 30-μm nylon net, which allows hyphae to pass through but not roots, was used to separate these compartments. Roots grew in firm contact with the nylon net and developed a dense surface area. The nylon net can be cut out and easily removed to allow collection of root exudates at the interface.

Air-dry calcareous soil (Luvisol) was sieved (2 mm) and moistened to 10% of field capacity with a full-strength, complete nutrient solution (for soil texture and fertilisation see Kothari et al. 1990). Soil was sieved again and sterilised with gamma irradiation (25 kj kg^{-1}) to eliminate soil microorganisms. Gamma irradiation was chosen to avoid Mn toxicity that has been observed when the soil is autoclaved.

The treatments were sterilised soil, inoculated with either rhizosphere microorganisms together with *Glomus mosseae* (Nicol. and Gerd.) Gerdemann and Trappe (MO + VAM), or rhizosphere microorganisms other than VAM (MO − VAM), and an uninoculated control. The inoculum was mixed uniformly with the soil of the central compartment where maize plants were grown. For MO + VAM, each pot received 100 g inoculum containing about 3000 infective propagules and other microorganisms obtained from VAM maize roots (plants were cultivated previously for 3 months, inoculated with the VAM fungus and kept under nonsterile conditions). The MO − VAM treatment received an equivalent amount of microorganisms from the non-VAM maize roots (plants were grown as before but inoculated with soil suspension without VAM). The microorganisms of the inoculated treatments were different in number and composition (see Posta et al. 1994), which might further affect root exudation in various ways. The control received 100 g sterilized inoculum mixed with the soil before sterilisation. In other pots filled with sterile soil and maintained without plants for 3 weeks to serve as a control for soil-borne low molecular weight compounds, no organic acids, amino acids, sugars and phenolics could be detected.

Maize (*Zea mays* L. cv. Alize) seeds were surface-sterilised with 30% H_2O_2 for 10 min and washed several times with sterile distilled water. Seeds were germinated for 2–3 days on filter paper soaked with a $CaSO_4$ solution. Four germinated seedlings were planted in the central compartment of each pot and thinned to two plants. All pots were maintained in a growth chamber (28/25° C and 16/8 h day/night regime). Water was supplied daily using string wicks, and after 3 weeks was added manually to the top of each pot to maintain soil moisture content close to field capacity (ca. 20% w/w).

Plant harvest, analysis and collection of root exudates

Plants were harvested 3 or 6 weeks after emergence. Each pot was opened from one side, the soil in the outer (hyphal) compartment was removed carefully and the nylon net cut out to expose the interface between the root and hyphae compartments. Agar sheets of 1% agar with or without antibiotics (30 mg/l Cefotaxime + 20 mg/l Trimethoprim) were placed on the exposed root-soil interface of the opened side (about 250 ml/525 cm^2 area on each side of the pot). The pots were closed and covered with a black plastic sheet to reduce evaporation and light effects. Root exudates were collected at the beginning of the light period. The agar sheets were removed after 3 or 6 h and immediately frozen (−20° C) for further analysis.

Plants were harvested and root sections were randomly collected and washed to evaluate the percentage of roots infected with VAM fungi (Phillips and Haymann 1970). Dry weights of roots and shoots were determined after drying at 70° C for 48 h. Ground plant samples of roots and shoots were dry ashed at 550° C and digested with nitric acid. The concentration of P was determined colorimetrically (Gericke and Kurmies 1952), micronutrient concentrations by atomic absorption spectrophotometry, and the N concentration in the shoot using a Heraeus Macro-N analyser.

In another experiment, plants were harvested after 7 weeks of growth. Root exudates were collected on agar sheets supplemented with polyethylene glycol (20 mM PEG 4000) as osmoticum with or without antibiotics; these agar sheets were incubated for 6 h as described above. PEG at this conncentration prevented water loss from the agar sheet to the drying soil for at least 6 h.

Extraction of root exudates

Agar sheets were thawed at 45° C, blended in distilled water using an Ultra Turrax and centrifuged (16000 rpm, 4° C, 30 min). The supernatant was filtered through a Blue Ribbon filter paper, evaporated to dryness at 45° C and redissolved in 15 ml distilled water. The concentrated extract was passed through a micropore filter (0.45 μm), and 10 μl of chloroform was added to each sample to limit microbial growth. Samples were stored at −20° C until analysis.

Analysis of root exudates

Exudates were separated into neutral, basic and acidic fractions using a combination of cation and anion exchange resins using a method similar to that of Schwab et al. (1983).

The concentrated fractions were analysed for carbohydrate content using the anthrone method (Ebell 1969), reducing sugars were determined by *p*-hydroxybenzoic acid hydrazide (Blakeney and Mutton 1980), and amino acids were quantified using the ninhydrin reaction (Pin Lee and Takahashi 1966) and identified using an amino acid analyser. Phenolic content was determined on crude extracts by hydrolysed with 2 N HCl for 30 min at 100° C and extracting 3 times with ethyl ether. The ether was evaporated at 40° C and the phenolic acids resuspended in HPLC-grade methanol. Total phenolics were determined using Folin-Denis reagent (Swain and Hillis 1959). Phenolic acids were quantified by HPLC (Sykam, S 1100) using a Lichrospher column (Lichrospher-100, RP 18, 5 μ, 250×4 mm) with a linear gradient of 5% acetic acid and methanol (flow rate 1 ml/min) run for 50 min, 0–100% methanol and UV detection.

Organic acids were determined from the acidic fraction after derivatisation to fluoro-organic ethyl esters (Epstein and Cohen 1981) and GC analysis (Netting and Milborrow 1988).

Results

Mycorrhizal root infections in the MO+VAM plants were 24% and 71% after 3 and 6 weeks of growth, respectively (Table 1). No VAM fungal infection was observed in the other two treatments. Shoot and root dry weights did not vary significantly between plants after 3 weeks. After 6 weeks of growth, shoot dry weight and shoot:root ratios in VAM plants were significantly greater than in the other two treatments (Table 1).

Concentrations of Zn, Cu and Mn in the shoots, and Cu and Mn in the roots were only slightly affected in 3-week-old plants (Table 2), but after 6 weeks there were significant effects attributable to the high level of VAM infection (Table 2). The Zn and Cu concentrations were significantly higher in the roots and shoots of VAM plants than in non-VAM plants after 6 weeks. Nonmycorrhizal and control plants had higher Mn concentrations in roots and shoots than the VAM plants at week 6. Shoot nitrogen concentrations were not affected by the different treatments (Table 3), but P concentrations were significantly higher, particularly in root and shoots of the VAM plants after 6 weeks (Table 3).

It was difficult to estimate the exact weight and length of roots exposed to the agar sheets, and total exudates were calculated per net. About 25% of the total root length in each pot was distributed close to the net on each side, and thus about 50% were not exposed to the agar sheets. Total carbohydrates collected from the 3-week-old plants during 3 h of incubation did not vary

Table 1 Shoot and root dry weights, their ratios and percent of root infection with vesicular-arbuscular mycorrhiza (VAM). Maize plants were grown in soil inoculated with rhizosphere microorganisms together with VAM fungus *(MO+VAM)* and rhizosphere microorganisms only *(MO−VAM)* and in a sterile soil *(Control)*. Values within a single column followed by different letters are significantly different (Duncan test, $P \leq 0.05$). Mean values ±SD of four replicates (two plants per pot) are given

Treatment	Shoot dry weight (g/pot)	Root dry weight (g/pot)	Shoot:root dry weight ratio	Percent root infection
3-week harvest				
MO+VAM	5.6±1.4 c	0.69±0.17b	8.2±0.6 a	24.2±7.1 b
MO−VAM	5.5±0.7 c	0.74±0.04 b	7.3±0.8 ab	0.0
Control	5.5±1.3 c	0.87±0.15 b	6.3±0.5 bc	0.0
6-week harvest				
MO+VAM	43.0±6.2 a	5.9 ±1.4 a	7.6±1.1 ab	70.9±2.4 a
MO−VAM	36.7±4.3 b	6.9 ±2.4 a	5.8±1.7 c	0.0
Control	36.8±5.4 b	5.3 ±1.0 a	7.0±0.4 abc	0.0

Table 2 Micronutrient concentrations in shoots and roots of maize plants grown in different soil treatments for 3 and 6 weeks. Values within a single column followed by different letters are significantly different (Duncan test, $P \leq 0.05$). Mean values ±SD of four replicates are given

Treatment	Micronutrient concentration (μg g^{-1} dry wt.)					
	Zn		Cu		Mn	
	Shoot	Root	Shoot	Root	Shoot	Root
3-week harvest						
MO+VA	154± 7 a	389±56 ab	20±1 a	30±2 b	120±16 b	394± 40 b
MO−VA	121±12 b	437±52 a	16±4 b	26±5 bc	93±14 c	363± 69 b
Control	116±10 b	435±34 a	17±5 ab	44±5 a	130±26 b	554±122 a
6-week harvest						
MO+VA	161±15 a	443±22 a	17±3 ab	27±4 bc	132± 7 b	159± 12 d
MO−VA	125±23 b	297±31 c	10±1 c	15±2 d	160±16 a	210± 15 c
Control	133±22 b	342±13 bc	11±1 c	23±1 c	178±13 a	322± 60 b

Table 3 Nitrogen and phosphorus concentrations in shoots and roots of maize plants grown in three soils subjected to different treatments. Values within a single column followed by different letters are significantly different (Duncan test, $P \leq 0.05$). Mean values ± SD of four replicates are given

Treatment	Nutrient concentration (mg g^{-1} dry wt.)		
	N	P	
	Shoot	Shoot	Root
3-week harvest			
MO+VAM	41±1 a	4.5±0.4 a	2.3±0.1 a
MO−VAM	42±1 a	4.5±0.4 a	2.0±0.1 b
Control	41±3 a	4.4±0.1 a	2.0±0.1 b
6-week harvest			
MO+VAM	24±2 b	3.3±0.2 b	1.7±0.2 c
MO−VAM	23±3 b	2.0±0.3 c	1.0±0.1 e
Control	23±2 b	1.9±0.3 c	1.2±0.1 d

Table 4 Total carbohydrates and reducing sugars (glucose equivalents) collected on agar sheets supplemented with antibiotics from maize roots as affected by three different treatments. Exudates were collected after 3 h of incubation from plants grown for 3 and 6 weeks. Values within a single column followed by different letters are significantly different (Duncan test, $P \leq 0.05$). Mean values ± SD of four replicates are given

Treatment	Carbohydrates (mg/net)	Reducing sugars (mg/net)
3-week harvest		
MO+VAM	9.62±0.43 d	4.30±0.38 b
MO-VAM	12.71±2.36 cd	5.70±0.22 a
Control	11.20±1.07 d	4.83±0.93 ab
6-week harvest		
MO+VAM	20.34±2.96 a	4.98±0.65 ab
MO−VAM	17.79±3.41 ab	4.63±0.67 b
Control	15.64±2.95 bc	5.02±0.87 ab

significantly between treatments (Table 4). For 6-week-old plants, total carbohydrates were lower in the control plants than the VAM plants. Total carbohydrates collected after 6 h of incubation were significantly (two-fold) lower than those collected after 3 h, as transpiration by the shoots induced a mass flow of water and organic solutes from the agar sheets into the drying soil (data not shown). This phenomenon was also observed when reducing sugars, amino acids, phenolics and organic acids were determined after 6 h incubation. The amounts of reducing sugars were not significantly affected by the different treatments after 6 weeks growth (Table 4).

The amounts of amino acids recovered from both harvests (3 and 6 weeks) were low relative to the other organic compounds (Table 5). Mycorrhizal plants grown for 3 weeks released lower amounts of amino acids during 3 h of incubation than non-VAM plants, but this was not true for plants grown for 6 weeks (Table 5). Glutamate and aspartate dominated in the root exudates, and glycine and alanine were detected in small amounts in exudates from all plants (data not shown).

Total phenolics in root exudates were not significantly different between treatments at both harvests, but more phenolics were recovered from 6-week-old than 3-week-old plants (Table 5). Four phenolic compounds detected in hydrolysed root exudates at both harvests were identified as protocatechuic, *p*-hydroxybenzoic, vanillic and syringic acids (Table 6). A peak corresponding to *p*-coumaric acid had a retention time similar to the standard, but a different λmax. These phenolics were detected in the exudates of all plants at both harvests and no treatment effects were observed (data not shown).

Organic acids determined in the exudates of plants at both harvests identified as oxalic, succinic, fumaric, malic, tartaric and citric acids (Table 7). Oxalic and succinic acids predominated, whereas only small amounts of citric acid were released from the roots. Three-week-old VAM roots exuded lower amounts of organic acids than non-VAM roots, but with 6-week-old plants, the exudate did not differ significantly between treatments.

Table 5 Total amino acids (glycine equivalents) and phenolics (*p*-hydroxybenzoic acid equivalents) collected on agar sheets supplemented with antibiotics from maize roots as affected by three different treatments. Exudates were recovered from 3- and 6-week-old plants after 3 h of incubation. Values within a single column followed by different letters are significantly different (Duncan test, $P \leq 0.05$). Mean values ± SD of four replicates are given

Treatment	Amino acids (μg/net)	Phenolics (μg/net)
3-week harvest		
MO+VAM	138±17 c	444± 60 b
MO−VAM	196±16 a	441± 57 b
Control	165±12 b	310±107 b
6-week harvest		
MO+VAM	175±13 ab	1182±141 a
MO−VAM	166±24 b	1200±268 a
Control	172±16 ab	1360± 72 a

Table 6 Identification of phenolics of root exudates of 3- and 6-week-old maize seedlings grown under three different soil treatments as compared with known standards. Mean values ± SD of three samples are given

Phenolic	Retention time		λ max	
	Standard	Exudate	Standard	Exudate
Protocatechuic acid	477± 7	484± 6	275	280–285
p-Hydroxybenzoic acid	625± 4	590±28	255	260
Vanillic acid	793± 3	797±12	260, 290	260, 280
Syringic acid	903±14	947±21	275	280
p-Coumaric acid	1097± 4	1117±25	315	255, 280

The influence of the rhizosphere microorganisms on the recovery of root exudates in agar sheets is shown in

Table 7 Organic acids recovered from agar sheets supplemented with antibiotics from maize roots as affected by three different treatments. Exudates were collected after 3 h of incubation from plants grown for 3 and 6 weeks. Values within the last column followed by different letters are significantly different (Duncan test, $P \leq 0.05$). Mean values of four replicates are given

Treatment	Acid (μg/net)						
	Oxalic	Succinic	Fumaric	Malic	Tartaric	Citric	Total
3-week harvest							
MO + VAM	175	163	40	39	56	4	476 b
MO − VAM	269	161	88	68	46	23	654 a
Control	229	203	77	100	28	0	636 a
6-week harvest							
MO + VAM	146	132	27	140	19	0	463 b
MO − VAM	162	171	30	51	20	0	434 b
Control	101	130	51	78	37	0	397 b

Table 8 Total carbohydrates, amino acids (glycine equivalent) and phenolics (*p*-hydroxybenzoic acid equivalents) recovered from agar sheets containing PEG with or without antibiotics as affected by two treatments. Exudates were collected after 6 h of incubation from plants grown for 7 weeks. Values within a single column followed by different letters are significantly different (Duncan test, $P \leq 0.05$). Mean values ± SD of three replicates are presented

Treatment	Antibiotics	Carbohydrates (μg/net)	Amino acids (μg/net)	Phenolics (μg/net)
MO + VAM	+	26900 ± 4780 a	230 ± 2 a	1814 ± 32 a
	−	7100 ± 2820 c	68 ± 45 d	675 ± 298 b
MO − VAM	+	20200 ± 1630 b	167 ± 12 b	2051 ± 144 a
	−	5300 ± 2010 b	115 ± 28 c	304 ± 46 b

Table 8. Supplementation of the agar with PEG as an osmoticum prevented mass flow of water and solutes from agar into the soil and allowed collection of root exudates for 6 h. Carbohydrates, amino acids and phenolics collected from root exudates of 7-week-old plants were several times higher when antibiotics were incorporated in the agar sheets (Table 8). Total carbohydrates and amino acids, but not phenolics, were recovered in significantly higher amounts from VAM than from non-VAM plants.

Discussion

Maize plants responded positively to mycorrhizal infection by increases in shoot dry weight, Zn, Cu and P concentrations, confirming many earlier reports (Pacovsky 1986; Koide 1991; Kothari et al. 1991; Li et al. 1991; Jakobsen et al. 1992). Mn contents in both roots and shoots were significantly lower in VAM than in non-VAM plants harvested after 6 weeks (Table 2), confirming earlier reports on maize (Kothari et al. 1991; Posta et al. 1994), red clover and soybeans (Arines et al. 1989; Pacovsky 1986). It has been proposed that VAM fungi modify root exudation, thereby decreasing Mn acquisition by VAM plants, either by depressing the number of Mn-reducing bacteria (Kothari et al. 1991; Posta et al. 1994), or by increasing the number of Mn-oxidizing bacteria in the rhizosphere (Arines et al. 1992).

Total carbohydrates, reducing sugars, amino acids, phenolics and organic acids recovered from 6-week-old plants did not differ significantly between treatments (Tables 4, 5, 7). In our experiments, root exudates were collected at the beginning of the photoperiod after watering the soil to field capacity. The quantities of exudates collected after 6 h of incubation from 3- and 6-week-old plants were significantly lower than after 3 h (data not shown). This suggests that with this technique the incubation period for collection of root exudates should not exceed 3 h to avoid a net flux of water and solutes from the agar sheets to the soil and roots. It was possible to collect root exudates for up to 6 h without any water loss using PEG as osmoticum. In this experiment, more root exudate was collected when antibiotics were added to the agar sheets (Table 8). However, PEG was not used in further experiments because centrifugation and resin exchange was insufficient to remove this compound completely from solution. PEG also interfered with some peaks detected in root exudates by GC and HPLC.

Phenolics recovered from hydrolysed root exudates were identified as protocatechuic, *p*-hydroxybenzoic, vanillic and syringic acid by retention times and maximum absorption (Table 6). Anderson and Pedersen (1983) using retention times and absorbance ratios (λ 254/280) identified phenolic acids in extracts from a cell suspension culture of maize as gallic, *p*-hydroxybenzoic, vanillic and syringic acids.

Our results demonstrate that under optimal growth conditions, VAM and non-VAM maize plants release similar amounts of exudates. In citrus seedlings inoculated with VAM fungi, exudation of reducing sugars and amino acids was lower in VAM than in non-VAM plants, and generally decreased as soil and root P increased (Dixon et al. 1988). Higher rates of root exudation (amino acids, sugars and phenolics) may also be the result of Zn deficiency (Cakmak and Marschner 1988). A particular role for VAM in Zn acquisition is well documented and was also confirmed in our study (Table 2). In our experiments, however, the maize plants were fertilised with Zn and thus Zn in the shoot dry matter was also high in the non-VAM plants.

Mean exudation rates calculated for maize plants (per h per g root dry wt.) grown for 6 weeks, assuming that 25% of total root length was distributed close to the nylon net on each side and that only one side of these roots (i.e. 12.5% of total root surface) was exposed to the agar sheets, were 2.25 mg reducing sugars, 534 μg phenolic acids, 209 μg organic acids and 79 μg amino acids for VAM plants. Exudation rates of non-VAM plants were 1.79 mg reducing sugars, 464 μg phenolics, 168 μg organic acids and 64 μg amino acids. In our work, non-VAM plants released higher amounts of sugars (1.5-fold), phenolics (18-fold) and amino acids (2-fold) than maize plants grown by Jones and Darrah (1993) for 10 days in sterile static cultures where released C was removed daily and 86% of the exuded C was reabsorbed by the plants. In our experiment, root exudates were collected from soil-grown plants on agar sheets supplemented with antibiotics similar to sterile growth culture. The much higher release of phenolics in our studies is presumably caused by the soil as substrate, which induces higher release of exudates, in particular phenolics (D'Arcy 1982).

Some exudates on the agar sheets may be reabsorbed by the plants and it is, therefore, important to collect root exudates for short periods only. In another study by Kraffczyk et al. (1984), root exudates of maize plants grown in nutrient solutions consisted of 65% sugars, 33% organic acids and only 2% amino acids; phenolics were not determined. Similar proportions of these organic compounds were present in the maize exudates collected with the agar-sheet technique. In soil-grown plants, some of the root exudates were presumably consumed by the rhizoplane microorganisms, and some organic compounds [6–10% of the net fixed carbon as reported by Koch and Johnson (1984)] are diverted to maintain the VAM fungus. Soil microorganisms normally utilise part of the root exudate during their release and, therefore, underestimation of these compounds may occur. Helal and Sauerbeck (1989) estimated that approximately 19% of the total photosynthetic carbon of various plant species was released into the rhizosphere as organic material. Some of this (15%) was transformed by rhizosphere microorganisms into CO_2, while only a small fraction (4%) remained in the soil, mainly as microbial biomass (2.5%). Supplementing the agar sheets with antibiotics was therefore very important since it limited the growth of rhizosphere microorganisms in the collection medium, and increased recovery of solutes in exudates (Table 8).

The results of our study show that mycorrhizal infection did not significantly affect either the amounts or the composition of root exudates of maize plants grown at optimal P level. *G. mosseae* and other soil microorganisms did not play an important role in the amounts of root exudates collected from soil-grown maize plants using agar sheets supplemented with antibiotics. This technique is a promising approach for recovering root exudates of soil-grown plants provided that efforts are made to relate exudation rates to either root length or root dry weight.

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