Loss of exudates from the roots of perennial ryegrass inoculated with a range of micro-organisms

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Abstract

To determine the effect of microbial metabolites on the release of root exudates from perennial ryegrass, seedlings were pulse labelled with $^{14}$C-CO$_2$ in the presence of a range of soil micro-organisms. Microbial inoculants were spatially separated from roots by Millipore membranes so that root infection did not occur. Using this technique, only microbial metabolites affected root exudation. The effect of microbial metabolites on carbon assimilation and distribution and root exudation was determined for 15 microbial species. Assimilation of a pulse label varied by over 3.5 fold, dependent on inoculant. Distribution of the label between roots and shoots also varied with inoculant, but the carbon pool that was most sensitive to inoculation was root exudation. In the absence of a microbial inoculant only 1% of assimilated label was exuded. Inoculation of the microcosms always caused an increase in exudation but the percentage exuded varied greatly, within the range of 3–34%.

Introduction

Our understanding of rhizosphere dynamics is still no more than elementary (Bowen and Rovira, 1992). To try and understand plant-soil interactions, simple model systems must be developed in which environmental parameters can be systematically altered and controlled. In this way, the complexity of such model systems may be increased to incorporate more environmental variables to simulate rhizosphere processes.

Microbial activity in the rhizosphere is stimulated by root exudation as exudates are readily assimilated by the soil biomass (Lynch and Whippes, 1990). In turn, the soil microflora tend to affect root function, be they infective or non-infective (Bowen and Rovira, 1992). Such complex root-microbe interactions are impossible to study in soil systems.

Meharg and Killham (1991) developed microcosms in which the effect of microbial metabolites on root exudation could be investigated. The microcosms allowed for the manipulation of experimental variables such as: microbial/plant growth substrate (e.g. nutrient status, osmotic potential, microbial inhibitors); plant stage of development; inoculant density; inoculant growth phase; and spatial relationship between roots and inoculants. Root exudation was determined by $^{14}$C-CO$_2$ pulse labelling of seedling shoots and recovering exuded label from the growth substrate.

The technique, even in the presence of microbial inoculants (which will mineralize a proportion of the exudate) enables a minimum recovery of 90% of root exudates (Meharg and Killham, 1991). The aim of this study was to determine, using the microcosm approach developed by Meharg and Killham (1991), the effect of microbial metabolites from a variety of micro-organisms (in vivo) on plant growth (as a function of ability to assimilate a pulse label) and on root exudation (as a percentage of total assimilated carbon) in perennial ryegrass seedlings.
Materials and methods

The experimental procedure is essentially the same as Meharg and Killham (1991). Briefly, the microcosms were constructed from Perspex sheets, with one half of the microcosm containing a well into which molten agar was poured (composition, quarter strength Hoaglands solution with 1% agar). Once the agar was set, a Millipore membrane, with a microbial inoculum filtered onto its upper surface, was placed on top of the agar. Inoculum was prepared in batch culture as in Meharg and Killham (1991). On top of this membrane a sterile Millipore filter was positioned. The seedling root system was then placed in the centre of the assembly, with the shoot threaded through a groove in the Perspex chamber. Another membrane was placed on top of the root system, and on top of this the other half of the Perspex microcosm was added to complete the assembly. The microcosms were autoclaved before assembly, and all procedures were carried out aseptically. Perennial ryegrass seeds were surface sterilized by successive shaking for 15, 30 and 90 minutes with 2% sodium hypochlorite, and then washing twice with 50 mL sterile, distilled water. The sterilized seeds were germinated for 9 days on yeast nutrient agar (5 g peptone; 5 g glucose; 0.5 g yeast extract; 15 g agar powder; 1 L distilled water) to ensure that the seedlings remained sterile. Sterile seedlings were then transferred to the microcosms. Fungal and bacterial inocula were isolated from soil and cultured on Czapek-Dox and Bunt and Rovira's agar slopes, respectively.

Assembled microcosms were placed in 250 mm vacuum desiccators. The seal on the desiccator was smeared with high vacuum grease, and the hole into which the stop cock was normally placed was plugged with non-absorbent cotton wool. After 5 days growth in the desiccator, under a fluorescent light bank (intensity 86 μM m$^{-2}$ s$^{-1}$), the desiccator was connected, by plastic tubing, to a Buchner flask with the neck of the flask fitted with a Suba seal (size 70). Into the flask, 10 mL phosphoric acid (1 M) and 4 mL [$^{14}$C]-CO$_2$ (1.85 MBq mL$^{-1}$) were injected to give an atmospheric CO$_2$ concentration 140 ppm above ambient. The plants were grown in the presence of the label for 48 hours before they were harvested. The [$^{14}$C] content of the microcosms was determined as in Meharg and Killham (1991). Each microbial inoculum was replicated 4 times. Data were analyzed using a one-way analysis of variance after arcsine transformation of percentage data.

Results and discussion

The results of this experiment show that microbial metabolites have a considerable effect on carbon assimilation, distribution and root exudation in perennial ryegrass seedlings (Table 1). The proportion of label exuded from the plant root varied between 1% for uninoculated microcosms to 34% for microcosms inoculated with *Penicillium notatum*. The term exudate in this study refers to soluble carbon lost from living cells and soluble lysates from dead cells. It should be noted that the percentage exudate recovered from the microcosms must be considered as a minimum figure, as a proportion of the exudate will be mineralized by the inocula. From the results of Meharg and Killham (1991), the percentage of exudate mineralized within the microcosm was expected to be less than 10%. All microbial inocula, except *Arthrobacter* sp., *P. notatum* (strain A) and *Aspergillus niger*, caused a significant increase (at the 5% level) in root exudation compared to the uninoculated microcosms. Carbon loss from plant roots in most inoculated microcosms represented a considerable percentage of the carbon budget of the plants.

Distribution of the label within the roots was not greatly dependent on inoculation, with the percentage of the label remaining within the root varying between 18–34%, with this figure being 27% for the uninoculated microcosms. Label remaining in the root was not significantly different (at the 5% level) in any of the inoculated microcosms compared to the uninoculated microcosm. The percentage of label remaining within the plant shoots showed a general trend of decreasing with increasing loss of exudates from the plant roots (Table 1). The percentage of label remaining within the root varied between 39–75%, with the corresponding figure for the uninoculated microcosm being 71%. A number of inocula (*Penicillium notatum* (a), *Fusarium solani*, *Mucor rouxii*) did significantly decrease percentage label remaining in the shoot compared to the uninoculated at the 5% level of significance. Inoculation of the microcosms also affected the assimilation of the label with plant activity varying between 0.13–0.41 MBq with uninoculated plants assimilating 0.32 MBq.

Metabolites produced by plant roots may be detrimental or beneficial to plant growth (Gillespie-Sasse et al., 1991). It is not always necessary for microbes to parasitize to detrimentally affect plant growth (Lynch, 1983). Microbial metabolites are varied in nature and may include toxins such as hydrogen sulphide,