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The co-existence between DSE and AMF symbionts affects plant P pools through P mineralization and solubilization processes

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ABSTRACT

The capacity of dark septate endophytes (DSE; *Phialocephala turiciensis, Acephala applanata, P. glacialis* and *Phaeomollisia piceae*) to solubilize inorganic phosphate (P) and to mineralize the organic form was studied. We analysed the effect of DSE strains on P uptake by *Trifolium repens* in the presence or absence of arbuscular mycorrhizal fungi (AMF). Phosphatases were observed both in the absence of the host plant and the organic resource, showing that the P mineralization process is not induced by the enzyme substrate or the host. DSE were more efficient at mineralizing organic P. Independently of the presence of AMF, DSE increased the pool of P in the soil, with significant differences being found in P levels among the different DSE. In contrast, plant P uptake was increased by AMF. The P content of plants increased with the co-inoculation of AMF and *P. turiciensis* or *P. piceae*. We hypothesize a close relationship between DSE and AMF in relation to P availability and uptake in plants. Whereas DSE increase the pool of P in the rhizosphere, AMF are responsible for P transfer to the host, with co-colonization of plants by DSE and AMF showing a synergistic outcome.

Introduction

Vascular plants host a great variety of micro-organisms, with the colonization of roots by fungal endophytes and arbuscular mycorrhizal fungi (AMF) being a common feature of the plant kingdom. Whilst AMF are important components of the rhizospheric microbial community, another type of root-dwelling fungal symbiont, the so-called dark septate endophytes (DSE), have been identified as one of the most abundant and widespread groups of plant root colonists. DSE

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represent a form taxon composed of many different fungal species with regularly septate and melanised mycelia (Jumpponen and Trappe, 1998; Mandyam and Jumpponen, 2005). Among the DSE, species belonging to the genus *Phialocephala* are abundant, showing a world-wide distribution, and include the well characterized *Phialocephala fortinii* – *Acephala applanata* species complex (Grünig et al., 2008). DSE have been found to increase or reduce the growth of their host plants, arousing controversy regarding their symbiotic roles, and have also been shown to affect plant nutrition and both phosphate (P) and nitrogen (N) assimilation (Jumpponen and Trappe, 1998; Jumpponen, 2001; Barrow and Osuna, 2002; Newsham, 2011). The dual colonisation of plant roots by DSE and AMF has been recognised since the work of Peyronel (1924). However, reports showing tripartite interactions among DSE, AMF and plants are scarce, with previous authors emphasizing the need for integrating research on mycorrhizal and fungal root endophytes (Scervino et al., 2009; Porras-Alfaro and Bayman, 2011; Reininger and Sieber, 2012). One such study shows that AMF can be influenced by the metabolism of *Dreschlera* sp., a DSE, with exudates from the latter being found to modulate the mycorrhizal state in the two stages of the life cycle of the symbionts (Scervino et al., 2009). During the pre-symbiotic stage, the DSE modulates AMF growth, promoting hyphal branching and affecting the development of the host plant (Scervino et al., 2009). Later on, once the symbiosis with a host root has been successfully established (the symbiotic phase), the effect of microbial exudates in the rhizosphere affects the host plant indirectly, modifying the mycorrhizal status of the plant (Sampedro et al., 2004; Lioussanne et al., 2009).

P is an essential and limiting nutrient for plant growth. It is found in inorganic and organic forms, some of which are unavailable to plants. Only a few soil fungi are capable of solubilizing inorganic P sources, a process commonly achieved through the acidification of the environment by proton pumping or the release of organic acids. Alternatively, P release from organic sources can be achieved by the action of nonspecific phosphomonoesterase enzymes, which are usually termed phosphatases (Seshadri et al., 2004). Once free in the soil solution, P is taken up directly by plants and microbial symbionts, such as DSE and AMF. The AMF directly allocate P to the plant in the arbuscules. The P released in the rhizosphere directly affects the plant symbiosis and might be involved in DSE-AMF interactions. To date, there have only been a few studies reporting how interactions between these micro-organisms change phosphatase enzyme activities. These activities may affect the solubilization and mineralization of P by DSE and its availability to plants, either directly or mediated by AMF (Barrow and Osuna, 2002; Osuna and Barrow, 2009). In the present work, we describe the solubilization and mineralization of inorganic and organic P sources by four DSE (*P. turiciensis, A. applanata, P. glacialis* and *Phaeomollisia piceae*). Additionally, we show a positive effect of two of these DSE strains on P uptake by clover (*Trifolium repens*). The potentially crucial influence of DSE-AMF interactions on phosphatase activity in the rhizosphere is discussed.

Materials and methods

Fungal strains

Stock cultures of the four DSE were maintained on slants of malt extract agar medium at 4 °C in darkness and were used as inoculum sources. The development of typical DSE structures with an absence of pathogenic effects have been confirmed for the four DSE by resynthesis assays with *Lolium multiflorum* and *Lycopersicum solanum* (Schadt et al., 2001). An AMF, *Gigaspora rosea* (BEG No. 9), was also used in plant interaction assays. *G. rosea* was maintained in open pot systems using a sterile mixture of soil: perlite:vermiculite (1:1:1) as the growth substratum and *Sorghum vulgare* as the host under controlled conditions in a glasshouse (Scervino et al., 2009). Wet sieving was used to isolate spores of *G. rosea* from 5 g of soil (Gerdemann, 1955). The spores were sterilized according to Mosse (1962) and used as pure inoculum in the plant interaction assays.

Measurements of P mineralization and solubilization

P mineralization and solubilization were tested in liquid media according to Scervino et al. (2011). The cultures were maintained under controlled conditions (20 °C in darkness) and P availability was checked as described below (see P measurements). DSE mycelia were macerated with sterile distilled water (1 ml) to obtain a homogeneous suspension. The mycelial suspension (100 μl) from each strain was mixed with National Botanical Research Institute's Phosphate liquid medium (200 ml; Nautiyal, 1999) at pH 6.5 (buffer MES), containing glucose (10 g l^{-1}), $MgSO_4$ (0.12 g l^{-1}), KCl (0.2 g l^{-1}), $MgCl_2$ $6H_2O$ (5 g l^{-1}) and $(NH_4)_2SO_4$ (0.1 g l^{-1}), supplemented with ampicillin (0.1 g l^{-1}) and amended with $Ca_3(PO_4)_2$ (5 g l^{-1}) as the inorganic P source, or sodium phytate (1 g l^{-1}) as the organic P source. There were five replicates of each treatment. After 28 days, when they had reached stationary phase, each culture was filtered through a cellulose membrane (0.45 μm) and the mycelia were oven dried (80 °C) to calculate dry weight. The supernatant was also collected and frozen at −20 °C until use.

In-plant interaction assays under controlled conditions

Clover plants (*T. repens*) inoculated with pieces of agar overgrown by mycelium of DSE from 4 week old in the presence and absence of *G. rosea* were cultured in pots (100 ml capacity) with a sterile mixture of soil:perlite (2:1 v:v) and maintained in a culture chamber with an incandescent cold light (Sylvania, 400 E m^{-2} s^{-1} of 400–700 nm), with 16/8 hr of light/darkness at 25 °C and 50 % relative humidity. The pieces of agar containing DSE and the AMF spores, according to treatments, were placed 3 cm below the clover plants to allow roots contact with fungal mycelia.

Total C in the growing medium was 12.08 g kg^{-1}, total N was 1.10 g kg^{-1}, total P was 34.20 mg kg^{-1}. The cationic exchange capacity of the medium was 13.4 cmol kg^{-1} and its pH value was 6.5. Plants were grown for 60 d before being harvested, when roots and shoots were separated. A fraction of the roots was retained to measure the frequencies of the

symbionts using the statistical program Mycocalc (http://www2.dijon.inra.fr/mychintec/Mycocalc-prg/download.html) according to Newman (1966) and Trouvelot et al. (1986).

P measurements

Soluble P levels in liquid cultures of DSE and extractable solutions from plant material and growth substrates were estimated according to Scervino et al. (2010) using Spectroquant Phosphate-Test (Art.-Nr. 1.14842 Merck). Extractable solutions were obtained according to Sadzawka et al. (2007) for plant material and Self-Davis et al. (2000) for soil-perlite medium. The soil water-soluble P was estimated using the Water Extractable Phosphate method (WEP) described by Kovar and Pierzynski (2009).

Extracellular phosphatase activity

The activities of acid (at pH 5.5) and alkaline (at pH 9.0) phosphatases were measured using p-nitrophenylphosphate as the substrate in aliquots from supernatants of 28-d-old liquid cultures (Pawar and Thaker, 2009) and rhizosphere soil-perlite medium from plant interaction assays (Gómez-Guiñan, 2004). An enzymatic unit (EU) was defined as the amount of enzyme produced per gram of dry mycelia that hydrolysed 1 μmol p-nitrophenylphosphate per minute in either 1 ml of supernatant or 1 g of soil-perlite medium. P release per EU was expressed as $\mu g\ P\ EU^{-1}$.

Statistical analysis

The data were analysed by one and two way ANOVAs using Statistica 7.0 software. After normality assumptions were tested, differences identified by ANOVA were compared with Tukey's HSD test.

Results

P transformation and phosphatase activities in cultures

P release and enzymatic activities of solutions were measured without DSE in each experiment. No enzyme activities or traces of P were found in the media used in any experiments.

P solubilization and mineralization

Liquid cultures amended with $Ca_3(PO_4)_2$ or sodium phytate and inoculated with each DSE were prepared in order to analyse P release. No transparency of the liquid cultures was apparent with the naked eye. However, a low amount of soluble P in liquid culture was detected by spectrophotometry. Two way ANOVA showed significant ($P \leq 0.05$) interactions between DSE and P form (organic or inorganic) on P release to the culture medium. The data showed that DSE were more efficient at releasing P from phytate (0.11–0.22 $\mu g\ PO_4^{-2} \times \mu l^{-1} \times g^{-1}$) than from the inorganic source (0.007–0.04) (Fig 1). When cultured with calcium phosphate, *P. turiciensis* and *A. applanata* released 0.01 $\mu g\ PO_4^{-2} \times \mu l^{-1} \times g^{-1}$ mycelia, while *P. glacialis* and *P. piceae* released 0.04. In the presence of phytate, *P. piceae* released 0.22,

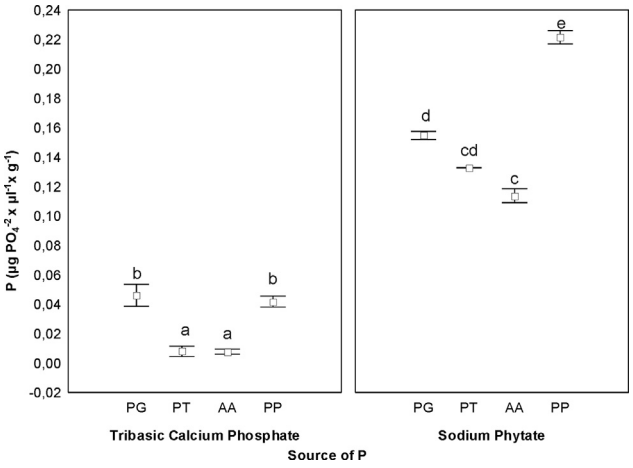

Fig 1 – P release from tribasic calcium phosphate or sodium phytate to the culture media by DSE in *in vitro* assays. *Vertical bars* denote 95 % confidence intervals. Values with the same letter are not significantly different (Tukey's test; $P > 0.05$) between DSE. Abbreviations: PT, *Phialocephala turiciensis*; AA, *Acephala applanata*; PG, *Phialocephala glacialis*; PP, *Phaeomollisia piceae*.

while *P. glacialis*, *P. turiciensis* and *A. applanata* released 0.155, 0.130 and 0.115, respectively (Fig 1).

Extracellular phosphatase activity in culture media

Acid phosphatases

Liquid cultures with or without the organic P source were used to analyse phosphatase activities of DSE. Due to the lack of interaction of the two factors (two way ANOVA: DSE × P form, $P \leq 0.05$), the main effects of DSE and P source on acid phosphatase activity were tested (Fig 2A and B). These analyses showed that the enzymatic activities of *P. turiciensis* and *A. applanata* (20.12 and 23.23 EU) were higher than those of *P. piceae* and *P. glacialis* (4.93 and 6.41 EU), irrespective of the form of P supplied (Fig 2A). On the other hand, when data for all DSE were combined, the enzymatic activity in the presence of the organic P source was higher than the activity with the inorganic source (16.65 and 10.70 EU, respectively; Fig 2B).

Alkaline phosphatases

Alkaline phosphatase activity was detected in the presence and absence of sodium phytate as the enzymatic substrate. Two way ANOVA (factors: DSE and P form) showed that some combinations of DSE and P sources produced an increase in the enzymatic activity of the media. The alkaline phosphatase activities of *P. piceae* and *P. glacialis* were higher in the presence of the organic P source than the inorganic source (ranges 4.386–12.235 EU and 0.256–0.354 EU, respectively). The other DSE maintained the same enzymatic activities, irrespective of the form of P supplied (Fig 3).

P release per EU with sodium phytate as the enzymatic substrate

P release per EU of acid or alkaline phosphatase ($\mu g\ PO_4^{-2} \times EU^{-1}$) released to the culture media was compared for

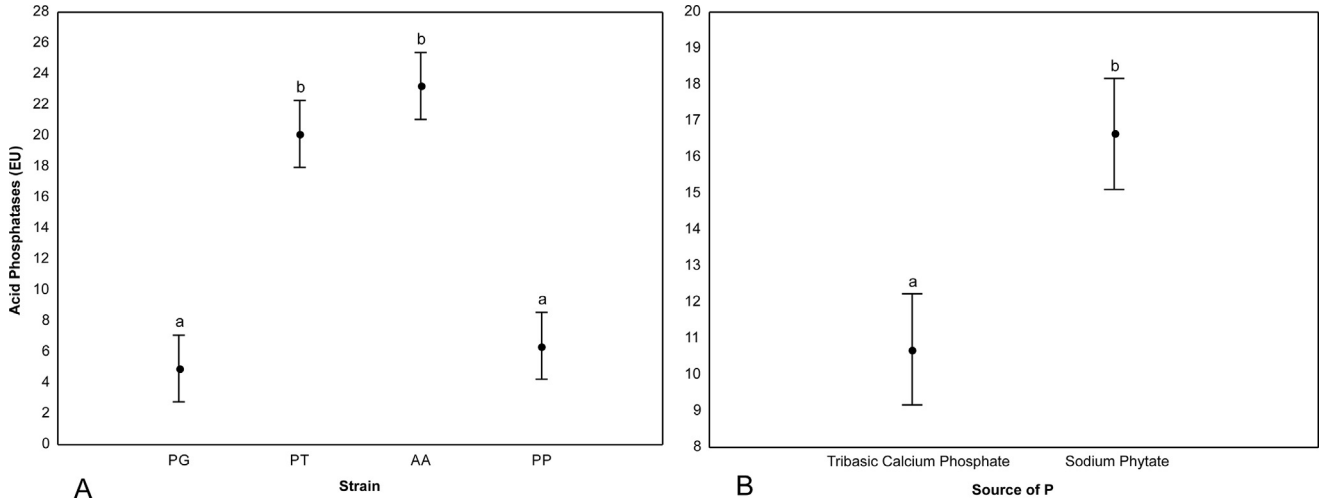

Fig 2 – Principal effects in the acid phosphatase activity experiment independently of P source and DSE treatments. (A) Acid phosphatase activity independent of P sources and (B) acid phosphatase activity independent of DSE. Main effect: $F_{4,93} = 17.4$, $P = 0.029$. Error bars, notation and abbreviations as in Fig 1.

each DSE by one way ANOVA. These analyses indicated that there were significant ($P \leq 0.05$) differences between DSE in their capacities to release P (Table 1). The P concentration released from phytate per EU showed no significant differences for alkaline or acid phosphatases, except for *P. piceae*, for which the release of P per EU of alkaline phosphatases (5.152×10^{-2} μg $PO_4^{-2} \times EU^{-1}$) was higher than for acid phosphatases (1.953×10^{-2} μg $PO_4^{-2} \times EU^{-1}$; Table 1).

In vivo *experiments*

Root length of T. repens *and AMF colonization*

DSE inoculation had no effect on the root length of *T. repens* in the presence or absence of AMF. Similarly, the frequencies of AMF hyphae and arbuscules were not affected by the DSE ($P = 0.612$ and $P = 0.624$, respectively). The frequencies of AMF colonization in roots co-inoculated with AMF and *P. turiciensis* (65.3 %), *P. glacialis* (61.4 %), *A. applanata* (71.3 %) and *P. piceae* (77.3 %) were not significantly different from plants inoculated solely with AMF (68.3 %). Similarly, the frequencies of arbuscules in roots co-inoculated with AMF and *P. turiciensis* (33.4 %), *P. glacialis* (26.0 %), *A. applanata* (32.5 %), *P. piceae* (26.3 %) were also not significantly different from plants inoculated only with AMF (27.5 %).

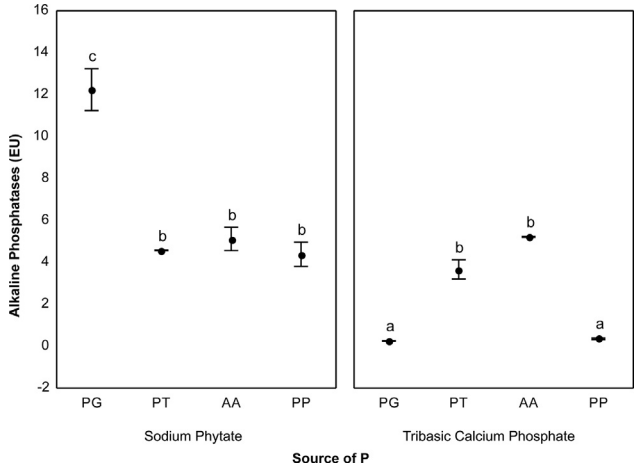

Fig 3 – Alkaline phosphatase activities of DSE in *in vitro* assays with organic (sodium phytate) or inorganic (tribasic calcium phosphate) sources of P. Error bars, notation and abbreviations as in Fig 1.

DSE colonisation in plants inoculated with AMF

Two way ANOVA indicated that the frequency of DSE in roots was not affected by AMF. Irrespective of AMF colonization, the frequencies of *A. applanata* and *P. glacialis* hyphae in roots (Table 2) were higher than those of *P. turiciensis* (Table 2). The presence of AMF also did not affect the frequency of sclerotia, with a decrease in sclerotia only being found for *P. turiciensis* co-inoculated with AMF ($P \leq 0.05$).

P measurement in soils

The effects of DSE and AMF on the release of P from soil solution (WEP) was analysed in *in vivo* experiments. ANOVA showed that P concentration in the soil was unaffected by the presence of AMF ($P \geq 0.05$), but that each of the four DSE

Table 1 – P release per UE of acid or alkaline phosphatase. Values with the same letter are not significantly different ($p \geq 0.05$) as determined by Tukey's test between DSE.

DSE	Phosphatase activity ($P \times UE^{-1}$)	
	Acid	Alkaline
Phialocephala glacialis	1.802×10^{-2ab}	1.276×10^{-2ab}
Phialocephala turiciensis	5.696×10^{-3a}	2.901×10^{-2b}
Acephala applanata	4.963×10^{-3a}	2.260×10^{-2ab}
Phaeomollisia piceae	1.953×10^{-2ab}	5.152×10^{-2c}

Table 2 – Development of DSE mycelia independent of AMF. Main effects: $F_{8,74} = 13.10$ $p = 0.007$. Values with the same letter are not significantly different ($p \geq 0.05$) as determined by Tukey's test between DSE.

DSE	Frecuency of DSE mycelia (I %)
Phialocephala glacialis	45.62[b]
Phialocephala turiciensis	31.6[a]
Acephala applanata	59.31[b]
Phaeomollisia piceae	17.25[ab]

significantly increased total soil WEP compared with controls. *A. applanata*, *P. turiciensis*, *P. piceae* and *P. glacialis* released 19.90 ± 2.9, 20.56 ± 0.59, 17.33 ± 0.56 and 17.32 ± 0.7 mg P × l^{-1} × g^{-1} soil respectively, in comparison with the total WEP in the control (13.20 mg P × l^{-1} × g^{-1}; Fig 4).

Extracellular phosphatase activity in the rhizosphere

Two way ANOVA showed no interactive effect of DSE and AMF on extracellular phosphatase activity ($P \geq 0.05$). The main effects indicated significant differences ($P \leq 0.05$) between the DSE species on the activities of both alkaline and acid phosphatases (Fig 5). Irrespective of AMF inoculation, the presence of alkaline phosphatases in soils inoculated with *P. turiciensis* and *P. glacialis* (147.86 and 152.89 EU) were higher than in soils with *A. applanata* and *P. piceae* (136.76 and 131.59 EU). The alkaline phosphatase activities of the latter two DSE were not significantly different from the control (Fig 5). Only *P. glacialis* elicited an increase in acid phosphatase activity (254.38 EU) relative to controls and other DSE (Fig 5).

P uptake by plants

There was a statistically significant interaction between DSE and AMF on plant P uptake. P content increased when plants

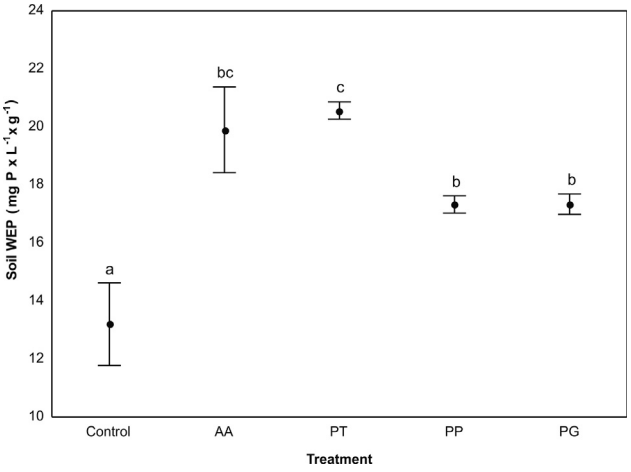

Fig 4 – Principal effect of DSE treatments, independently of AMF inoculation, on P concentration in soil solution (WEP). Main effect: $F_{33,58} = 10.7$, $P = 0.012$. Error bars, notation and abbreviations as in Fig 1.

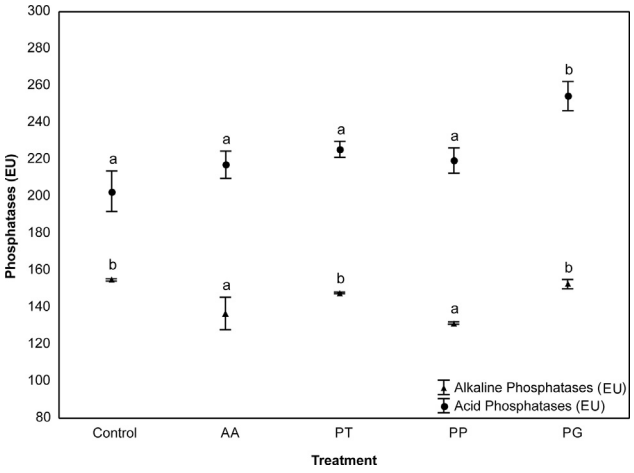

Fig 5 – Extracellular phosphatase activities in the rhizosphere of *Trifolium repens*. Error bars, notation and abbreviations as in Fig 1.

were co-inoculated with AMF and either *P. turiciensis* or *P. piceae* (Fig 6A). In contrast, P concentration was lower in plants co-inoculated with AMF and *P. glacialis* compared with plants inoculated with AMF alone (Fig 6A). P translocation from root to shoot was studied by analysing the ratio of P between the two plant parts. Shoot:root P ratio was higher in plants co-inoculated with *P. piceae* and AMF than in plants inoculated with AMF alone (Fig 6B). This ratio was lower in plants co-inoculated with *P. glacialis* and AMF, compared with plants inoculated with *P. glacialis* alone (Fig 6B). There were no differences in shoot:root P ratios between plants co-inoculated with *A. applanata* or *P. turiciensis* and AMF, compared with plants inoculated with AMF alone (Fig 6B).

Discussion

In the present study, we analysed the *in vitro* abilities of four DSE isolates to solubilize and mineralize inorganic and organic P sources, and examined the effects of DSE on P mineralization and uptake by *T. repens* plants with or without AMF colonisation. To our knowledge, this is the first report showing that DSE are responsible for solubilising P in the rhizosphere. The isolate of *P. piceae* used here came from needles of *Picea abies*, whilst *P. glacialis* had been isolated from roots and needles. The latter species has an intermediate position between *P. piceae* and the *P. fortinii*-*A. applanata* species complex (PAC) (Grünig et al., 2009). PAC have so far only been isolated from belowground tissues of trees or stem bases close to the ground. Based on 18S rDNA and ITS sequences, the PAC species complex is phylogenetically well separated from *P. piceae* and *P. glacialis* (Grünig et al., 2007, 2009; Tellenbach et al., 2011). In contrast, the two PAC strains are closely related, but can nevertheless be classified as belonging to two separate species.

Low levels of inorganic P solubilisation by fungi were found when DSE were cultured in liquid medium. The ability of the DSE isolates to solubilise P in liquid culture, although subtle,

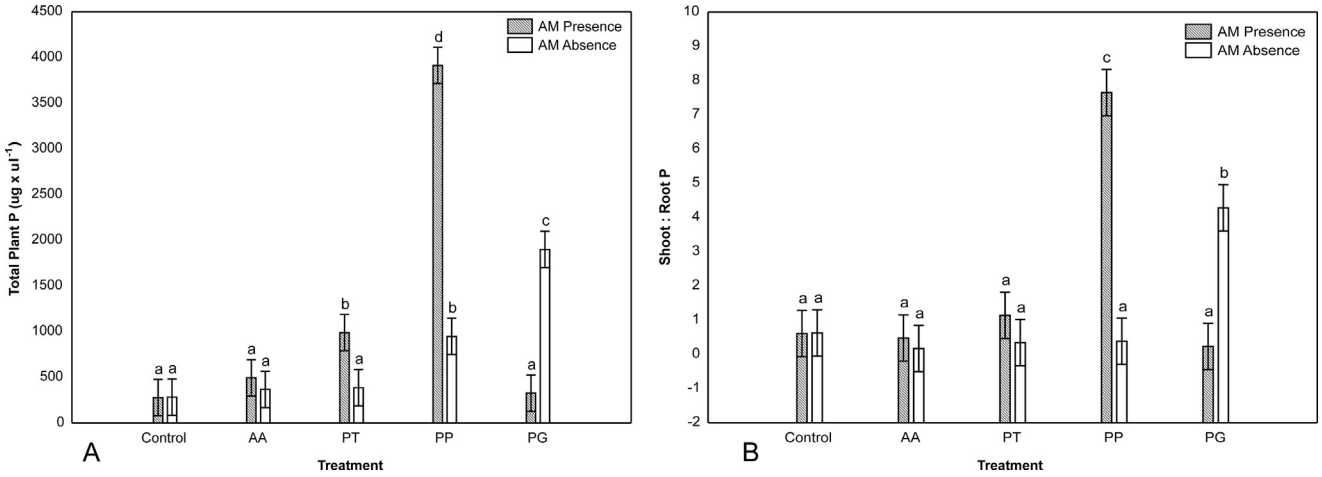

Fig 6 – P in clover plants inoculated with DSE alone (open columns) or co-inoculated with DSE and AMF (filled columns). (A) Total plant P content and (B) shoot:root P ratio. Error bars and notation as in Fig 1. Adjacent filled and open columns are not significantly different (Tukey's test; $P > 0.05$).

indicates the potential of these fungi to promote plant growth, and suggests that they might be used in agriculture to promote productivity, forming part of a plethora of strategies of interaction known as the "additive hypothesis" (Bashan and Levanony, 1990).

We also evaluated the *in vitro* phosphatase activities of DSE isolates grown in the absence of an organic P source to test if these fungi produce phosphatases by an alternative to the substrate-responsive pathway. The DSE isolates tested showed extracellular phosphatase activity (both acid and alkaline) in the absence of the host plant and the organic resource. When grown in the presence of an inorganic P source, we only observed the production of alkaline phosphatases by *A. applanata* or *P. turiciensis*. These results suggest that the induction and production of alkaline phosphatases by DSE differs between taxa. Similarly, Kapri and Tewari (2010) reported that both extracellular alkaline phosphatases and organic acids are produced by *Trichoderma* spp. in response to the presence and absence of tricalcium phosphate, which is in accordance with the participation of constitutive and inorganic P-induced mechanisms related to P solubilisation.

Although our data showed that the four DSE isolates produce phosphatases and that they can mineralise or solubilise P through metabolic or co-metabolic processes respectively, they appear to be more efficient at releasing P from organic sources such as phytate than from insoluble inorganic sources (*c.f.* Newsham, 2011). This might have a link with the interactive role of DSE with plants, since root exudates might be a main source of P, such as phytate, for these fungi and their role in the soil P cycle (Singh and Mukerji, 2006; Uren, 2001).

Analyses of phosphatase activity showed that the four DSE isolates showed similar P release per EU for acid enzyme activity as that reported by Yadav and Tarafdar (2003). However, as previously reported by Aseri et al. (2009), alkaline phosphatase activity differed among the isolates, with *P. glacialis* having a higher activity compared with that of the other three DSE isolates.

Tripartite interactions between DSE, *T. repens* and the AMF *G. rosea* were studied. The frequencies of intraradical AMF mycelia and the abundance of arbuscules did not change in presence of the DSE, suggesting that there were no antagonistic interactions between these micro-organisms in the system studied. Scervino et al. (2009) previously reported that another DSE (*Dreschlera* sp.) altered the mycorrhizal status of the plant, indicating that the relationship between DSE and AMF must be carefully evaluated. Furthermore, the colonisation of *T. repens* plants by *G. rosea* did not affect DSE development. Independently of AMF infection, each DSE species tested in our study produced different levels of infection in the root system. On the other hand, only one fungal combination (*P. turiciensis* and *G. rosea*) decreased the frequency of DSE sclerotia. The intensity of *T. repens* root infection by intraradical DSE mycelium was not modified by the presence of AMF, showing that both symbionts coexist in several environments (Priyadharsini et al., 2012; Khodke, 2013).

Our results showed that, independently of AMF, DSE increased the pool of P in the soil, and that the level of P varied according to the DSE isolate. The AMF inoculated did not produce changes in the P pool in rhizospheric soil containing inorganic or organic forms of P. However, co-inoculation with DSE and AMF modified the plant P content. These findings support the role of extraradical AMF mycelia in P transport from soil to root (Ohtomo and Saito, 2005). Furthermore, some treatments also showed that P transport to the plant was either increased or decreased by the presence of both micro-organisms (*P. turiciensis* + AMF, *P. piceae* + AMF and *P. glacialis* + AMF), which was apparently dependent on the DSE infection level. It is possible that *P. turiciensis* and *P. piceae* might solubilize inorganic P and/or mineralize organic P more efficiently in our experimental glasshouse conditions than the other strains tested.

We observed an increase in alkaline phosphatase activity in the rhizosphere of *T. repens* when plants had been inoculated with DSE isolates. Similar observations were made by

Tarafdar and Rao (1995) in a tripartite system with *Trifolium*, DSE and *G. rosea*. Alkaline phosphatases are not produced by plants, and so such increases are possibly related to higher microbial activity in the rhizosphere (Chonkar and Tarafdar, 1981). The enzymatic activity was higher in *P. glacialis* when this fungus was grown axenically in liquid medium with organic P sources. This result suggests that *P. glacialis* is particularly efficient in producing phosphatase enzymes, both in axenic culture and in tripartite systems, and might therefore participate actively in organic P mineralisation. On the other hand, the activity of acid phosphatases was higher in *P. turiciensis* and *A. applanata* in liquid medium compared with the other DSE isolates tested. Yadav and Tarafdar (2001) showed that rhizospheric acid phosphatases released in the soil diminish their activity by five times when the P level is increased from 10 mg l^{-1} to 50 mg l^{-1}, suggesting that these enzymes are inhibited by the presence of soluble P in the soil due to be a competitive phosphatase inhibitor.

Although an increase in phosphatase activity might be related to enhanced plant P uptake (Dodd et al., 1987; Tarafdar and Jungk, 1987), our data suggest that this increase might be more dependent on the interaction among micro-organisms (DSE and AMF) and plants than on the amount and availability of enzymes in the rhizosphere. This might be related to the efficiency of P uptake by AMF, which overshadows the influence of the enzymes on P mineralisation and P uptake by plants. In this sense, numerous studies have shown that mycorrhizal roots are more efficient at P uptake than non-mycorrhizal roots (Klironomos, 2003; Bush, 2008).

According to Cabello et al. (2005) and Kohler et al. (2007), DSE isolates such as *P. turiciensis* and *P. piceae* in tripartite assays show increases in P uptake, though for *P. glacialis* such increases are not dependent on the presence of AMF. Our results provide evidence supporting an effect on plant nutrition and P uptake efficiency of microbial interactions in the rhizosphere (Bryla and Koide, 1998; Olsen et al., 1999).

Vaz et al. (2012) failed to find synergistic effects between a DSE (*Drechslera* sp.) and an AMF (*Glomus* sp.) in *Sorghum* plants. As both symbionts (DSE and AMF) did not affect the root development of *T. repens*, the reasons for the changes in plant P may be due to increased bioavailability of P in the rhizosphere, or the effect on the development of the DSE mycelium. In addition, our results showed that clover plants inoculated with *P. piceae* and AMF presented not only a higher level of total P, but also a higher P shoot:root ratio. This suggests that P translocation in *T. repens* from root to shoot is promoted by DSE strains, particularly in the presence of mycorrhizal fungi, thus showing a possible synergistic relationship between both fungal symbionts.

In conclusion, the co-existence between DSE and AMF appears to affect the pool of P in *T. repens* plants. DSE isolates apparently participate in this process by mineralising and solubilising P. Since both DSE and AMF utilise organic and inorganic P sources commonly found in soil, their interaction with plants may lead to an increase in plant P content. We thus propose a synergistic relationship involving both micro-organisms that may promote plant growth. However, additional research is required to confirm this tripartite interaction.

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