Practical exploitation of mycorrhizal fungi in agricultural systems

By I BRITO[1], M CARVALHO[1], L ALHO[1], M CASEIRIO[2] and M J GOSS[3]

[1]*University of Évora, ICAAM, Apartado 94, 7002 - 540 Évora, Portugal*
[2]*University of Évora, Crop Science Dep., Apartado 94, 7002 - 540 Évora, Portugal*
[3]*University of Guelph, Kemptville Campus, Kemptville K0G 1J0, Ontario, Canada*
Corresponding Author Email: ibrito@uevora.pt

Summary

Improving the sustainability of agricultural systems requires a more efficacious use of soil resources. Mycorrhizas are known to contribute to host plant P acquisition and protection against both biotic and abiotic stresses, such as soil-borne diseases and toxic metal ions. However, practical exploitation of the mutualistic relationship is rarely considered in agricultural systems, allegedly owing to the cost of inoculation and the requirement for timely colonisation. To overcome these limitations, the presence of an extensive extraradical mycelium (ERM) from indigenous arbuscular mycorrhizal fungi (AMF) could be used as the preferential source for colonisation of a crop plant. Colonisation of crop roots starting from an intact ERM takes place faster and generally forms a more effective mycorrhizal association than when initiated from other propagules such as spores and root fragments. We report on the ability of an intact ERM developed by indigenous AMF population on mycotrophic plants (Developers) to significantly improve the AMF colonisation of wheat, subterranean clover and maize allowing for a better performance of the crop. This mechanism allowed the protection of wheat and subterranean clover from excessive Mn concentration in the shoots or in the roots, as in the case of the clover, leading to a greater growth of the crop (2.7 and 4.7 times respectively). Using the same strategy to promote the AMF colonisation of maize, the crop was able to exhibit high levels of colonization, even up to 45 kg $P.ha^{-1}$ of applied P, and the plants took advantage in terms of P use efficiency.

Our results indicate that the use of intact ERM as preferential AMF propagule is a valid strategy to increase the role of this symbiosis under marginal or more intensive cropping systems, through simple adaptations to both crop rotations and tillage practices.

Key words: Arbuscular mycorrhizal fungi, extraradical mycelium, Mn protection, P fertilization, agricultural system

Introduction

Despite the generally recognised importance of arbuscular mycorrhizal fungi (AMF) under natural systems for nutrient acquisition and biocontrol, its intentional use in agricultural systems has been marginal. The importance of AMF in crop nutrient acquisition and especially phosphorous (P) is debatable due to the negative effect of applied P fertilizers on the colonisation progress and on the efficiency of the symbiotic association (Kahiluoto *et al*., 2000, 2001). The potential of mycorrhiza as a biocontrol agent is also limited by the time required to achieve an adequate level of AMF colonisation, and the high financial costs associated with the large-scale application of commercial inoculum (Sikora *et al*., 2008).

The extraradical mycelium (ERM) of mycorrhiza might be important for enhancing the roles of AMF under field conditions. Root colonisation initiated from an intact ERM starts earlier and develops faster than colonisation events initiated by other types of propagule, such as spores and root fragments (Martins & Read, 1997; Fairchild & Miller, 1988). Brito *et al*. (2014), in a pot experiment under glasshouse, developed a strategy to improve bioprotection of wheat against manganese (Mn) toxicity, by previously growing a tolerant mycotrophic plant to develop the ERM in the soil, which was kept intact when the crop was sown. This key experiment indicated the potential for arable mycotrophic weed species to benefit crop production, if deployed in conjunction with soil management strategies that aim to preserve an intact ERM in the soil. Here we summarize the results of three different experiments in order to propose a strategy to exploit the beneficial use of native AMF within the cropping systems both for protection against toxic metals and nutrient acquisition.

Material and Methods

A two-stage greenhouse experiment was conducted in 8 L pots containing a sandy loam Cambisol, where toxic levels of Mn were previously detected (22.6 mg $Mn.kg^{-1}$ DTPA - diethylenetriaminepentaacetic acid). In Stage 1, Mn tolerant plants species with different levels of mycotrophy (Developers), *Silene gallica* L.(Myc-) and *Ornithopus compressus* L. (Myc+), were grown for 6 weeks. At the end of stage 1, the Developer plants were killed by herbicide (6 mL per pot of a solution containing 1.3 $g.L^{-1}$ of glyphosate as Roundup® Supra™). After 7days the soil in half of the pots was disturbed by sieving through a 4 mm sieve (ERM of mycotrophic developers disrupted), and the other half were maintained undisturbed (ERM of mycotrophic developers intact). In Stage 2, wheat or subterranean clover were grown in pots under the various treatments. Subterranean clover was inoculated with an appropriate and effective strain of rhizobia. Wheat was supplemented with nitrogen (N; 67 $mg.kg^{-1}$ of soil as NH_4NO_3). At 21 days after planting (DAP) for wheat and 42 DAP for subterranean clover, shoot dry weight (dw) and concentrations of Mn in wheat shoots and roots of subterranean clover were determined. AMF colonisation rate was measured at 21 DAP for wheat and clover (AC; assessed according to McGonigle *et al*., 1990), but considering only the presence of arbuscules. For subterranean clover the nodule dry weight and % N in the plant was also measured at 42 DAP.

Using the same approach, that is the previous growth of an ERM developer plant, another pot experiment was performed. The same soil was used but the Mn toxicity depressed by liming, applying the equivalent of 2 $ton.ha^{-1}$. *Lolium rigidum* L., a highly mycotrophic plant, was used as the ERM Developer. After 6 weeks *Lolium* plants were killed by cutting the steam just below the soil surface. In half of the pots the soil was disturbed (ERM disrupted) and kept undisturbed (ERM intact) in the other half. Maize seedlings were then planted and P was applied at four different rates, 0, 6, 12 or 18 mg $P.kg^{-1}$ of soil as $NH_4H_2PO_4$ (equivalent to 0, 15, 30 or 45 kg $P.ha^{-1}$). The level of N was adjusted to 67 mg of $N.kg^{-1}$ of soil, as NH_4NO_3. Other nutrients were also applied: B (0.76 mg of $B.kg^{-1}$ of soil as BORAX 20% [w/w]), Zn (3.2 mg of $Zn.kg^{-1}$ of soil as $ZnSO_4$) and K (32 mg of $K.kg^{-1}$ of soil as K_2SO_4). After 21 days of growth the maize was harvested and shoot dry weight and arbuscular colonization rate were evaluated.

Results

The arbuscular colonisation of the wheat at 21 DAP increased significantly in the treatment comprising Undisturbed soil following *Ornithopus* (Fig. 1). This was also the treatment where shoot dw of wheat was significantly greater and the Mn concentration in the shoot was significantly smaller than in other treatments (Fig.1).

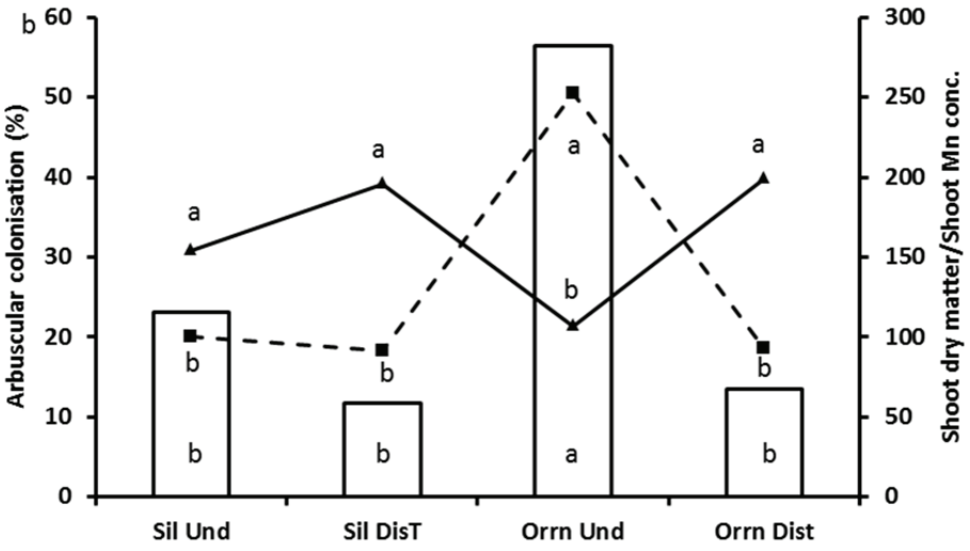

Fig. 1. Effect of the Developer plant (*Silene* or *Ornithopus*, denoted 'Sil and 'Orn', respectively) and soil disturbance (Undisturbed and Disturbed soil denoted 'Und' and 'Dist', respectively) on wheat root AMF colonisation (% AC; bars), shoot Mn concentration (mg.kg^{-1}; ▲) and dry weight (mg.plant^{-1}; ■) at 21 days after planting. Treatments with the same letters are not statistically different at $P \leq 0.05$.

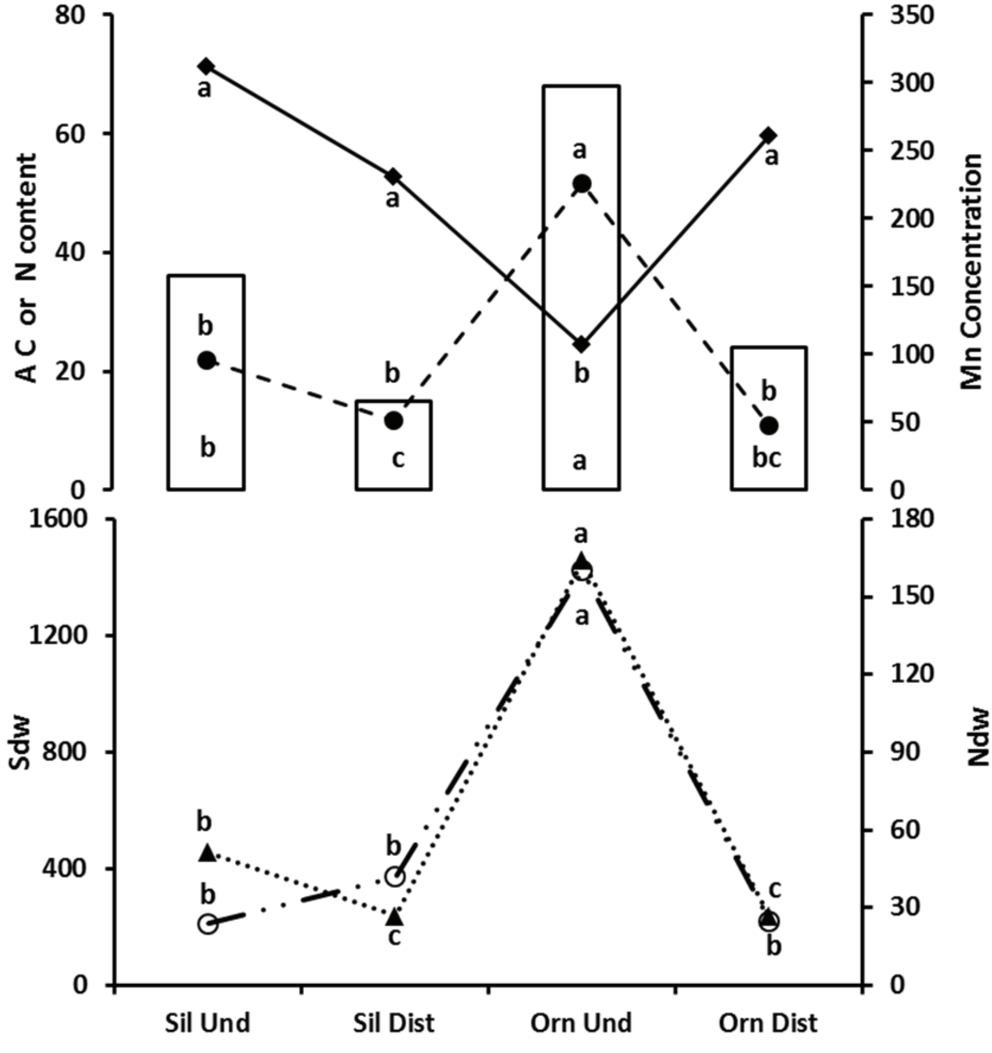

Fig. 2. Effect of the Developer plant (*Silene* or *Ornithopus*, denoted 'Sil and 'Orn', respectively) and soil disturbance (Undisturbed and Disturbed soil denoted 'Und' and 'Dist', respectively) on subterranean clover root arbuscular colonisation (% AC; bars) at 21 days after planting, plant N content (mg N.plant^{-1}; ●), Mn concentration in the roots (mg.kg^{-1}; ♦), shoot dry weight (Sdw mg plant^{-1}; ▲), and nodule dry weight (Ndw µg nodule^{-1}; ○) at 42 days after planting. Treatments with the same letters are not statistically different at $P \leq 0.05$.

The same trend was observed for clover, with AC at 21 DAP and shoot growth, N content and nodule dry weight at 42 DAP being significantly greater in the *Ornithopus* Undisturbed treatment (Fig. 2). The Mn concentration in the roots of the clover was significantly reduced in this treatment (Fig. 2).

The AC of maize was significantly greater in the undisturbed-soil treatment irrespectively of the level of P applied to the soil (Fig. 3). Also, as P level increased the AC of maize root decreased for both soil disturbance treatments (Fig. 4). For the average of P level, maize shoot dw at 21 DAP was significantly greater in the undisturbed-soil treatment, and the P level for maximum growth was equivalent to 30 kg.ha^{-1} in this treatment. In the disturbed-soil treatment maximum growth was achieved at 45.kg ha^{-1} (Fig. 3).

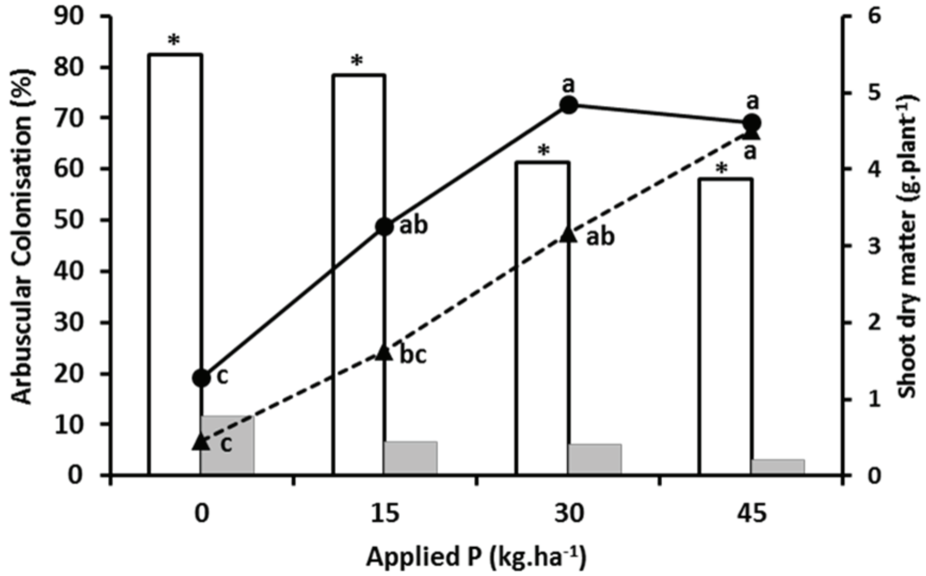

Fig. 3. Effect of applied P on maize root arbuscular colonisation (% AC; open bars - undisturbed soil; grey bars - disturbed soil) and shoot dry matter (g.plant^{-1}; ● undisturbed soil; ▲ disturbed soil), at 21 days after planting. Treatments with the same letters are not statistically different at $P \leq 0.05$. * indicate significant differences ($P \leq 0.05$) in AC.

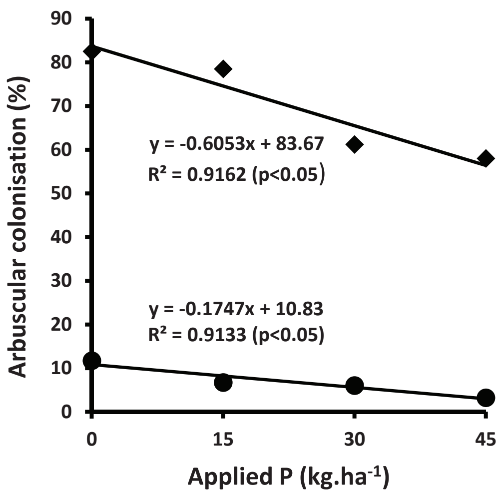

Fig. 4. The relationship between the Applied phosphorus (P) and the Arbuscular colonisation of maize (%) 21 days after planting.

Discussion

The AMF colonisation of the three tested crops (wheat, subterranean clover and maize) was significantly greater when an intact ERM was present in the soil at the time of planting, in agreement with Martins & Read (1997), and Fairchild & Miller (1988). Under conditions where Mn toxicity was a major limitation to the growth of wheat plants, an intact ERM (soil Undisturbed following *Ornithopus*) resulted in a high level of arbuscular colonisation (four times more than in Disturbed soil treatment) and greater plant dry matter (×2.7 more).

Similarly, with large soil Mn concentrations, the AMF colonisation of the subterranean clover supported by an intact ERM facilitated a reduction in root Mn concentration, greater weight of root nodules, improved shoot N content and shoot dw (4.7 times). Comparing the difference between intact and disrupted ERM treatments as propagule source for wheat and subterranean clover, the beneficial effect of the Developer plant and intact-ERM was greatest for the clover. This may be associated with the clover dependence on the natural source of nitrogen from biological fixation, as opposed to wheat which was supplemented with inorganic nitrogen.

The efficacy of AMF in protection against Mn toxicity was significantly enhanced when AMF colonisation was initiated by an intact ERM, previously developed by a tolerant mycotrophic plant in association with the indigenous population and this could overcome the perceived limitations of AMF for bio-protection (Sikora *et al*., 2008). The probability of having adequate AMF species to protect against stress may well be greater among the indigenous populations that are pre-adapted, and this would avoid the necessity of applying commercially available inoculants.

The plants improved ability to acquire P is usually attributed to AMF. However, the benefit of AMF in the context of more intensive cropping systems is questioned by the irrelevance of P acquisition through the symbiosis and the negative effect of P fertiliser on root colonisation by mycorrhiza (Kahiluoto *et al*., 2000, 2001). In our study, under more favorable soil conditions (e.g. limed soil), despite the negative effects of P fertilizer on AMF colonisation, when it was initiated by an intact ERM the % AC was still high (58%), up to 45 $kg.ha^{-1}$ of P application, and the maximum shoot dw was achieved at a lower P application (30 $kg.ha^{-1}$). Comparing the treatments in maize with the ERM intact or disrupted the latter required 15 kg $P.ha^{-1}$ more to achieve similar shoot dry weights. These results challenge the general perception that AMF are not important when considerable amounts of P are applied and show that an intact ERM as the preferential AMF propagule is a valid strategy to increase the potential of this symbiosis under more intensive cropping systems. Several benefits can accrue to plants from their association with arbuscular mycorrhizal fungi, depending on the environmental conditions (Gupta *et al*., 2000). Given that in most cropping systems the plants of each crop cycle experience different kinds of biotic and abiotic stresses, a well-colonised crop can have additional advantages under field conditions, even in more intensive cropping systems.

An intact ERM as the preferential AMF propagule can greatly enhance the benefits of AMF in agricultural systems under both marginal and more favorable production conditions. Within an agricultural system an ERM can be developed in association with the natural vegetation (weeds) or by different elements of the crop rotation (including cover crops), and kept intact by the adoption of appropriate tillage practices.

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