# NO-TILL PROVIDES THE OPPORTUNITY TO MANAGE UNDERGROUND INTERACTIONS BETWEEN ARBUSCULAR MYCORRHIZAL FUNGI, WEEDS AND CROP PLANTS UNDER MEDITERRANEAN CONDITIONS

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## Abstract

Early colonization of crop roots by arbuscular mycorrhiza (AM) is considered beneficial but its importance likely depends on the possible stresses faced by the host plant. Manganese toxicity is one such stress that AM can alleviate. Colonization initiated by extraradical mycelium (ERM) is faster than other sources of inoculum. No-till creates the possibility of encouraging inoculation via this source. At seeding time the ERM available for colonizing plants under no-till would have developed in association with previous crops or those weeds that germinated after the first autumn rain. However, the long, hot and dry summer under Mediterranean conditions might reduce the effectiveness of the ERM to colonize the new crop. The hypothesis that an intact ERM developed by weeds can affect the earlier AM colonization of wheat and alleviate Mn toxicity was tested in a pot experiment. Two mycotrophic (Ornithopus compressus L., Lolium rigidum Gaudin), and one non-mycotrophic (Silene galica L) weed species were grown for 7 weeks before being controlled with Glyphosate (the ERM remaining intact) or by mechanical disturbance (which also disrupted the ERM). Wheat was then planted and allowed to grow for 21 days. AM colonization, plant dry weight and shoot nutrient content were evaluated for both weeds and wheat. When an intact mycelium was present at the seeding of wheat (treatments with Ornithopus compressus and Lolium rigidum controlled by Glyphosate) there was a threefold increase in the AM colonization rate and growth of the crop compared with results for all the other treatments. The enhanced growth of wheat was associated with an alleviation of Mn toxicity, consistent with the hypothesis. However, there was a significant difference of the wheat growth after Ornithopus and Lolium (1.9 times), suggesting functional diversity within mycotrophic weeds and crops.

Key words: Weeds, arbuscular mycorrhiza, soil disturbance, Mn toxicity, wheat

#### Introduction

Arbuscular mycorrhizal fungi (AMF) are an important component of the soil biota in most agroecosystems. Many benefits are considered to accrue to plants from their association with AMF (1). The importance of mycorrhiza is greater under marginal biotic or abiotic conditions, such as poor soil fertility or toxic levels of micronutrients (2). There is considerable functional diversity between AMF (3; 4) and their lack of specificity confers a great advantage for the success of mycorrhization in mixed plant communities.

The extraradical mycelium (ERM) of AMF is a more effective source of inoculum than spores given that active hyphae of mycorrhiza can confer the double advantage of more timely colonization (5) and enhanced energetics (6). The earlier and faster that plant roots are colonized the more effective are the mycorrhiza formed as rapidity of infection seems to be more important in stimulating plant growth than is the capability of colonized roots (7; 8).

Sources of ERM in the soil can be either the mycelium associated to the previous crop or associated to the weeds that develop between cropping seasons. Under a Mediterranean climate both sources may be present. The mycelium of the previous crop can remain infective after the long and dry summer (9). Germination of weeds occurs several weeks before the crop is seeded, when temperatures are more favorable for AM spore germination, and their AM extraradical mycelium has time to develop before the crop is sown. Under appropriate management systems, by integrating chemical weed control and no-till seeding the mycelium can be kept intact creating a unique opportunity to enhance early colonization of the next crop (10).

We hypothesized is that an early AMF colonization, based on an intact ERM, could be an especially important agronomic tool under situations where AMF confer protection against a potentially lethal agent, such as Mn toxicity. Therefore, the objectives of this work were to investigate the importance of ERM developed by the weeds and possible AMF functional diversity associated to the different weed species.

# **Material and Methods**

The two-stage experiment was set up in a greenhouse using 8L pots. In Stage 1 different weeds were grown for 7 weeks, after which the plants were controlled either by

herbicide (ERM kept intact) or soil disturbance (ERM disrupted). In Stage 2 wheat was grown for 21 days.

The weed species grown in Stage 1 were *Silene galica* L., *Lolium rigidum* Gaudin and *Ornithopus compressus* L. These weed species are widespread in soils associated with Mn toxicity and have different levels of mycotrophy, ranging from highly mycotrophic (*Lolium* and *Ornithopus*) to non-mycotrophic (negative control, *Silene*). An additional control treatment, where weed growth was prevented prior to sowing the wheat, was included to evaluate AMF colonization of wheat predominantly from spores and to discriminate between any effect of weeds on the protection of wheat from Mn toxicity by soil nutrient depletion and the impacts of earlier AMF colonization of the wheat in treatments where an intact ERM was present. Hereafter this control treatment is referred as "No weeds".

For Stage 2 of the experiment wheat (*Triticum aestivum* L., var. Ardila) was selected as the test host plant, being an important modern high yielding variety available for this Mediterranean region. Live weeds were never present during this stage as they were fully susceptible to the herbicide or disturbance treatments.

Shoot and root dry weight, arbuscular colonization (AC) and concentration of Mn in the shoots were measured for both weeds and wheat. As the dry weight of wheat shoot material was only enough for one Mn determination there are no replicas of this assessment. Therefore it was not possible to perform a statistical analysis for this parameter but because we used almost all the available material, the value obtained can be considered the value of the population. The measurements for the weeds were carried out on plants grown in an extra set of 5 pots during Stage 1.

The treatments were arranged in factorial combination and the experimental design was a complete randomized block with 5 replicas.

The soil used in this experiment was a sandy loam Cambisoil. It was collected in the autumn from the top 20 cm of a grassland field. Basic fertility assessment showed that the air-dried and sieved (4 mm) soil contained 7 ppm of P (Olsen) and 22.6 ppm of Mn (DTPA), 1.1% OM and had a pH (water) of 6.0.

#### Results and discussion

The shoot growth of the weeds was similar but *Lolium* produced significantly more root material than the other species (Fig. 1). The arbuscular colonization (AC) of the weeds was significantly different between plant species (Table 1). *Silene* was confirmed as a

non-mycotrophic plant, and *Ornithopus* was the weed that was most colonized by AMF. However as the mass of the *Lolium* root system was significantly larger than that of *Ornithopus* (Fig. 1) the two were expected to provide at least a similar amount of AMF inoculum for the next crop. The accumulation of Mn in the weeds shoots was significantly greater in *Silene* but difference between *Lolium* and *Ornithopus* was not significant (Fig. 2).

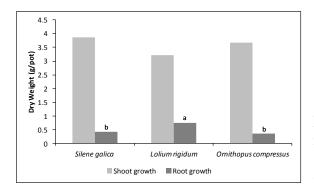


Figure 1 – Weeds growth (shoot and root) in the first stage of the experiment. The same letter indicates no significant differences between treatments (p>0.05).

Table 1 – Arbuscular colonization of the weeds at the end of stage one of the experiment (7 weeks growth). The same letter indicates no significant differences between treatments (p>0.05).

| Weed species          | % Arbuscular colonization |
|-----------------------|---------------------------|
| Lolium rigidum        | 48 a                      |
| Ornithopus compressus | 73 b                      |
| Silene galica         | 0 c                       |

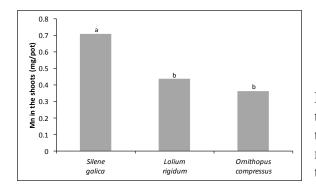
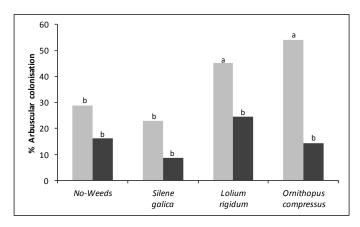


Figure 2 – Mn extraction by the shoot of the weeds at the end of the first stage of the experiment. The same letter indicates no significant differences between treatments (p>0.05).

Arbuscular colonization (AC), shown in Figure 3, and growth, shown in Figure 4, of wheat were significantly affected by the weed species previously present, the disturbance of the soil and the interaction between the two factors.

Soil disturbance only affected AC of wheat when a mycotrophic weed had been growing previously (Fig. 3), which clearly indicated that ERM is a better source of AM inoculum for timely AM colonization. This earlier AM colonization of the wheat significantly improved its growth (Fig. 4). Despite the fact that the AC of wheat grown after *Lolium* was not significantly different from the one after *Ornithopus*, in undisturbed soil the wheat growth after *Ornithopus* was significantly greater. This seems to indicate a functional diversity of the AMF associated with the roots of the weeds, which was transferred to the wheat



 Effect of the Figure 3 treatments (previous weed present and soil disturbance) wheat arbuscular on colonization at 21 days growth. Grey bars Undisturbed soil; Black bars – Disturbed soil. The same letter indicates significant no between differences treatments (p>0.05).

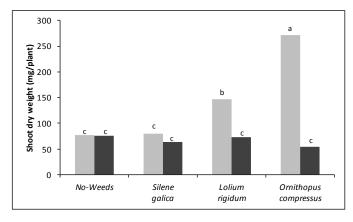


Figure 4 - Effect of the treatments (previous weed present and soil disturbance) on wheat shoot growth at 21 days. Grey bars – Undisturbed soil; Black bars – Disturbed soil. The same letter indicates no significant differences between treatments (p>0.05).

As the growth of the wheat in the No-weeds treatment, where no soil nutrient depletion had occurred, was significantly reduced relative to treatments with an ERM present at sowing, that is in the undisturbed soil after *Lolium* and *Ornithopus* (Fig. 4), the benefits of earlier and greater mycorrhizal colonization of the wheat cannot be due to improved acquisition of nutrients, such as P. The presence of an intact ERM

from the previous weeds resulted in reduced Mn content in the wheat (Fig. 5). Thus Mn concentrations in the wheat shoots were greater when no weeds had been present, the soil was disturbed or *Silene*, the non-mycotrophic weed, had been grown. The Mn concentration in the wheat shoots was significantly and negatively related to the growth of the crop (Fig. 6).

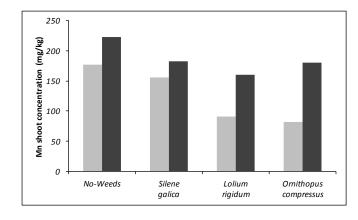


Figure 5 - Effect of the treatments (previous weed present and soil disturbance) on the concentration of Mn in the wheat shoots at 21 days growth. Grey bars — Undisturbed soil; Black bars — Disturbed soil.

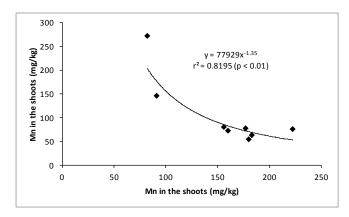


Figure 6 – Relationship between Mn concentration in wheat shoots and dry matter production at 21 days

When the benefit from AMF is the protection of crop plants against a nutrient toxicity like Mn, the presence of an intact ERM in the soil before the seeding of the crop appears to be crucial. The only practical approach to keeping intact the mycelium developed by weeds that germinate after the first autumn rains, well before sowing for small-grain cereals seeding, is to apply herbicides and adopt no-till seeding.

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