WEED MANAGEMENT UNDER NO-TILL TO INCREASE THE BENEFITS OF THE TRIPARTITE SYMBIOSIS

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Abstract

In southern regions of Europe subclover (*Trifolium subterraneum* L.), inoculated with rhizobia, is commonly sown in acid soils that are marginal for arable crop production. The rate of success of these pastures is small both in terms of plant growth and nodulation with the introduced rhizobia. Manganese toxicity is frequently considered to be a contributory factor. Nevertheless, other native legumes, particularly *Ornithopus*, are commonly found to grow satisfactorily under these conditions. Knowing that *Ornithopus* is a highly mycotrophic plant, we hypothesized that preserving the extraradical mycelium (ERM) of mycorrhizal fungi associated with its roots at the time for seeding subclover would allow for a better performance of subclover through an enhanced tripartite symbiosis (legume-rhizobia-mycorrhizal fungi). To test this hypothesis a pot experiment was conducted in a soil where manganese toxicity was known to occur. *Ornithopus compressus* and *Silene gallica* (a non-mycotrophic plant) and two different weed control methods were used to promote different levels of clover AM colonization. The weeds were allowed to growth for 6 weeks and controlled by glyphosate or mechanically with soil disturbance, prior to the seeding of inoculated subclover (*T. subterraneum* cv. Nungarin). The only treatment where the ERM was kept intact was *Ornithopus* controlled by glyphosate. Three weeks after planting, arbuscular mycorrhiza (AM) colonization of the clover under this treatment was significantly greater (3 times) than all the other treatments. In consequence, there was significant improvement in shoot dry weight (5 times), nodule dry weight (7 times) and N accumulation (4 times) of clover. The results were consistent with the hypothesis that the roots of the clover seedlings connected to the intact ERM developed by *Ornithopus* promoting the tripartite symbiosis .

Introduction

Under semiarid conditions in southern regions of Europe, subclover (*Trifolium subterraneum* L.) is a common pasture species sown during autumn and usually inoculated with rhizobia. Major constraints to pasture productivity include rainfall irregularity, soil acidity with associated Al and Mn toxicity and soil P availability. The rate of success of these pastures is small both in terms of plant growth and nodulation by the introduced rhizobia. Nodulation and rhizobia inoculation are frequently problematic (1). Nevertheless, other native legumes, like *Ornithopus*, are commonly found to grow well under these marginal soil conditions.

It is well known that arbuscular mycorrhizal fungi (AMF) can confer several benefits to the host plant, particularly under marginal soil conditions (2). Such benefits are more evident if the infection starts from an intact extraradical mycelium (ERM) (3) when AMF colonization is faster (4).

A synergistic effect can exist between mycorrhiza and rhizobia with the leguminous host crop through this tripartite symbiosis (5, 6). *Ornithopus* is known to be a mycotrophic plant (7). We hypothesized that the ability of this species to be successful under these conditions is associated with an interaction between AMF and rhizobia and if the ERM associated with the roots of *Ornithopus* could colonize the roots of clover seedlings, an earlier AM colonization of the clover could result and a similar degree of protection from Mn toxicity attained. This early colonization of subclover might be achieved if the *Ornithopus* were allowed to grow and develop an ERM before being controlled by herbicides immediately before seeding the subclover with a minimum soil disturbance technique, such as no-till.

Material and Methods

A two stage experiment was conducted in 8L pots containing a sandy loam Cambisoil, 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. In Stage 1 of the experiment different weeds were allowed to grow for 6 weeks after which they were controlled either by herbicide (ERM kept intact) or mechanically with soil disturbance (ERM disrupted). In Stage 2 of the experiment clover (*T. subterraneum* cv. Nungarin) was grown for 6 weeks.

Weed species grown in Stage 1 of the experiment were *Silene gallica* L., and *Ornithopus compressus* L.. These weed species are widespread in soils with Mn toxicity and have different levels of mycotrophy: *Ornithopus* being highly mycotrophic and *Silene* being non-mycotrophic and used as a negative control in the experiment. An additional control treatment, in which no weeds were allowed to grow prior to the clover, was included to evaluate AMF colonisation of clover predominantly from spores and to differentiate between effects of weeds in the protection of subclover against Mn toxicity by soil nutrient depletion and early AMF colonisation of the clover. Hereafter this control treatment is referred as “No weeds”. For Stage 2 of the experiment, 6 seedlings of clover were introduced into the pots previously inoculated with a dense suspension of an effective strain of *Rhizobium leguminosarum* bv. Trifolii. After one week the number of plants per pot was adjusted to 5, with two of these plants being sampled after 3 weeks to determine the arbuscular colonization of the clover, while the three remaining plants were allowed to grow for a total of 6 weeks.

Live weeds were never present during Stage 2 of these experiments as they were fully susceptible to the herbicide or mechanical disturbance treatments.

Shoot and root dry weight, AMF colonisation, assessed by the presence of arbuscules (AC), and concentration of Mn in the shoots were measured for both weeds and clover. The values for the weeds were obtained from plants grown in an extra set of 8 pots. In addition, evaluations for the subclover included number and dry weight of nodules and shoot N content.

The treatments were in factorial combination and the experimental design was a complete randomized block with 4 replicates.

Results and Discussion

The growth of the two weeds in Stage 1 was not significantly different, but the Mn extraction by *Silene* was significantly larger than by *Ornithopus* but arbuscular colonisation (AC) in *Ornithopus* was much greater than in *Silene* (Table 1). The arbuscular colonisation of *Silene* was almost zero, confirming that this weed is non-mycotrophic.

There was a significantly interaction between the weeds previously grown and the soil disturbance treatments in relation to the arbuscular colonisation of the subclover at three weeks stage (Fig. 1). The positive effect of *Ornithopus* on the AM colonization of the subclover under the herbicide treatment was offset when the soil was mechanically disturbed to control this weed. For the other weed treatments, where no ERM was developed (No weed and *Silene*), the disturbance of the soil had no significant effect on the AM colonisation of the clover. This clearly indicates that the ERM developed by *Ornithopus* and kept intact in the undisturbed treatment was a better source of inoculum in relation to the timing of colonisation of the following subclover plants.

This interaction was also reflected in the shoot dry weight of the subclover (Fig.1) and the nodule dry weight (Fig. 2) six weeks after the planting of the clover. The enhanced AMF colonisation of the clover at an early stage when an intact ERM was present (*Ornithopus* undisturbed treatment) was an important advantage both in terms of plant (Fig. 3) growth and root nodule formation (Fig.4).

The same interaction between weeds and soil disturbance treatments was observed for the nitrogen balance (Table 2). The N accumulation by the subclover after *Ornithopus* undisturbed treatment was significantly larger than all the other treatments and this cannot be explained by either an increase of the $N\text{-}NO_3$ in the soil after the weeds or a greater depletion of $N\text{-}NO_3$ of the soil during the growth of the subclover. In fact the amount of $N\text{-}NO_3$ in the soil at the seeding of the subclover (After weed – Table 2) was largest in the No Weed treatment and after the growth of *Silene* and *Ornithopus* values were similar. Therefore, the significantly larger N balance observed for the clover after *Ornithopus* undisturbed treatment can only be explained by a bigger contribution of N derived by symbiotic N_2 fixation. This is consistent with the size of the nodules (Fig. 2) and there was a significant correlation between these two variables (Fig. 5). Our results clearly indicate that the tripartite interaction between AMF - rhizobia and legume was impacted by the earlier AM colonisation of the subclover (due to the presence of an intact ERM associated with the roots of *Ornithopus*) leading to an improvement of the growth and activity of the rhizobia and the subsequent growth of the subclover. The mechanism associated with the role of AMF in this work is not yet explained. However, it cannot be an improvement of acquisition of immobile nutrients (e.g. P) by the subclover, because its growth after the No Weed treatment (where no previously depletion of nutrients from the soil occurred) was not enhanced. It has been reported that Mn toxicity is associated with the soil used in this experiment (8). Brito *et al.* (9) showed that the presence of an intact ERM in the soil at the seeding of the wheat can alleviate Mn toxicity and significantly improving the growth of that crop. In our work the Mn concentration in the shoots of the subclover were not affected by the treatments (data not shown). It is still an open question if the AM colonisation could have had any effect on the protection of the nodules from an excess of Mn which could explain the greater symbiotic activity observed.

Under marginal soil conditions the presence of an intact ERM at the seeding of subclover can significantly improve the growth of the pasture. This ERM can be developed in association with the roots of appropriate weeds present prior to sowing the pasture and kept intact if no-till seeding techniques are used.

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Table 1. Weed growth parameters. Values in columns with the same letter are not significantly different (p>0.05).

	Weed Shoot Dry Weight (g/pot)	Arbuscular colonization (%)	Mn in the shoots (mg/pot)
Silene	9.73 a	0.7 b	1.63 a
Ornithopus	6.99 a	67.0 a	0.72 b

Table 2. Soil and plant N balance for the 6 experimental treatments. Values in columns with the same letter are not significantly different (p>0.05). Dist. – Disturbed; Undist. – Undisturbed.

		Soil N after weed (1)	Soil N after clover (2)	N uptake (3)	N balance (3)+(2)-(1)
			(mg N-NO3/pot)		
No weed	Undist.	75.2	90.6 a	55.4 b	70.8 b
No weed	Dist.		72.6 b	30.3 b	27.8 c
Silene	Undist.	7.7	26.1 c	47.8 b	66.3 b
Silene	Dist.		36.8 c	42.4 b	71.7 b
Ornithopus	Undist.	12.9	12.0 c	155.7 a	154.8 a
Ornithopus	Dist.		36.8 c	41.1 b	64.9 b

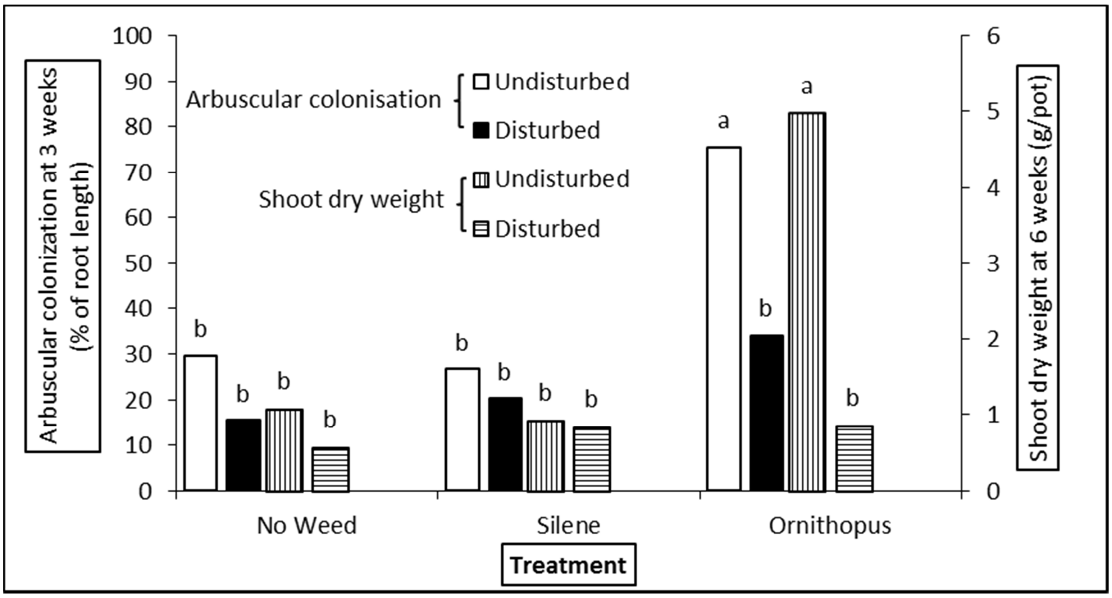

Figure 1. Arbuscular colonisation after 3 weeks and shoot dry weight after 6 weeks in subclover. Bars with the same letter are not significantly different (p>0.05).

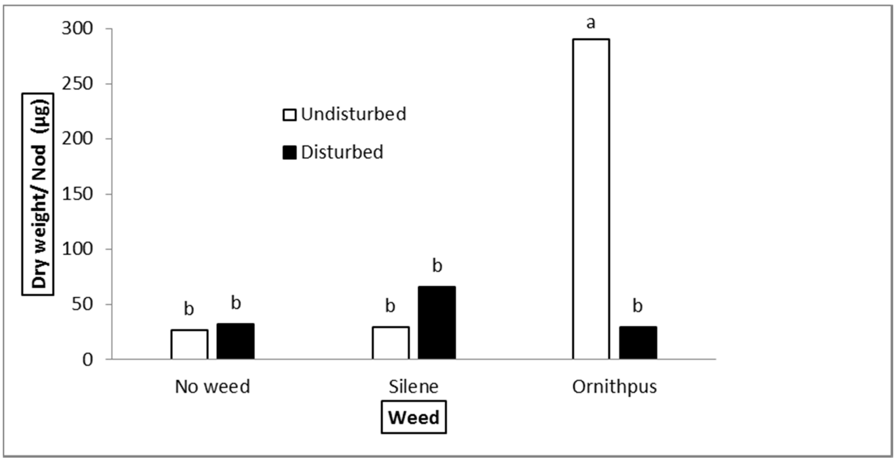

Figure 2. Average nodule dry weight (µg/nodule) of the first 10 nodules present on subclover roots. Bars with the same letter are not significantly different (p>0.05).

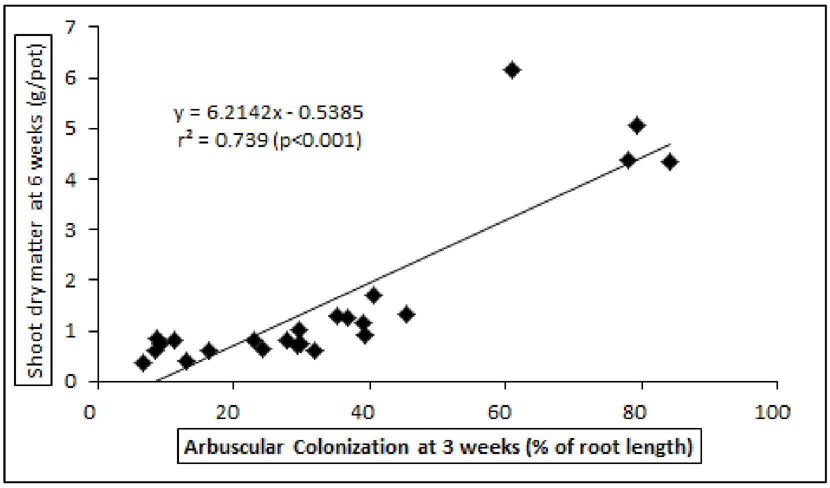

Figure 3. Relationship between arbuscular colonisation after 3 weeks and shoot dry weight after 6 weeks of subclover.

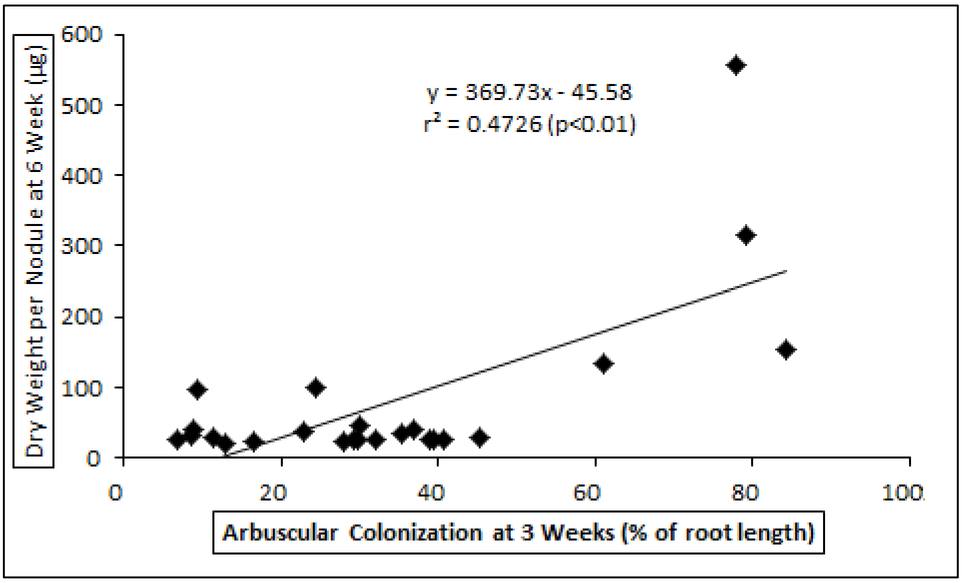

Figure 4. Relation between Arbuscular colonisation after 3 weeks and mean dry weight of nodules after 6 weeks in subclover.

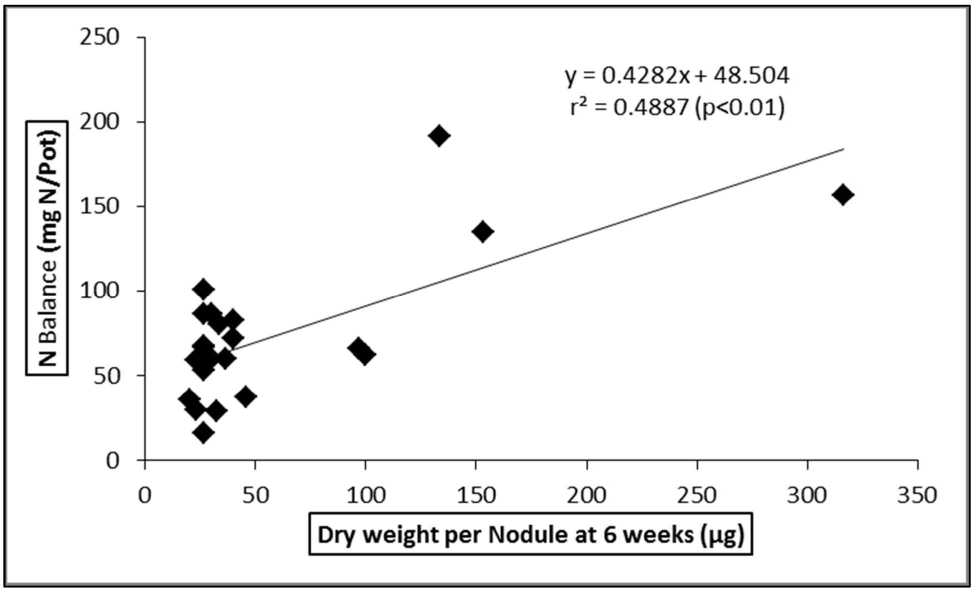

Figure 5. Relation between the dry weight per nodule and the N balance estimated for the subclover growth.