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Influence of arbuscular mycorrhizal fungi (AMF) on plant competition for growth of a legume and a grass plant species

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ABSTRACT

AMF has considerable influence on plant nutrition uptake, competition between the legume (*Trifolium incarnatum*) and a grass (*Anthoxanthum odoratum*) is influenced by the presence of Arbuscular Mycorrhizal Fungi (AMF) in this study. AMF has the ability to change the nutrient distribution between plants which may lead to changes in competitive relationships in plant communities. Pot experiments with a replacement design were conducted to test the influence of AMF isolates and a non AMF control on the competitive relationships of those plant pairs. Key consideration for the study was AMF influence on growth of legume and grass plants when grown individually or in combination of two plants and measured by the dry weight of biomass. Results revealed that AMF stimulates the plant growth of legume and negative effect on grass plant; number of root nodules of legume plant has positive effect in monoculture & mixture. While in competition, legume plant increased biomass in absence of AMF but the relative yield of the grass plant decreased or remains equal and AMF affects the competitive relationship between legume and grass plant.

Key Words: Arbuscular mycorrhizal fungi, Legume, Grass, Growth and Nutrient competition

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I. Introduction

Arbuscular Mycorrhizal Fungi (AMF) is an important component of soil communities in many temperate and tropical ecosystems. It is associated with most species of terrestrial plants (Allen *et al.* 1995). AMF has considerable influence on plant nutrition uptake. It enhances the uptake of phosphorus and also other immobile nutrients which are required by plants (Smith and Read, 1997).

With AMF associations plants may receive more than 50% of phosphorus (Jakobsen *et al.* 1992). The growth and composition of plant communities are greatly influenced by plant competition (Aerts, 1999). Abiotic factors such as nutrient availability and climate influence plant Competition. Plant competition also influenced by interactions with other organisms such as herbivores, pathogens and mutualists (Hetrick *et al.* 1994; Van der Putten and Peters 1997; Hart *et al.* 2003). This investigation focused on the influence of Arbuscular Mycorrhizal fungi (AMF) on plant competition.

Different plant species do not profit equally from AMF. Some plants are capable of acquiring more nutrients from the AMF than others (Smith and Read, 1997). The symbiosis between plants and AMF is non-specific. Competing plant species can be interconnected by mycorrhizal hyphal networks. Carbon and nutrients may transfer from one plant to another via such hyphal networks; it has even been reported by several authors (Grime *et al.* 1987; Newman 1988; Simard *et al.* 2002). Hence, AMF has the ability to change the nutrient distribution between plants which may lead to changes in competitive relationships in plant communities.

Different studies have investigated the effect of the presence of AMF on plant competition (Fitter 1997; Hamel *et al.* 1992; Hartnett *et al.* 1993; Hetrick *et al.* 1994; West 1996; Marler *et al.* 1999). This experiment focused specifically on competition between legumes and other plant species. Legumes are of particular interest because they form symbioses with two group ground organisms that influence plant nutrition and AMF and Rhizobia. Rhizobia are bacteria that able to convert atmospheric nitrogen into ammonium that can be used with legumes (Werner, 1992). Legumes are often highly dependent on AMF to supply extra phosphorus required for nitrogen fixation by plants. At the low phosphorus levels, nitrogen fixation can be partly or completely inhibited in the absence of AMF.

This study tested whether competition between the legume (*Trifolium incarnatum*) and a grass (*Anthoxanthum odoratum*) is influenced by the presence of AMF. Pot experiments with a replacement design, and tested the influence of AMF isolates and a non AMF control on the competitive relationships of the plant pairs.

This poses the question whether AMF influence growth of legume and grass plants when grown individually or in combination of two plants, and measured by the dry weight of biomass. Thus, few null hypothesis was accounted during the study: a) there will be no change in the yield of legume plants measured in the dry weight of biomass when AMF is added compared to when no AMF is added, b) there will be no change in the yield of grass plants measured in the dry weight of biomass when AMF is added compared to when no AMF is added, c) there will be no change in the number of root nodules of legume plant in monoculture or in combination when AMF is added compare to when no AMF is added, and there will be no change in competitive interaction in the yield of legume and grass plants measured in the dry weight of biomass; AMF is added compared to when no AMF is added.

II. Materials and Methods

Plants: Legume - *Trifolium incarnatum* (supplier Bijenhuis Wageningen) and Grass- *Anthoxanthum odoratum* (supplier Cruydt-hoeck Nijberkoop) was used for this study

Soil: Droevendaal soil (Typic Endoaquoll) with poor nutrient quality was used and the soil pH was 6.08. The soil was collected at geocoordinate 52.03°N and 05.67°E, Wageningen, Netherlands. Soil contains 1.2 mg Nkg⁻¹ soil in total (0.7 mg N-NH₄ kg⁻¹ soil, 0.5 mg NO₃+NO₂kg⁻¹soil).

Inoculation: AMF inoculated on top of 3 species of AMF with 20 ml.

Timing of treatments: Plants germination started on the 7th of August, plants transplanted to bigger pots on the 17th of August and AMF added on the 4th of September and plants were harvested on the 28th of August.

Treatments: The following treatments were applied for each plant species: with/without Arbuscular Mycorrhizal Fungi (AMF). For each treatment there were four replicates.

To investigate the effects of inoculation with AMF on the plant growth, several measurements were carried out in the 6th week after planting. The measured response variables consisted of dry weight biomass of both the shoot and the root and root nodules.

Competition between *T. incarnatum* and *A. odoratum* was investigated with two plants per pot in the monocultures and 1+1 plant per pot in the mixtures. With AMF, and a control, and four replicates, this resulted in a total of 24 pots.

After harvest, sand was removed and plants were washed. Then plants were divided into shoots and roots. After drying shoots and roots were weighed.

The Relative Yield Total (RYT), which is a measure of the relative productivity of a mixture, and the Aggressively Index, which is a measure of relative performance of the two plant species, compared with each other, were calculated by equations 1 and 2 respectively (de Wit, 1960; McGilchrist & Trenbath, 1971):

$$\text{RYT} = (Y_{ij}/Y_{ii}) + (Y_{ji}/Y_{jj}) \dots \dots \dots \text{Eqn. 1}$$

$$\text{Aggressivity index} = (Y_{ij}/Y_{ii}) - (Y_{ji}/Y_{jj}) \dots \dots \dots \text{Eqn. 2}$$

Here, Y_{ij} and Y_{ii} are the shoot yields of *T. incarnatum* in mixture and monoculture respectively. Similarly, Y_{ji} and Y_{jj} are the shoot yields of *A. odoratum* in mixture and monoculture. The factor (Y_{ij}/Y_{ii}) and (Y_{ji}/Y_{jj}) are relative yields of the plant species in mixture. The lower the aggressively index, the more competitive *T. incarnatum* is compared with *A. odoratum*.

AMF dependency of each plant species in monoculture or in mixture was calculated (Van der Heijden, 2002; Eqn. 3).

$$\text{AMF Dependency} = 1 - (b/a), a \geq b, -1 + (a/b), a < b \dots \dots \dots \text{Eqn. 3}$$

Where 'a' is the average biomass of the treatments with AMF, and 'b' is the biomass in the non-AMF treatment.

Statistical analysis: SPSS program was used for all statistical analysis and the significance level was set at 0.05. Differences between AMF treatments were calculated for each plant species in monoculture and in competition separately, using a one-way analysis of variance (ANOVA). Differences between AMF treatments in RYT and aggressively index were also calculated with a one-way ANOVA.

III. Results

The total biomass of the plant was counted in all plants and the root nodules also counted for *T. incarnatum*. Biomass of *T. incarnatum* in monoculture was higher with AMF than without AMF (Figure 01). The number of root nodules of *T. incarnatum* in monoculture was also higher with AMF added compared to the when no AMF is added (Figure 02), While the biomass of *A. odoratum* in monoculture was lower with AMF than without (Figure 03).

But in mixture with *T. incarnatum* biomass of *A. odoratum* was hardly affected by AMF and the biomass *T. incarnatum* in mixture with *T. incarnatum* was higher in case of without AMF but with AMF the biomass of *T. incarnatum* was found low (Figure 04). However, the number of root nodules of *T. incarnatum* was much higher with AMF than without AMF (Figure 05). Competitive relationships were not influenced by AMF. The RYT was not significantly different between the treatments ($F=0.06$, $P=0.814$, one-way ANOVA; Figure 06) and the aggressively index was not significantly different in competition ($F=2.623$, $P=0.156$; one-way ANOVA; Figure 07) and was not significantly different from the without AMF treatment.

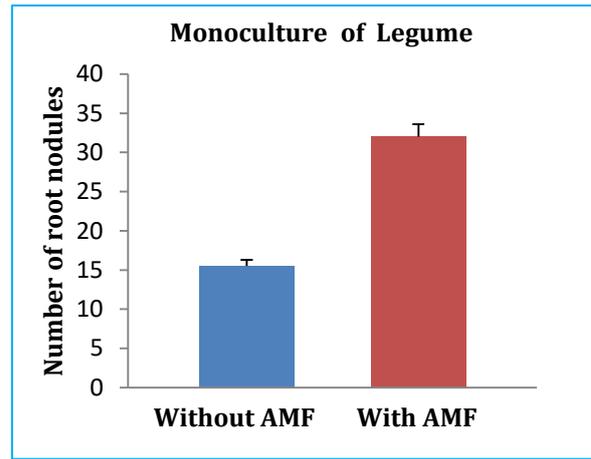
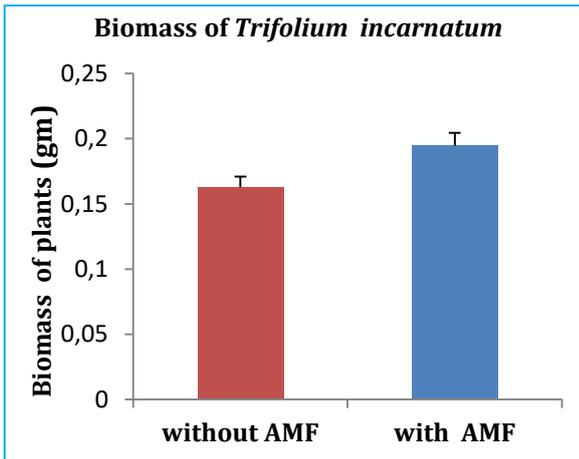


Figure 01. Biomass of *T. incarnatum* in monoculture. Figure 02. Root nodules of *T. incarnatum* in monoculture.

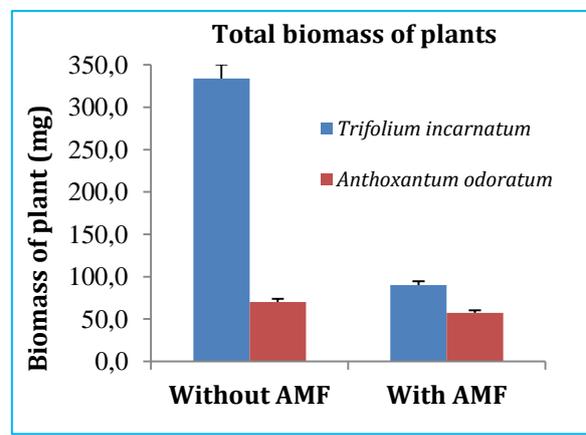
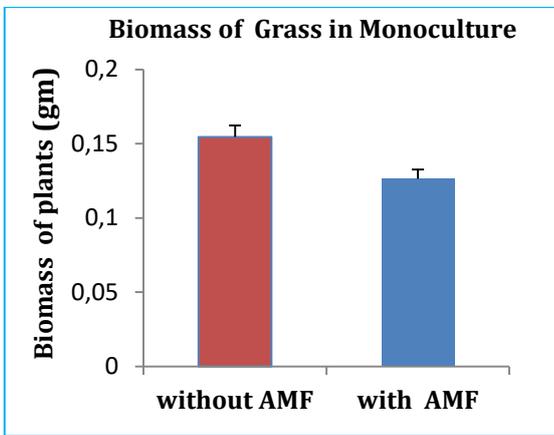


Figure 03. Biomass of *A. odoratum* in monoculture. Figure 04. Biomass of *T. incarnatum* & *A. odoratum* in mixture.

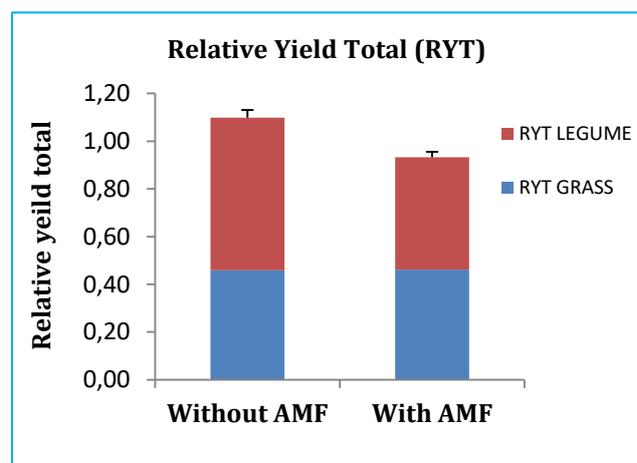
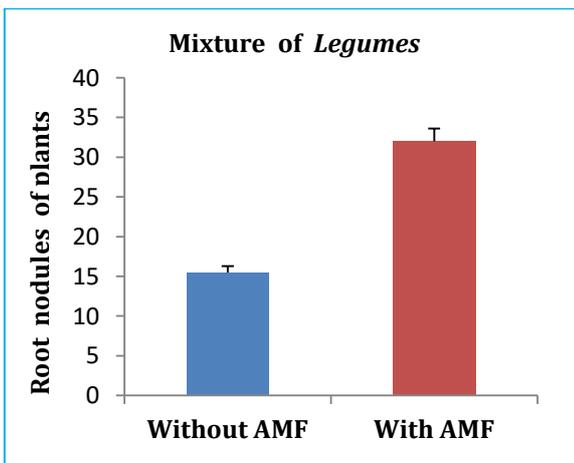


Figure 05. Number of root nodules in mixture.

Figure 06. RYT in the competition between plants.

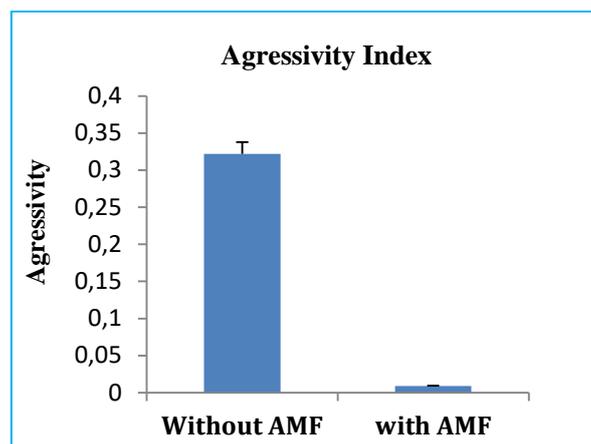


Figure 07. Agressivity Index for the plants.

IV. Discussion

The experiment revealed that AMF influence the plant growth, i.e., the biomass of *T. incarnatum* is favoured by the fungi. This is in accordance with first hypothesis that the biomass of legume plant increases with the presence of AMF and the number of root nodules of *T. incarnatum* is also positive to AMF compare to without AMF.

A. odoratum was negatively responsive to AMF in monoculture. This result is in agreement with other study studies that observed AMF have negative effect on some plants species (Verbruggen et al. 2011; Veiga et al. 2011). This is not unexpected as plants are often unresponsive or negatively responsive to AMF in pot experiments where root development is space limited (Graham and Abbott, 2000). Mycorrhizal growth depressions are attributed to AMF parasitism, where carbon (C) demand from the fungus exceeds the benefits of increased nutrient uptake (Veiga et al. 2011).

But grass plant remained unresponsive in competition with the presence of AMF. These results show that plant responses to AMF in monocultures cannot serve as a prediction for how AMF affect plants in competition with other species.

During competition, relative yield of legume plant increased yield in absence of AMF while the relative yield of the grass plant decreased or remain equal. From the figure of aggressivity index, it can be stated that competitive relationship changed in favour of legumes (aggressivity become lower) in presence of AMF. This is in accordance with our 4th hypothesis that AMF had influence on the competitive relationship between *T. incarnatum* and *A. odoratum*. These results are in agreement with other studies. Several researchers observed AMF to alter competitive relationship between plants (Hamel et al. 1992; Hartnett et al. 1993; Hetrick et al. 1994; West 1996; Tanja et al. 2007).

V. Conclusion and recommendation

This experiment revealed that AMF stimulates the plant growth of *legume* and negative effect on grass plant, and number of root nodules of legume plant has positive effect in monoculture and mixture. In case of competition between plants, *legume* plant increased biomass in absence of AMF while the relative yield of the grass plant decreased or remain equal, and AMF affects the competitive relationship between *legume* and grass plant.

Further studies will be required to determine the effect of AMF on the biomass production of *T. incarnatum* and *A. odoratum* require further investigation in rather larger scale, while factors that affect the competitive relationship need to investigate more deliberately.

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