

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.

Keywords: Arbuscular Mycorrhizal Fungi, Legume, Grass, Plant Nutrient Competition

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 & 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 & 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.

Materials and Methods

Plants: Legume - *Trifolium incarnatum* (supplier Bijenhuis Wageningen) and Grass-*Anthoxanthum odoratum* (supplier Cruydt-hoeck Nijberkoop)

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

AMF: Inoculated on top (3 species of AMF, added 20 ml)

Timing of treatments: Plants germination start: 7th of August, plants transplanting to big pots: 17th of August, AMF addition: 4th of September and Plant harvest: 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.

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 1). 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 2), While the biomass of *A. odoratum* in monoculture was lower with AMF than without (Figure 3).

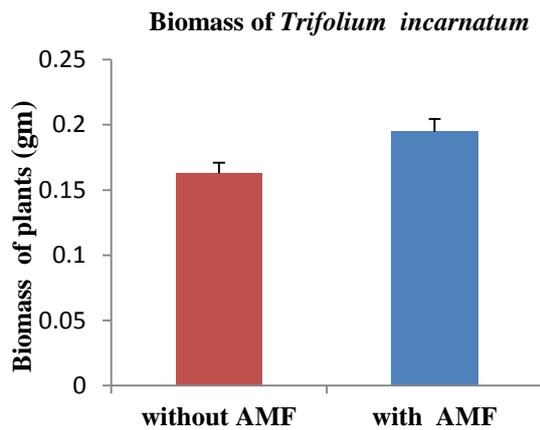


Figure 1. Biomass of *T. incarnatum* in monoculture

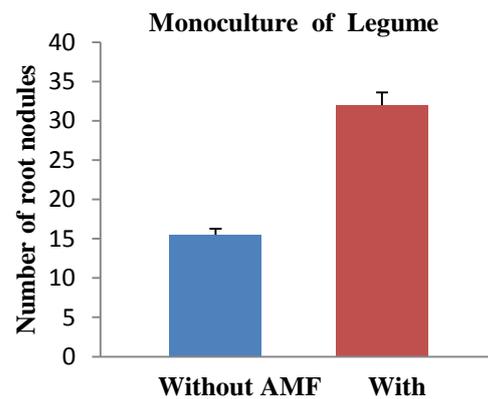


Figure 2. Root nodules of *T. incarnatum* in monoculture

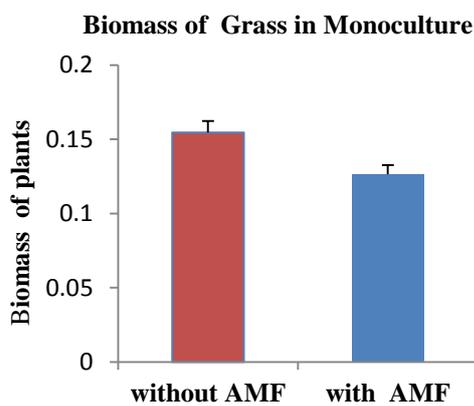


Figure 3. Biomass of *A. odoratum* in monoculture

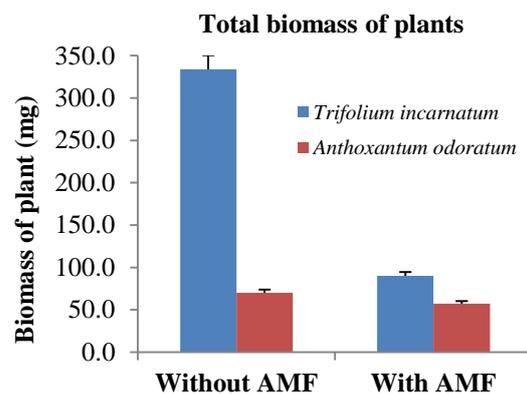


Figure 4. Biomass of *T. incarnatum* & *A. odoratum* in mixture

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 4). However, the number of root nodules of *T. incarnatum* was much higher with AMF than without AMF (Figure 5). 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 6) and the aggressiveness index was not significantly different in competition ($F=2.623$, $P=0.156$; one-way ANOVA; Figure 7) and was not significantly different from the without AMF treatment.

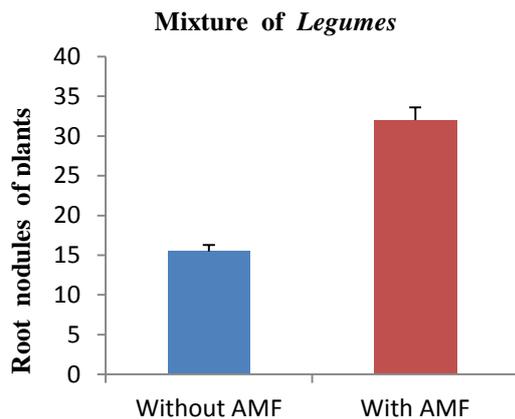


Figure 5. Number of root nodules in mixture

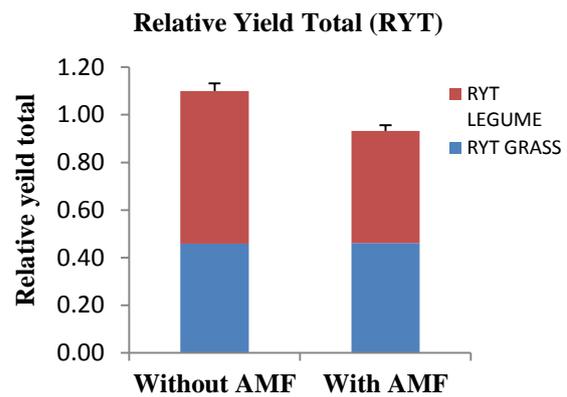


Figure 6. RYT in the competition between plants

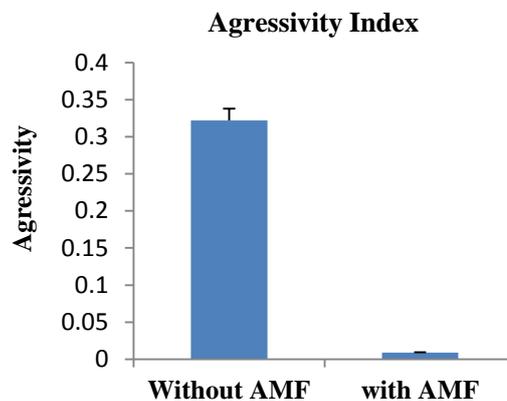


Figure 7. Agressivity Index for the plants

Discussion

The study 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 & 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).

Conclusion

This experiment revealed the following conclusions:

1. AMF stimulates the plant growth of *legume* and negative effect on grass plant.
2. Number of root nodules of legume plant has positive effect in monoculture & mixture.
3. In competition, *legume* plant increased biomass in absence of AMF while the relative yield of the grass plant decreased or remain equal, and
4. AMF affects the competitive relationship between *legume* and grass plant.

Recommendations

Further study will be required to determine:

1. The effect of AMF on the biomass production of *T. incarnatum* and *A. odoratum* require further investigation in large scale, and
2. Factors that affect the competitive relationship need to investigate more.

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