

## Effects of Arbuscular Mycorrhiza Fungi on Growth Characteristics of *Dactylis glomerata* L. under Drought Stress Conditions

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### Abstract

Limited information is available regarding the selection of effective mycorrhizae and the exploitation of their beneficial effects on the enhancement of the forage production of *Dactylis glomerata* under the predicted warmer and drier conditions in the Mediterranean region. The objective of this study was to test the effects of *Glomus intraradices*, *Glomus mosseae* and their mix inoculation on growth characteristics and dry matter production of *Dactylis glomerata* in relation to full and limited irrigation. The experiment was conducted in Orestiada, Northeastern Greece. Limited irrigation significantly decreased yield and yield components of *Dactylis glomerata* over the full irrigation. Drought stressed plants had significantly higher root dry weight as a response for better survival under water deficit conditions. The Arbuscular mycorrhizal fungi (AMF) inoculated plants had significant higher shoot dry weight, tiller height and number of leaves in comparison to the non-inoculated plants. On the contrary, under drought stress conditions all AMF plants had lower root dry weights than control plants. Among the studied mycorrhizae species, *Glomus intraradices* performed better than *Glomus mosseae* and their mixture as it increased S/R ratio, tiller height and number of leaves.

**Keywords:** forage, *Glomus intraradices*, *Glomus mosseae*, Mediterranean region, orchardgrass

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### Introduction

Water availability is one of the most ominous abiotic factors limiting the productivity of forage crops in the Mediterranean region. It is predicted that due to climatic change, precipitation will significantly reduce in the Mediterranean region (Bates *et al.*, 2008). The predicted warmer and drier climate conditions will considerably affect the grassland ecosystems' goods and services (Cheddadi *et al.*, 2001). Consequently, significant reductions in productivity are expected and, it is therefore important to adopt appropriate management techniques in order to maintain forage production under the more severe drought conditions.

In the warm and dry Mediterranean environments, arbuscular mycorrhizal (AM) symbiosis could play an important role in alleviating the effects of drought on crop yield. Arbuscular mycorrhizal fungi (AMF) symbiosis could significantly improve plant resistance to drought via increased dehydration-avoidance and increased tolerance (Ruiz-Lozano and Azcón, 2000). However, its efficacy should be linked to the plant AMF functional

compatibility. The root colonization by the mycorrhiza increases active absorptive surface area and stimulates water uptake even in water stress conditions. The AMF symbiosis could increase the drought tolerance via the increased soil water movement to the plant roots (Ruiz-Lozano, 2003), and the ability of the mycorrhizal plants to mediate the osmotic stress. These osmotic regulations are related to the sugar level adjustments in the plant shoots (Wu and Xia, 2006). AMF plants postpone declines in water potential during drought stress (Porcel and Ruiz-Lozano, 2004). The ability of the fungus to enhance the water uptake and transport it in the plant, could explain these effects. What is more, the effects of AMF to the drought - plant relation also extend to the soil environment by promoting soil aggregation and thus improving the overall soil water conditions to the rhizosphere (Rillig *et al.*, 2002).

*Dactylis glomerata* L. is a widespread perennial grass species, distributed throughout Eurasia including the Mediterranean basin (Lumaret, 1988). It is considered as one of the most important forage species due to its high productivity and nutritive value. Although its populations

and varieties thrive in a multitude of environments and many of them have been reported as well adapted to drought stress conditions (García and Lindner, 1998; Stewart and Ellison, 2010), little information is available regarding the effects of AMF colonization on its growth characteristics.

The beneficial effect of mycorrhizal colonization on many field crops has been well documented. However, limited information is available concerning the selection of effective mycorrhizae and exploitation of their beneficial effects on enhancing the forage production of *Dactylis glomerata* in the Mediterranean region. The understanding of growth characteristics and dry matter production as affected by AMF colonization is important for the sustainable utilization of *D. glomerata* swards under the predicted future warmer and drier conditions. The general objective of this study was to test the effects of various AMF on growth characteristics and dry matter production of *Dactylis glomerata* in relation to different water availability.

### Materials and methods

The experiment was conducted at the farm of the Democritus University of Thrace, in Orestiada, Northeastern Greece (41° 33' N, 26° 31' E), at an altitude of 33 m above sea level. The climate of the area is characterized as sub-Mediterranean. The mean annual precipitation is 515 mm, and the mean annual temperature is 14.2 °C.

Seeds of the perennial grass species *Dactylis glomerata* L. collected from grasslands in the surrounding area during the summer of 2011, were sown into 40 plastic pots (10 lt volume) full of surface soil (0-20 cm) from the Democritus University farm mixed with sand, in March 2012. The soil is silty clay with pH 7.5 (1:2 water), organic matter 20.5 g kg<sup>-1</sup>, electrical conductivity 0.04 S m<sup>-1</sup>, total N 1.11 g kg<sup>-1</sup>, N-NO<sub>3</sub> 9 mg kg<sup>-1</sup>, P (Olsen) 13.2 mg kg<sup>-1</sup>, and available K 178 mg kg<sup>-1</sup> (Koutroubas *et al.*, 2012). The total seeding density was seven seeds per pot. Two weeks after the emergence of the seeds, some seedlings were removed from every pot in order to acquire a planting density of two seedlings per pot. The pots were then placed in an open area and subjected to a full irrigation treatment for a month prior to the onset of the experiment.

Two different irrigation treatments were applied once the experiment commenced: (1) up to full capacity and (2) 33% of full capacity. AMF application treatments were defined as: (1) control (no addition of AMF); (2) *Glomus intraradices* Schenck and Smith inoculation; (3) *Glomus mosseae* (Nicol and Gerd) Gerdemann and Trappe (Gm) inoculation; (4) mix of *Glomus intraradices* and *Glomus mosseae* inoculation. In the AMF inoculated pots, 8 ml of 10% w/v water-inoculum solution (25 spores g<sup>-1</sup>) was applied 3 cm below the depth of seeding in each pot before sowing. The pots were arranged in a completely randomized block (block in irrigation levels) with five replications. All pots were randomized within each irrigation treatment biweekly. Hand weeding was done when required.

The number of tillers per plant, the length of tillers from the base to the tip, the number of leaves per plant and the length of the leaf lamina between the ligule of the youngest fully expanded to the tip of the emerging leaf were measured twice a week, during the growing season (May-June).

Subsequently, Leaf Elongation Rate was calculated: Leaf Elongation Rate (LER)=(L2-L1)/t, where L1 is the leaf length at the beginning and L2 is the leaf length at the end of a time (t), (Lemaire and Agnusdei, 2000). The plants from all the pots were removed at late June 2012 and oven-dried at 60 °C for 48 h. Shoot and root samples were weighed in order to determine shoot dry weight (gr plant<sup>-1</sup>) and root dry weight (gr plant<sup>-1</sup>) and to calculate the S/R ratio.

Root samples were cleared and stained and mycorrhizal colonization was evaluated according to Trouvelot *et al.* (1986). It expressed as percentage of mycorrhization (M%) and percentage of arbuscules in the root system (A%).

Data for the shoot dry weight, root dry weight, the S/R ratio, the leaf growth rate, mycorrhizal colonization and the percentage of arbuscules in the root system were analyzed as completely randomized block design with two irrigation treatments and four AMF colonization treatments (Quinn and Keough, 2002) with five replications. Data for the number of tillers, the length of tillers and the number of leaves per plant were analyzed as completely randomized block design in time with two irrigation treatments and four AMF colonization treatments (Quinn and Keough, 2002). General linear models procedure (SPSS 18 for Windows) was used for ANOVA. The LSD at the 0.05 probability level was used to detect the differences among means (Steel and Torrie, 1980).

### Results and discussion

Mycorrhizal colonization did not vary with water stress

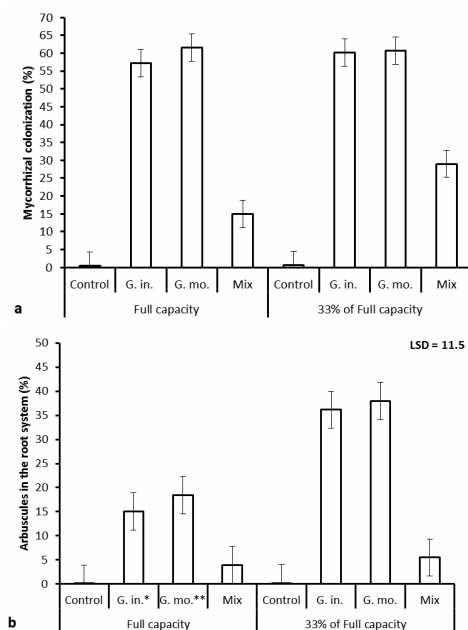


Fig. 1. (a) Percentage of mycorrhizal colonization (Mean  $\pm$  S.E.) and (b) percentage of arbuscules (Mean  $\pm$  S.E.) in *Dactylis glomerata* plants grown under well-watered conditions (up to full capacity) and drought stressed (33% of full capacity)

\* *Glomus intraradices*

\*\* *Glomus mosseae*

treatment (Fig. 1a). Similar results are common in studies involved potted plants (Auge, 2001). AMF development could be influenced not only by soil water content but also by differences in host and soil factors (Bethlenfalvai *et al.*, 1988). Generally, the colonization of both the studied mycorrhizae species was satisfactory and superior to that of their mixture. Both water stress and mycorrhizae application affected significantly the percentage of arbuscules in the root system (Fig. 1b).

Under limited irrigation the percentage of arbuscules in the root system increased, while reduced arbuscules (%) were found in plants inoculated with the mix of mycorrhizae species.

Water stress and mycorrhizae application affected significantly shoot dry weight, root dry weight, S/R ratio and the number of leaves per plant of *Dactylis glomerata*

(Tab. 1). Additionally, significant differences for leaf growth rate and number of tillers per plant were recorded only between the irrigation treatments, while for tiller height only among the tested AMF colonization treatments. The dates of measurements affected significantly the number of leaves and the number of tillers, but did not affect the tillers' height. Significant interaction was observed between the irrigation and the mycorrhizae treatments only for root dry weight, S/R ratio, the number of tillers and tillers' height (Tab. 1), indicating that the water stress effect was not consistent in the mycorrhizae. Moreover, the interaction of irrigation treatment and dates of measurements was significant for the number of leaves and the number of tillers.

The shoot dry weight (across mycorrhizae treatments) and the S/R ratio of limited watered plants reduced

Tab. 1. Statistical significance of F ratios from the analysis of variance for dry weights of shoot and root, on S/R ratio and on growth parameters

Source of variation	df	A. b <sup>1</sup>	U.b <sup>2</sup>	A/U <sup>3</sup>	LGR <sup>4</sup>	No Leaves	No Tillers	Tiller Height
Water (W)	1	p≤0.05	p≤0.05	p≤0.05	p≤0.05	p≤0.05	p≤0.05	NS
Mycorrhiza (M)	3	p≤0.05	p≤0.05	p≤0.05	NS	p≤0.05	NS	p≤0.05
Date (D)	5					p≤0.05	p≤0.05	NS
WXM	3	NS	p≤0.05	p≤0.05	NS	NS	p≤0.05	p≤0.05
WXD	5					p≤0.05	p≤0.05	NS
MXD	15					NS	NS	NS
WXMxD	15					NS	NS	NS

F Test at p≤0.05

<sup>1</sup> Aboveground biomass, <sup>2</sup> Underground biomass, <sup>3</sup> Aboveground/Underground, <sup>4</sup> Leaf Growth Rate

Tab. 2. Effects of watering (across mycorrhiza application treatments) on dry weights of shoot and root, on S/R ratio and on growth parameters (Mean ± S.E.) of *Dactylis glomerata*

Treatment	Watered	Drought stressed	LSD <sub>0.05</sub>
Shoot dry weight (gr)	1.22±0.03 <sup>a</sup>	0.91±0.03 <sup>b</sup>	0.09
Root dry weight (gr)	2.68±0.24 <sup>b</sup>	4.56±0.25 <sup>a</sup>	0.71
S/R	0.65±0.03 <sup>a</sup>	0.24±0.03 <sup>b</sup>	0.09
Number of tillers	6.96±0.16 <sup>a</sup>	6.31±0.16 <sup>b</sup>	0.44
Tiller height (cm)	17.41±0.16 <sup>a</sup>	17.44±0.16 <sup>a</sup>	NS
Number of leaves	6.44±0.15 <sup>a</sup>	5.95±0.15 <sup>b</sup>	0.42
Leaf growth rate (cm/d)	0.18±0.01 <sup>a</sup>	0.14±0.01 <sup>b</sup>	0.03

Means within each row followed by the same letter are not significantly different (LSD Test, p≤0.05).

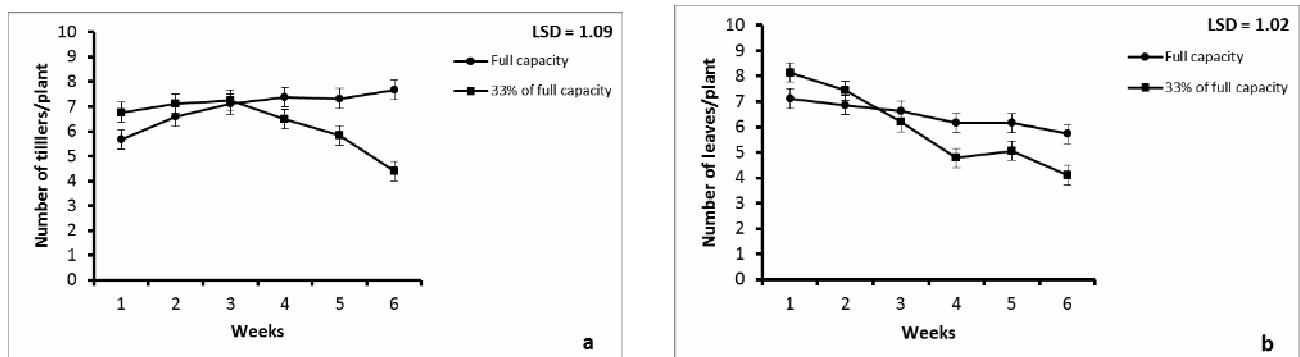


Fig. 2. Number of tillers (a) and number of leaves (b) of *Dactylis glomerata* for the two irrigation treatments (across AMF application treatments) during the experimental period

approximately 25% and 63% respectively, while the root dry weight increased almost 70% (Tab. 2). Similarly, the number of leaves, number of tillers and leaf growth rate were significantly lower for limited watered plants compared to well watered ones, while the tiller height did not significantly differ between them (Tab. 2). The number of tillers and number of leaves under limited irrigation reduced significantly after the fourth and third week respectively (Fig. 2).

The results of the present study suggest that limited irrigation decreases significantly yield and yield components in *Dactylis glomerata*. It has to be noted that the decreased leaf growth rate recorded at plants grown under drought stress conditions has been described as a protection mechanism of grasses grown under drought conditions (Volaire et al., 2009; Wang and Bughrara, 2008). Drought stressed plants had significantly higher root dry weight. This result is associated with the fact that many grass species growing in drought conditions tend to increase their roots in order to increase their active absorptive surface area for water uptake. This response is a water restriction tolerance mechanism for the better survival of grasses under water deficit conditions (Lopez et al., 2013). These results confirm the fact that soil moisture constitutes a critical factor for plant growth and herbage production in the Mediterranean region (Etienne, 1996).

The AMF inoculated plants (across the irrigation

treatments) had significant higher shoot dry weight compared to the non-inoculated plants, while there were no significant differences among the mycorrhizae application treatments (Tab. 3). Similarly, the AMF inoculated plants had higher tiller height and number of leaves in comparison to the non-inoculated plants, while the number of tillers and leaf growth rate did not significant differ between them (Tab. 3). Among the mycorrhizae treatments, the plants with *Glomus intraradices* had the highest tiller height and number of leaves although their tiller height did not differ significantly from these of *Glomus mosseae* and their number of leaves from these of their mix. On the contrary, the root dry weight of both *Glomus intraradices* and *Glomus mosseae* inoculated plants was the lowest although this of *Glomus mosseae* inoculated plants did not differ significantly from root dry weight of the mix and the non-inoculated plants. Additionally, the *Glomus intraradices* inoculated plants had the highest S/R ratio, while the mix and non-inoculated plants the lowest one. The opposite trend of the irrigation treatment main effect was observed for plants of the mix mycorrhizae species which had significantly higher root dry weight, lower number of tillers and tillers' height under well watered compared to limited watered conditions (Tab. 4).

Arbuscular mycorrhizal fungi (AMF) inoculation seems to improve *Dactylis glomerata* yield, due to the increase of shoot dry weight, S/R ratio, tiller height and number of

Tab. 3. Effects of mycorrhiza colonization (across watering treatments) on dry weights of shoot and root, on S/R ratio and on growth parameters (Mean  $\pm$  S.E.) of *Dactylis glomerata*

Treatment	Control	<i>Glomus intraradices</i>	<i>Glomus mosseae</i>	Mixed AM	LSD <sub>0.05</sub>
Shoot dry weight (gr)	0.94 $\pm$ 0.05 <sup>b</sup>	1.14 $\pm$ 0.05 <sup>a</sup>	1.11 $\pm$ 0.05 <sup>a</sup>	1.09 $\pm$ 0.05 <sup>a</sup>	0.14
Root dry weight (gr)	4.33 $\pm$ 0.35 <sup>a</sup>	2.64 $\pm$ 0.35 <sup>b</sup>	3.39 $\pm$ 0.35 <sup>ab</sup>	4.14 $\pm$ 0.37 <sup>a</sup>	1.00
S/R	0.38 $\pm$ 0.05 <sup>bc</sup>	0.64 $\pm$ 0.05 <sup>a</sup>	0.47 $\pm$ 0.05 <sup>b</sup>	0.30 $\pm$ 0.05 <sup>c</sup>	0.13
Number of tillers	6.36 $\pm$ 0.23 <sup>a</sup>	6.92 $\pm$ 0.24 <sup>a</sup>	6.61 $\pm$ 0.23 <sup>a</sup>	6.65 $\pm$ 0.24 <sup>a</sup>	NS
Tiller height (cm)	16.59 $\pm$ 0.22 <sup>c</sup>	18.26 $\pm$ 0.23 <sup>a</sup>	17.68 $\pm$ 0.22 <sup>ab</sup>	17.17 $\pm$ 0.23 <sup>bc</sup>	0.62
Number of leaves	5.49 $\pm$ 0.21 <sup>c</sup>	6.81 $\pm$ 0.23 <sup>a</sup>	6.06 $\pm$ 0.21 <sup>bc</sup>	6.44 $\pm$ 0.23 <sup>ab</sup>	0.59
Leaf growth rate (cm/d)	0.14 $\pm$ 0.01 <sup>a</sup>	0.18 $\pm$ 0.01 <sup>a</sup>	0.16 $\pm$ 0.01 <sup>a</sup>	0.16 $\pm$ 0.01 <sup>a</sup>	NS

Means within each row followed by the same letter are not significantly different (LSD Test,  $p \leq 0.05$ ).

Tab. 4. Effects of watering and mycorrhiza colonization on dry weights of shoot and root, on S/R ratio and on growth parameters (Mean  $\pm$  S.E.) of *Dactylis glomerata*

Treatments	Shoot dry weight (gr)	Root dry weight (gr)	S/R	Number of tillers	Tiller height (cm)	Number of leaves	Leaf growth rate (cm/d)
<b>Watered</b>							
Control	1.12 $\pm$ 0.07	1.93 $\pm$ 0.49 <sup>de</sup>	0.64 $\pm$ 0.07 <sup>b</sup>	7.71 $\pm$ 0.32 <sup>a</sup>	17.39 $\pm$ 0.32 <sup>bc</sup>	5.85 $\pm$ 0.30	0.16 $\pm$ 0.02
<i>Glomus intraradices</i>	1.23 $\pm$ 0.07	1.26 $\pm$ 0.49 <sup>c</sup>	1.01 $\pm$ 0.07 <sup>a</sup>	7.78 $\pm$ 0.35 <sup>a</sup>	18.37 $\pm$ 0.35 <sup>a</sup>	6.94 $\pm$ 0.34	0.20 $\pm$ 0.02
<i>Glomus mosseae</i>	1.35 $\pm$ 0.07	1.94 $\pm$ 0.49 <sup>de</sup>	0.73 $\pm$ 0.07 <sup>b</sup>	6.96 $\pm$ 0.32 <sup>ab</sup>	17.34 $\pm$ 0.32 <sup>bc</sup>	6.68 $\pm$ 0.30	0.18 $\pm$ 0.02
Mixed AM	1.19 $\pm$ 0.07	5.61 $\pm$ 0.49 <sup>ab</sup>	0.22 $\pm$ 0.07 <sup>cd</sup>	5.99 $\pm$ 0.32 <sup>c</sup>	16.63 $\pm$ 0.32 <sup>cd</sup>	6.30 $\pm$ 0.30	0.17 $\pm$ 0.02
<b>Drought stressed</b>							
Control	0.76 $\pm$ 0.07	6.74 $\pm$ 0.49 <sup>a</sup>	0.11 $\pm$ 0.07 <sup>d</sup>	5.62 $\pm$ 0.32 <sup>c</sup>	15.78 $\pm$ 0.32 <sup>d</sup>	5.12 $\pm$ 0.30	0.12 $\pm$ 0.02
<i>Glomus intraradices</i>	1.05 $\pm$ 0.07	4.02 $\pm$ 0.49 <sup>cd</sup>	0.27 $\pm$ 0.07 <sup>cd</sup>	6.05 $\pm$ 0.32 <sup>c</sup>	18.16 $\pm$ 0.32 <sup>a</sup>	6.67 $\pm$ 0.30	0.16 $\pm$ 0.02
<i>Glomus mosseae</i>	0.86 $\pm$ 0.07	4.82 $\pm$ 0.49 <sup>bc</sup>	0.22 $\pm$ 0.07 <sup>cd</sup>	6.26 $\pm$ 0.32 <sup>bc</sup>	18.03 $\pm$ 0.32 <sup>ab</sup>	5.44 $\pm$ 0.30	0.15 $\pm$ 0.02
Mixed AM	0.98 $\pm$ 0.07	2.66 $\pm$ 0.55 <sup>d</sup>	0.38 $\pm$ 0.07 <sup>c</sup>	7.31 $\pm$ 0.35 <sup>a</sup>	17.80 $\pm$ 0.35 <sup>ab</sup>	6.57 $\pm$ 0.34	0.15 $\pm$ 0.02
LSD <sub>0.05</sub>	NS	1.41	0.19	0.89	0.88	NS	NS

Means within each column followed by the same letter are not significantly different (LSD Test,  $p \leq 0.05$ ).

leaves under up to full and limited irrigation. This could be associated with the higher accumulation of phosphorus, nitrogen and potassium from the soil (Erman *et al.*, 2011; Jacobsen, 1994; Pearson and Jacobsen, 1993).

In contrast, under drought stress conditions all AMF plants had lower root dry weights than control plants. This result is in agreement with Di and Allen (1991) for *Agropyron* cultivars and Cavagnaro *et al.* (2008) for *Solanum lycopersicum* who also found that AMF colonization of plants decreased root biomass. It seems that the AMF inoculated plants cope with water deficit by other mechanisms and not by increasing the root biomass at the expense of shoot biomass. For instance, the AMF inoculated plants of *Agropyron* cultivars performed better under water stress due to their higher water use efficiency compared to non-inoculated ones (Di and Allen, 1991). According to Ruiz-Lozano *et al.* (1995), there is a differentiated effect on plant adaptation to drought among the AMF species. The seven fungal species of genus *Glomus* they studied had the particular ability to alter the physiological parameters that enhance the adaptation of *Lactuca sativa* L. cv. Romana to low soil water content. Additionally, the low root dry weight can be associated with the AMF symbiosis, which improved significantly the resistance of the inoculated plants to drought by reducing the need of an increase to the roots.

Among the studied mycorrhizae species, *Glomus intraradices* performed better than *Glomus mosseae* and their mixture as it increased S/R ratio, tiller height and number of leaves. In contrast to our results, Moradi and Salimi (2013) reported that *Glomus mosseae* was more effective for the growth parameters of *Poa* sp. This confirms that AMF inoculation has positive effects on various plants, but there are variations in response of the varieties, cultivars or species to various AMF species (Long *et al.*, 2010; Naher *et al.*, 2013; Tufenkci *et al.*, 2012; Wang *et al.*, 2008). The mix of these mycorrhiza species although it increased the growth parameters of *Dactylis glomerata* in comparison to the control, it was inferior to the other AMF application treatments. Moreover, the mix reduces drastically the root system of the plants under limited irrigation, which could detrimentally affect the plants growth under prolonged drought and the sward persistence. This is probably has to do with the interaction between the two mycorrhiza species that reduces their positive effect to plants. The variation upon the growth response is not unusual, with mycorrhizal growth response varying from positive to neutral or negative. These responses are influenced by the symbiotic partners, the genotypes, the developmental stages and the environmental conditions (Facelli *et al.*, 2010; Klironomos, 2003; Smith and Smith, 2011). In particular, the soil conditions could determine how well the non mycorrhizal plants grow in relation to mycorrhizal plants. Negative response also correlates with the higher symbiotic cost in relation to the photosynthetic supply (Jacobsen, 1999). However, the negative growth response as reported here, the reduced number of tillers, may be related to a hormonal response to AMF. Strigolactones, a newly described plant hormone are connected with the number of the tillers in plants (Foo and Reid, 2013). AMF and strigolactones interactions could result to a reduced number of tillers.

When plant roots were inoculated by two AMF species, increased the levels of the strigolactones in the shoot leading to tiller reduction.

## Conclusions

In conclusion, this study provided evidence that AMF have the potential to be beneficial on growth characteristics and dry matter production of *Dactylis glomerata* grown under different water availability conditions. The beneficial activity of AMF is most likely to be favored under drought stress. Therefore, further studies on *Dactylis glomerata*-AMF symbiosis in the field are needed in order this symbiosis to be tested under plant competition and grazing conditions. The increased number of leaves found in the inoculated plants is an indicator of ameliorated nutritive value (Parissi *et al.*, 2007) but the effects of AMF inoculation on the forage quality of *Dactylis glomerata* also need to be tested.

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