Growth and nutrition of *Tetraclinis articulata* (Vahl) Mast. cultivated in different rhizosphere soils collected from *Tetraclinis* stand

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Five representative plant species (*Withania frutescens* Pauquy, *Lavandula multifida* L., *Pistacia atlantica* Desf., *Olea europaea* L. subsp. *oleaster* (Hoffmanns. & Link) Negodi and *Tetraclinis articulata* [Vahl] Mast.) were selected from Moroccan *Tetraclinis* woodland in order to evaluate their mycorrhizal potential and to compare the impact of their rhizosphere soil on growth and nutrition of *Tetraclinis articulata* seedlings. We observed that roots of selected plants were highly colonized by Arbuscular Mycorrhizal (AM) fungi. *Lavandula multifida* roots showed the highest colonization percentage (100%). We recovered AM fungal spores from the rhizosphere soils of the five plant species and we found that the spores number from *L. multifida* and *T. articulata* rhizosphere was significantly different from that of other plants. We assessed the mycorrhizal potential of the indigenous soils by using the Most Probable Number (MPN) approach. We found that MPN in soils collected near the five plant species was significantly higher than the one in the bare soil. The average of MPN per 100 g of dry soil was 11.8 (from 5.6 to 25.0) in the bare soil and 228.5 (from 108.0 to 476.0) in *L. multifida* rhizosphere. This result indicates that the soil surrounding the target plants can be used as inoculum for mycorrhization. We conducted a nursery experiment in which *L. multifida* soil yielded a high mycorrhizal percentage in *T. articulata* plants, thus contributing to improve the plant growth. However, the highest formation of arbuscules in *T. articulata* grown in *Lavandula* soil did not significantly affect the growth or the nutrition of plants compared to *Tetraclinis* soil, except for potassium concentration. Our investigation clearly showed that *L. multifida* soil can be used as biofertilizer to inoculate nurseries for *T. articulata* production. This will greatly contribute to sustain *Tetraclinis* woodland.

Keywords. Indigenous soil inoculum, *Tetraclinis* woodland, shrub species, arbuscular mycorrhiza, nutrition.

Croissance et nutrition du thuya de Berbérie (*Tetraclinis articulata* [Vahl] Mast.) mis en culture dans différents sols rhizosphériques de peuplement de thuya. Cinq espèces végétales (*Withania frutescens* Pauquy, *Lavandula multifida* L., *Pistacia atlantica* Desf., *Olea europaea* L. subsp. *oleaster* (Hoffmanns. & Link) Negodi et *Tetraclinis articulata* [Vahl] Mast.), rencontrées au niveau de l'écosystème tétraclinaie au Maroc ont été sélectionnées afin d'évaluer leur potentiel mycorhizien et de comparer l'impact de leur sol rhizosphérique sur la croissance et la nutrition des jeunes plants de thuya (*Tetraclinis articulata*). Les racines des plantes sélectionnées ont été abondamment colonisées par les champignons mycorhiziens arbusculaires (AM). Les racines de *L. multifida* ont montré le pourcentage le plus élevé de mycorhization (100 %). Nous avons ensuite isolé les spores de champignons AM à partir du sol de ces cinq espèces de plantes et nous avons constaté que le nombre total de spores isolées de la rhizosphère de *L. multifida* et *T. articulata* a été sensiblement différent de celui des autres plantes. Le potentiel mycorhizien des sols indigènes a été évalué en utilisant l'approche du nombre le plus probable (MPN). Nous avons constaté que ce nombre était significativement plus élevé dans les sols récoltés près des cinq espèces de plantes, que celui dans le sol sans couvert végétal, soit une moyenne du MPN par 100 g de sol sec de 11,8 (compris entre 5,6 et 25,0) dans le sol nu et 228,5 (compris entre 108,0 et 476,0) dans la rhizosphère de *L. multifida*. Ce résultat indique que le sol entourant les plantes cibles peut être utilisé comme inoculum mycorhizien. En pépinière forestière, les plants de thuya mis en culture dans le sol sous la lavande ont montré les meilleurs pourcentages de mycorhization et ont pu améliorer leur croissance. Toutefois, la production

en arbuscules du thuya se développant dans la rhizosphère de la lavande n'a pas significativement affecté la croissance ou la nutrition des plants par rapport à ceux se développant dans un sol sous le thuya, sauf pour la concentration du potassium. Nos recherches ont montré clairement que le sol sous *L. multifida* peut être utilisé comme inoculum mycorhizien efficace dans la production de *T. articulata* en pépinières forestières, et peut participer ainsi au maintien de l'écosystème tétraclinaie.
Mots-clés. Inoculum indigene, peuplement de thuya, plantes arbustives, mycorhizes à arbuscules, nutrition.

1. INTRODUCTION

Moroccan forest ecosystems are predisposed to degradation because of demographic expansion of the local populations, which is coupled with increased requirements for cultivable lands, commercial exploitation of certain forest trees and climatic constraints (*i.e.* drought, erosion, fire, parasitic attacks, etc.). This degradation results in the reduction of biological diversity, leading over time to the alteration of physico-chemical and biological soil properties (Kennedy et al., 1995; Warren et al., 1996; Carrillo-García et al., 1999).

Arbuscular mycorrhizal symbiotic fungi are regarded as a "key" microbial group in ecosystems due to their capacity to promote the development of plants in degraded areas. The mycorrhizal propagules are involved in the functioning of the resource islands that are developed around plant roots (Azcon-Aguilar et al., 2003; Koide et al., 2004; Ouahmane et al., 2006a; Ouahmane et al., 2006b). In the forest land, the mycorrhizal effect can be obtained either by introducing a fungal symbiont previously selected for its ability to stimulate the growth of the host plant under given environmental conditions (Duponnois et al., 2005), or by the management of the native soil mycorrhizal potential (Duponnois et al., 2001). The success of the second approach is in keeping with the use of highly mycotrophic plant species (grasses and shrubs) that promotes dissemination of mycorrhizal propagules in the soil and enhances the ability of target plants to become inoculated. Barea et al. (1990) reported that in degraded zones, the mycorrhizal component may disappear or, at least, be severely depleted and so it may be necessary to reinforce or replace it by appropriate inoculation.

In Mediterranean areas, the regeneration of a great number of tree and shrub species shows a spatial pattern associated with established plants, suggesting a net positive balance in plant-plant interactions (Zamora et al., 2004). A good shrub cover is the best insurance to avoid soil erosion, to achieve natural regeneration of woodlands, and to boost the success of reforestation (Zamora et al., 2004).

Several studies support the potential for exploiting the natural diversity of mycorrhizal fungi as inoculum sources in revegetation programs. Azcon et al. (1997) showed that *Lavandula* plants must be mycorrhized in order to thrive in degraded soils from desertification-threatened areas in typical Mediterranean ecosystems, and the consequences of the mycorrhization of these plants with regards to a revegetation strategy are obvious.

Recently, Ouahmane et al. (2006b) observed that growth and mycorrhizal colonization of *Cupressus arizonica* and *L. multifida* were both improved when the two species were grown together. These results emphasize the role of "resource islands" and "nurse plants" of *Lavandula* species in the regeneration processes of forest trees such as *Cupressus* spp. Thus, the conservation of ecosystems is highly dependent on the composition of the soil microbial communities, especially, mycorrhizae.

In *Tetraclinis* woodlands, *T. articulata* is an endomycorrhizal-dependant species (Diaz et al., 1993; Abbas et al., 2006) and many shrub species are observed in the natural distribution area of this forest plant, including *Withania frutescens* Pauquy, *Lavandula multifida* L., *Pistacia atlantica* Desf., *Pistacia lentiscus* L., *Olea europaea* L. subsp. *oleaster* (Hoffmanns. & Link) Negodi. However, there are no studies on the potential of those species to produce arbuscular mycorrhizal (AM) inoculum for selected plant species to be used in revegetation strategies. In addition, plants growing under natural conditions differ in their ability to enrich the soil by the mycorrhizal propagules and the effectiveness of AM depends on the native host species (Azcón-Aguilar et al., 2003; Caravaca et al., 2005). Therefore, the aim of this study was to assess whether the most distributed shrub species in *Tetraclinis* woodlands could form a reservoir of mycorrhizal propagules at the root system layers of *T. articulata* and could be used as an efficient mycorrhizal inoculum for *T. articulata* production in forest nurseries.

2. MATERIALS AND METHODS

2.1. Study site

The experimental area is located in Khémisset, Morocco (33' 52 557 N, 005' 54 907 W, 279 m above sea level). The climate is similar to that expected

in semi-arid Mediterranean environments, with an average annual rainfall of 577 mm and a mean annual temperature of 17.8 °C. The substratum is schistose or siliceous with a neutral to neutral-alcaline pH and low amounts of nitrogen and phosphorus, respectively 0.50‰ and 3.8 mg·kg^{-1} of soil. *Tetraclinis articulata*, *P. atlantica*, *L. multifida*, *W. frutescens* and *O. oleaster* were the perennial shrubs the most widely distributed in this site. They were always recorded in the vicinity of *Tetraclinis articulata* adult and then selected as target species in the sampling area. The physico-chemical analysis of soil from those plants is summarized in **table 1**.

2.2. Field sampling

Soil samples were collected from the rhizospheres of the five target species (*W. frutescens*, *L. multifida*, *P. atlantica*, *O. oleaster* and *T. articulata*) in the experimental area. For each target species, five individual plants were randomly chosen and rhizosphere soil samples were taken. Each sample consisted of five sub-samples (100 cm^3) collected at a depth of 10-20 cm. Control samples were randomly collected from bare soil site, away from plant influence. Roots of target species (about 100 g fresh root per species) were randomly collected within the experimental area at 20 cm depth and conserved in alcohol (50%).

2.3. Mycorrhizal quantification

Roots were washed with tap water, clarified and stained according to the method of Phillips and Hayman (1970). They were then placed on a slide in a drop of polyvinyl alcohol-lactic acid-glycerol (PVLG) (Koske et al., 1983) for microscopic observation (Trouvelot et al., 1986). About 90 root pieces (1 cm) were observed per plant.

Mycorrhizal development was evaluated by the method of Trouvelot et al. (1986) and expressed as frequency of AM colonization (F%, percentage of root fragments showing fungal colonization), intensity of AM colonization (M%, which gives an estimation of the amount of root cortex that became mycorrhized and is referred to the whole root system) and arbuscule abundance (A%, which gives an estimation of the arbuscule richness in the whole root system).

AM fungal spores were extracted from the rhizosphere of each plant and from bare soils by wet sieving and decanting method, followed by sucrose centrifugation (Sieverding, 1991). Then the supernatant was poured through a 50 μm sieve and rinsed with tap water. Spores were counted using a stereomicroscope and grouped according to their morphological characteristics. The uniformity of the morphological groups was confirmed under an optical microscope and the different morphotypes were identified according to genus. Spore identification was assessed mainly using spore size and color, wall structure and hyphal attachment (Walker, 1983; INVAM, 1997).

2.4. Indigenous soil inoculum potentials

The mycorrhizal potential of the rhizosphere soil samples collected from the five target species and bare soil was measured by the well-known "Most Probable Number" method (MPN), using the dilution technique (Sieverding, 1991). Six dilutions of each soil were made by thoroughly mixing the original soil in 1:4 proportions with an autoclaved sandy soil (121 °C, 40 min, two times). After autoclaving, its physical and chemical characteristics were as follows: pH (H$_2$O) 6.7; clay 3.8%; coarse silt 0.8%; fine sand 34.3%; coarse sand 61.1%; carbon 1.89%; total nitrogen 0.08%; total phosphorus 134 mg·kg^{-1}.

Table 1. Physico-chemical characteristics of different studied sols — *Caractéristiques physico-chimiques des différents sols étudiés*.

	T. articulata	*L. multifida*	*P. atlantica*	*O. oleaster*	*W. frutescens*	Sol nu	P
pH	7.5 (± 0.03)	6.99 (± 0.06)	7.67 (± 0.07)	7.66 (± 0.08)	7.6 (± 0.1)	7.21 (± 0.01)	< 0.001
Clay (%)	27.51 (± 0.11)	34.25 (± 0.18)	34.17 (± 1.00)	25.70 (± 0.31)	31.25 (± 0.63)	26.83 (± 0.24)	0.006*
Silt (%)	41.36 (± 0.45)	40.36 (± 0.33)	21.33 (± 0.80)	25.3 (± 0.57)	30.00 (± 0.34)	25.36 (± 0.52)	< 0.001
Sand (%)	31.04 (± 0.37)	25.32 (± 0.29)	44.11 (± 0.18)	48.87 (± 0.50)	38.68 (± 0.47)	47.76 (± 0.43)	< 0.001
Organic matter (%)	3.38 (± 0.03)	4.55 (± 0.09)	2.75 (± 0.03)	5.46 (± 0.26)	2.83 (± 0.04)	1.77 (± 0.03)	< 0.001
Nitrogen (‰)	0.43 (± 0.02)	0.53 (± 0.24)	0.36 (± 0.03)	0.24 (± 0.01)	0.36 (± 0.03)	0.13 (± 0.03)	0.009*
Available phosphorus (mg·kg^{-1})	2.65 (± 0.28)	2.53 (± 0.11)	1.98 (± 0.04)	1.48 (± 0.37)	1.42 (± 0.03)	1.02 (± 0.09)	0.007*

*: Values are based on non-parametric Kruskal-Wallis H-test — *Valeurs de P sur la base du test non paramétrique de Kruskal-Wallis*.

Five replicates were prepared for each dilution. The seeds of *Sorghum vulgare* (surface sterilized with 10% sodium hypochlorite) were pre-germinated for 2-days on humid filter paper. One germinated seed was then transplanted into each of small plastic pots filled with 100 g of different soil dilutions, and pots were placed in the forest nursery. After one month, the entire root system of each seedling was collected, washed under tap water, cleared and stained by the method of Phillips and Haymann. Each entire root system was mounted on a microscope slide and observed at a 250 x magnification under a compound microscope to observe the presence of arbuscular mycorrhizal structures. Data were expressed as the number of AM fungal propagules in 100 g of dry soil and the confidence limits, superior or inferior at 95%, were assigned according to Fisher et al. (1970).

2.5. Growth and nutrition of *Tetraclinis articulata* seedlings

Each soil sample from the rhizosphere of the five target plants and of the bare soil was packed in 500 ml pots. Seeds of *T. articulata* were sown directly on top of the soil. The pots were arranged in a randomized complete block design with five replicates per treatment. Plants were grown in a forest nursery under natural conditions. After nine months, the heights and diameters of the stems were measured. *Tetraclinis articulata* plants were uprooted and the root systems gently washed with tap water. The extent of AM fungal colonization was assessed as described above. After drying (65 °C, 72 h), the dry weight of shoots was measured and samples of shoot tissues were burnt to ash at 600 °C for 3 h and digested in a high acid mix (HCl and HNO_3). The phosphorus was analyzed with a PerkinElmer spectrophotometer and the potassium by atomic spectrophotometry (GBC 906AA).

The total nitrogen was analyzed by Kjeldahl method at the LAMA-US 191 (Laboratory analysis of IRD Centre in Dakar, Senegal).

2.6. Statistical analysis

Statistical analyses were conducted using XLSTAT-2006 at 5 %. We used ANOVA parametric tests when the normality (Kolmogorov–Smirnov test) and homoscedasticity (Levene test) assumption were met and non-parametric tests (Kruskal-Wallis H-test) when the data lacked normality and homoscedasticity. Comparison among means was made using Student Newman-Keuls test calculated at $P < 0.05$. To assess the relationship between mycorrhizal parameters, the non-parametric Spearman's rank correlation coefficient was used. When significant correlations occurred a simple linear regression was developed.

3. RESULTS

3.1. Mycorrhizal quantification in the rhizosphere of the associated shrub species

Natural mycorrhizal root colonization. The examination of the roots from the main mature plants growing in the ecosystem (*L. multifida*, *W. frutescens*, *P. atlantica* and *O. oleaster*) showed that all of them were mycorrhizal and densely colonized. They all had typical mycorrhizal structures (coils, arbuscules, hyphae, and vesicles) but in terms of mycorrhizal intensity (M) and arbuscules, a few plants, like *L. multifida* and *P. atlantica*, showed some potential for mycorrhizal infectivity and could be more receptive to the settlement of mycorrhizal structures (**Table 2**). Furthermore, statistical analysis showed that no correlations were found between all mycorrhization parameters except for *P. atlantica*, where the root colonization was negatively correlated with the mycorrhizal intensity ($Rs = -0.975$, $P = 0.005$). Indeed, the linear regression showed that the two variables are sharply related (Estimate ($\pm$SE) = -0.786 ± 0.129; $t = -6.100$; $P = 0.009$; R^2 adjusted = 0.901).

Population of spores. The number of spores found in the root zones of *T. articulata*, *L. multifida* and *O. oleaster* was higher than in the other species with spores of three different genera of AM fungi (*Glomus*, *Scutellospora* and *Acaulospora*) observed in the different rhizospheres prospected. A significant difference was observed in the total number of AM fungal spores isolated from the rhizosphere soil of *L. multifida* and *T. articulata* with respect to the other soils. Also, the number of *Scutellospora* spores was slightly higher in *L. multifida* than in *T. articulata* soils (**Figure 1**). In our experiment, the number of *Glomus* spores was higher in all the rhizosphere areas.

Table 2. Assessment of root mycorrhization in different target plants — *Évaluation des paramètres de la mycorhization racinaire des différentes plantes cibles.*

Plant species	F (%)	M (%)	A (%)
Tetraclinis articulata	92.22[b]	21.78[bc]	14.30[b]
Lavandula multifida	100.00[a]	36.22[a]	33.85[a]
Pistacia atlantica	93.89[ab]	17.87[c]	35.72[a]
Olea oleaster	98.55[ab]	31.44[ab]	22.05[b]
Withania frutescens	93.85[ab]	31.82[ab]	21.04[b]

F: AMF colonisation — *colonisation par les CMA*;
M: intensity of mycorrhization — *intensité de mycorhization*;
A: arbuscules — *arbuscules*; Mean values in columns followed by different letters are significantly different (p ≤ 0.05) — *les valeurs moyennes dans les colonnes suivies de lettres différentes sont significativement différentes (p ≤ 0,05).*
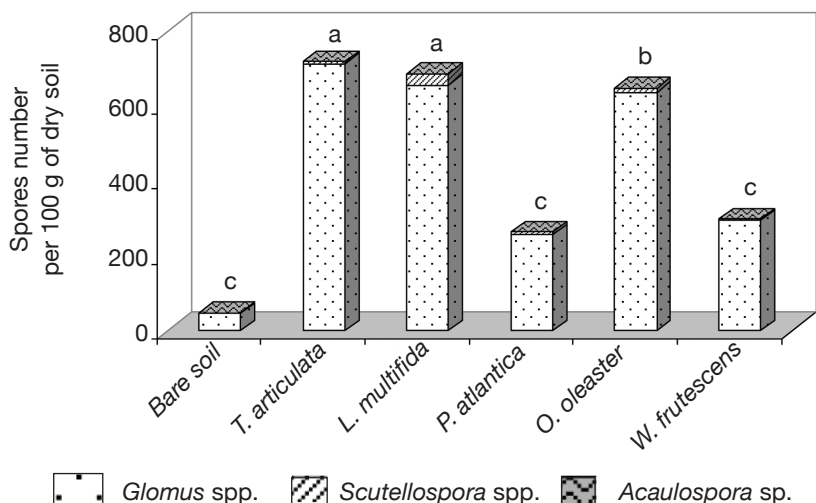
Figure 1. Arbuscular mycorrhizal fungal (AMF) spore abundance in the rhizosphere areas of *Lavandula multifida*, *Withania frutescens*, *Pistacia atlantica*, *Olea oleaster*, *Tetraclinis articulata* and bare soil — *Abondance des spores de CMA au niveau de la rhizosphère de* Lavandula multifida, Withania frutescens, Pistacia atlantica, Olea oleaster, Tetraclinis articulata *et du sol hors couvert végétal*.

Data followed by the same letter are not significantly different according to the one-way analysis of variance (p < 0.05) — *Les données suivies de la même lettre sont non significatives selon l'analyse de la variance à un seul facteur (p < 0,05).*

The main species collected were *Glomus aggregatum*, *Glomus constrictum* and *Glomus etunicatum* identified according to the classical phenotypic parameters (spore wall, coloration, etc..) (Palenzuela Jiminez Eulogio Javier, Estacion Experimental del Zaidin, Granada-Spain, pers. com.)

3.2. Indigenous soil inoculum potentials

The number of mycorrhizal propagules in soils collected near the five plant species was significantly higher than the one in the bare soil. The average of MPN per 100 g of dry soils tested was in the following order: *L. multifida* > *P. atlantica* > *T. articulata* > *O. oleaster* > *W. frutescens* > bare soil. This result indicates that *L. multifida* was the most important plant that was able to improve its rhizosphere soil in mycorrhizal propagules. Indeed, the average of MPN per 100 g of dry soil was 88.93% higher in *L. multifida* rhizosphere (228.5) than in the bare soil (11.8) (**Table 3**).

3.3. Growth and nutrition of *Tetraclinis articulata* seedlings on the rhizosphere soils collected from the different target plants and the bare soil

Mycorrhizal quantification. Data summarized in **table 4** showed that the mycotrophy intensity of the existing vegetation in the studied ecosystem differed from one plant to another. The intensity of AM fungal colonization was significantly higher in *T. articulata* growing in *L. multifida* and *T. articulata* rhizosphere soils than in the other soils. In terms of arbuscules the *L. multifida* soil showed however an enhancement in those structures considered, as a principal site of exchange between plant and fungus in the roots of *T. articulata*. Indeed, analysis showed that, only in *L. multifida*, a positive correlation was found between

Table 3. Indigenous soil inoculum potentials of AM fungi, assessed by the MPN method (MPN: Most Probable Number) — *Estimation des potentiels mycorhizogènes indigènes des sols par la méthode MPN (MPN : le nombre le plus probable).*

Soils	AM infective propagules per 100g of soil (95% confidence limits)
Bare soil	11.84 (5.6-25.4)
Tetraclinis articulata	106.14 (49.92-228.7)
Lavandula multifida	228.50 (107.88-494.17)
Pistacia atlantica	114.56 (53.89-246.88)
Olea oleaster	96.51 (45,39-207.92)
Withania frutescens	32.70 (15.36-70.35)

Table 4. Quantification of mycorrhizal extension of *Tetraclinis articulata* grown in the rhizosphere of different plant species and in the bare soil — *Quantification de l'extension mycorhizienne du thuya en culture dans la rhizosphère des différentes plantes cibles et dans le sol nu.*

Source inoculum	F (%)	M (%)	A (%)
Bare soil	43.03[c]	0.92[c]	0[b]
Tetraclinis articulata soil	83.51[a]	31.55[a]	10.90[b]
Lavandula multifida soil	81.82[a]	26.52[a]	56.58[a]
Pistacia atlantica soil	57.41[b]	2.52[c]	2.82[b]
Olea oleaster soil	41.57[c]	4.8[c]	0.17[bb]
Withania frutescens soil	78.18[a]	11.57[b]	0[b]

F: AMF colonisation — *colonisation par les CMA*;
M: intensity of mycorrhization — *intensité de mycorhization*;
A: arbuscules — *arbuscules*; Mean values in columns followed by different letters are significantly different (p ≤ 0.05) — *les valeurs moyennes dans les colonnes suivies de lettres différentes sont significativement différentes (p ≤ 0,05).*

mycorrhizal intensity and number of arbuscules ($Rs = 0.900$, $P = 0.037$). The linear regression showed that the two variables are highly related (Estimate ($\pm$SE) = 0.311 ± 0.057; $t = 5.454$; $P = 0.012$; R^2 adjusted = 0.878).

Growth and nutrition of *Tetraclinis articulata* seedlings. After nine months of *T. articulata* growing in the rhizosphere soil of *P. atlantica*, *O. oleaster* and *W. frutescens*, only the plant heights and nitrogen leaf contents were significantly higher compared to plants in the bare soil (**Table 5**). However, it is noteworthy that *T. articulata* and *L. multifida* soils were the most effective source of inoculum to improve stem diameter and phosphorus contents by *T. articulata* species. Differences between *Tetraclinis* plants grown in bare soil and rhizosphere soil from *Tetraclinis* or *Lavandula* were 145 and 145% for height, 90.4 and 174% for stem diameter, 739 and 809% for shoot fresh biomass, 22 and 25% for N concentration, 5.2 and 9.5% for P contents and 0 and 14% for K concentration, respectively. Only the K concentration in soil from *Lavandula* was significantly different from that one in *Tetraclinis* soil.

4. DISCUSSION

Because of the key ecological functions played by AM symbiosis (Jeffries et al., 2003), management of the native soil mycorrhizal potential through native and highly mycotrophic plant species is one of the main reclamation strategies to increase revegetation success (Duponnois et al., 2001; Azcon-Aguilar et al., 2003). A previous work has shown that *Tetraclinis* woodlands contain a high relative abundance of AMF spores (Abbas et al., 2006), and this ecosystem is characterized by a patchy distribution of individual plant species. Thus, this study was carried out to assess the role of shrubby plants mainly encountered in *Tetraclinis* woodlands in improving, by mycorrhizal colonization, the quality

of *T. articulata* production in forest nurseries. Indeed, many experiments in the Mediterranean area have shown the importance of native mycorrhizal potential as a source of AM inoculum for shrubs and trees. Caravaca et al. (2003b) noted that indigenous AM may be considered as a preferential inoculation strategy to guarantee the re-establishment success of native shrub species in a semi-arid degraded soil. Gasque et al. (2004) showed that *Stipa tenacissima*, for example, acts as a nurse plant providing a physical barrier to the water and sediment circulation down slope, which results in habitat improvement for the establishment of *Pinus halepensis*.

In the present study, soil samples were collected from the rhizosphere of five target species (*W. frutescens*, *L. multifida*, *P. atlantica*, *O. oleaster* and *T. articulata*) existing in the experimental area. Mycorrhizal status and effectiveness on *Tetraclinis* seedlings were analyzed. Our results showed that all roots of the target plants examined from the field were highly infected by AM fungi. These results agree fairly well with other survey studies carried out in Mediterranean areas (Requena et al., 1996; Requena et al., 1997), but natural effectiveness of the different target plants was not the same. *Lavandula multifida* and *P. atlantica* showed the best percentage of mycorrhizal intensity (M) and arbuscules (A), and seemed to be more receptive to the establishment of mycorrhizal structures. This feature seems to be important to improve growth and nutrition of *T. articulata* seedlings, because different AM fungal species have different effects on plant performance and nutrient uptake (Allen et al., 1995; Jeffries et al., 2001). Quantification of spore abundance in the rhizospheres of the target plants and in the bare soil showed a predominance of Glomeraceae species which is in agreement with a previous report based on morphological characters of Moroccan *Tetraclinis* woodland spores (Abbas et al., 2006). The dominance of *Glomus* species in a disturbed ecosystem can be explained by the ability of the Glomeraceae to colonize

Table 5. Assessment of growth, nutrition and AM colonization of *Tetraclinis articulata* seedlings growing in different rhizosphere and bare soils after 9 months in nursery — *Évaluation de la croissance, de la nutrition et de la colonisation racinaires par les CMA des jeunes plants de thuya se développant dans la rhizosphère des différentes plantes cibles et sur sol nu, après 9 mois de culture en pépinière.*

	Bare soil	*T. articulata*	*L. multifida*	*P. atlantica*	*O. oleaster*	*W. frutescens*
Height (cm)	6.67[b]	16.33[a]	16.33[a]	12.67[a]	13.5[a]	13.45[a]
Stem diameter (cm)	1.25[b]	2.38[ab]	3.43[a]	1.89[b]	2.00[b]	2.11[b]
Shoot biomass (mg fresh weight)	0.33[c]	2.77[ab]	3.00[a]	1.12[abc]	1.73[abc]	1.07[bc]
N (%)	1.99[d]	2.43[a]	2.49[a]	2.25[c]	2.15[b]	2.11[c]
C/N	22.5[c]	17.8[a]	17.8[a]	19.1[d]	20[bc]	21.2[ab]
P (mg·kg⁻¹)	1,390[c]	1,462.5[ab]	1,517.5[a]	1,352[bc]	1,390[bc]	1,300[c]
K (mg·kg⁻¹)	1,045[b]	1,045[b]	1,200[a]	1,054[b]	980[b]	990[b]

via fragments of mycelium or mycorrhizal root pieces (Biermann et al., 1983). The relative abundance of spores of AM fungi varies with the nature of target species, as sporulation can depend on the AM fungus, the host plant, the soil characteristics and the climatic conditions (Sieverding, 1991). Morphological description showed that only three to five AM fungal spore morphotypes were detected in the rhizosphere of the target plant species and in the bare soil, which can be a signal for degradation of the ecosystem. Indeed, high diversity in natural ecosystems may principally be based on a great variety of plant species (Sieverding, 1991). Thus, vegetable cover has a great benefit for AM propagules density that can be affected by its degradation.

In the current study, counting infective AM propagules from field samples showed that the five target species differ in their capabilities to enrich the soil with mycorrhizal propagules. *Lavandula multifida* was the most effective plant to provide a high number of infective propagules per unit of soil weight. Previous reports have already described that many plants from the Mediterranean area form arbuscular mycorrhizae association and have been classified as "obligatory mycorrhizal" or as "highly dependent on mycorrhiza" (Brundrett, 1991; Habte et al., 1991; Azcon et al., 1997; Caravaca et al., 2003a; Caravaca et al., 2003b; Ferrol et al., 2004). AM fungi can besides enhance the growth of native shrub species in the short term, which in turn creates a more favorable environment for the development of ecosystem processes (Caravaca et al., 2003b). For this reason, autochthonous plant species are widely used for reclaiming degraded lands in Mediterranean semi-arid areas (Caravaca et al., 2002).

Growth of *T. articulata* in the different rhizosphere soils showed different response levels but, generally, it was significantly higher in the rhizosphere of the target species than in the bare soils. The same result was found in glasshouse experiments by Ouahmane et al. (2006a), where the growth of *Cupressus atlantica* seedlings was significantly higher in the *C. atlantica* soil and in the shrub species soils than in the bare soils. Other studies on *L. multifida* and *Cupressus arizona* corroborated the above mentioned results and emphasized the role of "resource islands" and "nurse plants" of *Lavandula* species in the regeneration processes of forest trees such as *Cupressus* spp. (Ouahmane et al., 2006b).

The uptake of nitrogen was significantly stimulated when *T. articulata* was grown in different target soils. This result involves yet again the beneficial effect of AM fungi in this nutrient acquisition as found in previous studies (Tobar et al., 1994a; Tobar et al., 1994b; Azcon et al., 1997). *Lavandula multifida* and *T. articulata* soils showed the best percentage of this improvement. As those soils are poor in N content (Abbas et al., 2006), we can suppose that beneath the

rhizosphere of the two types of species there are other microorganisms, different from the AM fungi, involved also in nitrogen uptake.

With regard to P and K uptake, except for *Lavandula* and *Tetraclinis* soils, there was no significant difference detected between the other soils. Van der Heijden et al. (1998) demonstrated that the increase of AM fungal diversity led to an increased phosphorus content in plant material and, consequently, to a more efficient exploitation of soil phosphorus. In the soil, though, AM fungi are found as spores, hyphae or infected root pieces, and all these propagules can be considered as inoculum sources (Duponnois et al., 2001). It is possible that in our study the AM mycelia network was the main source of inoculum, as previously observed in semi-arid and arid ecosystems (Brundrett et al., 1991; Ouahmane et al., 2006a). However, the highest arbuscule production, in *T. articulata* growing in *Lavandula* soil, did not significantly affect plant growth or nutrition compared with *Tetraclinis* soil, except for K concentration. This nutrient is one of the most important inorganic solutes, and has an important role in processes such as water balance, cell extension and solute transport in the xylem (Porras-Soriano et al., 2009). So, under dry conditions, the K concentration improvement by AM inocula from *Lavandula* soil may be very important to maintain a high tissue water level, even under conditions of osmotic deficiency.

Recently, the diversity of the AMF community composition in roots of two plant species (*T. articulata* and *L. multifida*) that exist alone or co-occur in Moroccan *Tetraclinis* woodlands was characterized using molecular techniques (Bakkali et al., 2011). The highest diversity was found in the roots of *T. articulata* alone, followed by the associated *T. articulata-L. multifida* and finally *L. multifida* alone. Many AMF were shared between the two species, although a few of them had a preference for one or the other (Bakkali et al., 2011). Maybe particular plant-fungus combinations are flavored according to the ecological conditions. Therefore, further research, using molecular tools (PCR/sequencing), is needed to identify and select the most efficiency spore species to be used in inoculation tests of *T. articulata*. Also, it is important to address in *Tetraclinis* woodlands the relationship between AM fungi sporulation, AM mycelia network and morphotype of AM fungi associated to *T. articulata* roots.

Finally, more research input is also needed to test the effect in the field, with different ecological conditions, of AM propagules isolated from the *Lavandula* rhizosphere to enhance the development and nutrition of *T. articulata*.

The present study supports the beneficial effects from a shrub species established in *Tetraclinis* woodland. *Lavandula multifida* rhizosphere seems to be of great

interest and could be used as an efficient mycorrhizal inoculant for the production of *T. articulata* in forest nurseries.

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