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Effect of aromatic plant species on vesicular-arbuscular mycorrhizal establishment in *Pistacia terebinthus*

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Abstract

The inoculation of *Pistacia terebinthus* with vesicular-arbuscular mycorrhizal (VAM) fungi and the spread of the infection were studied using a mixed cropping system, under glasshouse conditions, with *Salvia officinalis*, *Lavandula officinalis* and *Thymus vulgaris* colonized by *Glomus mosseae* as an inoculation method. This method was compared with soil inoculum placed under the seed or distributed evenly in the soil. Indirect inoculation with all the aromatic plants tested significantly increased VAM root colonization of *P. terebinthus* compared with the use of soil inoculum, although the effect on plant growth was different for each one of the aromatic species used as inoculum source. Inoculation with *L. officinalis* and *T. vulgaris* were the best treatments resulting in high VAM colonization and growth enhancement of *P. terebinthus*.

Introduction

Pistacia spp. is naturally infected by indigenous vesicular-arbuscular mycorrhizal (VAM) fungi, although the symbiosis is not widespread in commercial plantations (Estaún et al., 1990) where it develops later in time (Estaún, 1991). Under controlled conditions *P. vera*, *P. atlantica* and *P. terebinthus* formed the symbiosis when inoculated with *Glomus mosseae* (Nicol. and Gerd.) Gerd. and Trappe (Estaún et al., 1990). To achieve early benefits the symbiosis should be established at seedling level, but the inoculation is difficult to accomplish because of management problems probably related to the geometry and lignification patterns of root development.

This work compares different methods for VAM inoculation including the use of pre-infected aromatic plants as an indirect system. These aromatic plants have been shown to be mycorrhizal dependent and achieve a high percentage of internal infection (Camprubi et al., 1990). The growth of aromatic plants in a dual inoculation system can produce an additional crop in the field.

Materials and methods

Seeds of *Thymus vulgaris* L., *Salvia officinalis* L. and *Lavandula officinalis* L. were surface sterilized with a 0.5% NaClO solution for 30 minutes, rinsed thoroughly with sterile distilled water and sown in sterile sandy soil (steamed twice at 100°C for 1 h with a 24 h interval). Three weeks after seed emergence, the seedlings were transferred to small individual containers (150 mL) and inoculated with 3 g of *Glomus mosseae* soil inoculum. The inoculum was rhizosphere soil from a white clover (*Trifolium repens* L.) stock culture containing spores and infected root fragments. After three months the roots of 10 plants from

each aromatic plant species were cleared in KOH and stained with trypan blue in lactic acid (Kormanik and McGraw, 1982) and mycorrhizal internal infection assessed using the gridline intersect method (Giovanetti and Mosse, 1980).

Seeds of *Pistacia terebinthus* were treated first with H_2SO_4 (96%) for 30 minutes, then soaked for 24 h in 20 ppm giberellic acid (GA_3) solution and sown also in sterile sandy soil.

P. terebinthus seedlings were planted individually in containers filled with 5 L steam-sterilized sandy soil of low P content (9 ppm available P (Olsen)). The following inoculation procedures were tested using 10 seedlings per treatment: noninoculated control (1), *G. mosseae* soil inoculum (15 g) placed under the seedlings (2), *G. mosseae* soil inoculum (25 g) homogenously distributed in the container (3), and two 3 month-old plants of *S. officinalis* (4), *L. officinalis* (5) and *T. vulgaris* (6) colonized by *G. mosseae*. The Percentage infection of these plants was, respectively, 54%, 52% and 47%, and they were placed at a 5 cm distance from the *P. terebinthus* plantlet in the same container. A filtrate of soil inoculum free from VAM propagules was added to the non-inoculated controls.

Containers were watered weekly with 25 ml of Hoagland's nutrient solution without P (Hoagland and Arnon, 1950) and the plants were maintained in glasshouse conditions.

After 16 weeks growth, plants were harvested. Dry weight of roots and shoots and the total root density (mg fresh root cm^{-3} soil) (Scheltema et al., 1987) of both plant species, *P. terebinthus* and the associated aromatic plants, were recorded. The root system was cleared and stained (Kormanik and McGraw, 1982) and the VAM infection quantified (Giovanetti and Mosse, 1980). Data were statistically analyzed by analysis of variance followed by Tukey's multiple range test ($p = 0.05$).

Results

Percentage of root colonization was significantly higher when *P. terebinthus* was indirectly inoculated through aromatic plants compared with the soil inoculum treatments (Table 1).

The growth of *P. terebinthus* was significantly enhanced by the inoculation with soil inoculum distributed throughout the container and by soil inoculum portions placed under the roots. No statistically significant differences were found between shoot dry weights of non-mycorrhizal plants and those of plants inoculated with pre-infected aromatic plants (Table 1) and no relationship was found between the percentage of VAM colonization and plant size.

The root : shoot ratio was lower in mycorrhizal plants compared with uninoculated controls except when inoculation had been achieved through *S. officinalis*. In this last treatment the lowest growth of *P. terebinthus* roots and shoots, and the lowest root density were recorded (Table 1).

S. officinalis plants had the highest root density and shoot growth while *L. officinalis* plants exhibited little shoot development and *T. vulgaris* had the lowest root density (Table 2) among the aromatic plants. The percentage of root VAM colonization of the aromatic plants was the same at the begining as at the end of the experiment.

Table 1. Effect of inoculation systems on growth of *P. terebinthus* and VAM inoculation by *G. mosseae*

Inoculum source	Shoot dry weight (g)	Root/shoot	Root density (mg cm^{-3})	Mycorrhizal root (%)
1	0.11 a	1.29 b	0.05 ab	–
2	0.25 b	0.77 a	0.07 b	19 a
3	0.29 b	0.75 a	0.05 ab	20 a
4	0.09 a	1.03 ab	0.03 a	39 b
5	0.21 ab	0.84 a	0.07 b	38 b
6	0.18 ab	0.76 a	0.05 ab	33 b

(1) Uninoculated control, (2) localized and (3) distributed soil inoculum, (4) mycorrhizal *S. officinalis*, (5) *L. officinalis* and (6) *T. vulgaris*. Figures in the same column followed by the same letter are not significantly different after Tukey's multiple-range test ($p = 0.05$).

Table 2. Size parameters and mycorrhizal colonization of the aromatic plants used as inoculum source of *G. mosseae*

Plant species	Shoot dry weight (g)	Root dry weight (g)	Root density ($mg\ cm^{-3}$)	Mycorrhizal root (%)
S. officinalis	2.43 ± 0.75	1.25 ± 0.52	1.21 c	53 ± 10
L. officinalis	1.33 ± 0.55	0.72 ± 0.30	0.85 b	57 ± 5
T. vulgaris	1.89 ± 0.85	0.48 ± 0.27	0.38 a	49 ± 6

Mean ± SD. Average of 20 plants.
Figures in the same column followed by the same letter are not significantly different after Tukey's multiple-range test ($p = 0.5$).

Discussion

The inoculation with aromatic plants as 'donor' is an effective technique to obtain a high mycorrhizal root colonization level. The cultivation of aromatic plants with *P. terebinthus* in the same container improves the mycorrhizal status of *P. terebinthus* roots, but not all the aromatic species had the same effect on growth of *P. terebinthus*. We tested three aromatic plant species adapted to the same environmental conditions suitable for *Pistacia* trees (periods of water stress, calcareous soils and extreme temperatures). In our experiment we found that *L. officinalis* and *T. vulgaris* were the best intercropping plants for inoculating *P. terebinthus* in the early stages of development. The third aromatic plant used, *S. officinalis*, was a good host plant to spread VAM infection but it depressed the growth and root density of *P. terebinthus* growing in the same container. This growth reduction can be explained by physical competition among the roots of each species for a limited supply of soil nutrients, that will be probably overcome when transplanted to field conditions with no longer physical limitation. The volume of soil available to plants grown in containers affects nutrient uptake (Baath and Hayman, 1984). *S. officinalis* had a more extensive root system than *P. terebinthus* and the other two species of aromatic plants.

The establishment, spread and persistence of inoculum must be considered as important items in the economics of field inoculation with VAM endophytes. All these factors will decide the quantities of inoculum needed, its placement in the field and whether inoculation will have to be repeated after harvest (Warner and Mosse, 1982). Mycorrhizal infection of plants in many natural vegetation systems arises when uninfected roots contact the VA mycorrhizal mycelium spreading from infected roots (Francis and Read, 1984). Inoculation of *P. terebinthus* with previously infected VAM plants was a more practical and efficient means to obtain VAM colonization in *P. terebinthus* roots by *G. mosseae*.

This study considers the possibility of introducing a secondary crop on a field to stimulate mycorrhizal development on pistachio. If aromatic plants are introduced in the field as a source of VAM inoculum for *Pistacia* orchards, and excellent intercropping system could be achieved with an additional crop of agricultural value in mediterranean semi-arid zones.

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