A field inoculation system for citrus nurseries using pre-cropping with mycorrhizal aromatic plants

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ABSTRACT
Lavandula vera, *Thymus vulgaris* and *Rosmarinus officinalis* plantlets, colonized with a selected *Glomus intraradices* isolate, were planted in a soil that had been used as a citrus nursery 5 years before. Four treatments were established: fumigated citrus nursery bed soil with and without aromatic plants, and nonfumigated citrus seedbed soil with and without aromatic plants. Six months after planting, aromatic plants were cut and seeds of Troyer citrange and Cleopatra mandarin were sown in the same field soils. The number of arbuscular mycorrhizal propagules increased significantly after crop rotation with aromatic plants, and this proved to be highly beneficial for the survival and growth of Troyer citrange and Cleopatra mandarin. Crop rotation with mycorrhizal aromatic plants would be a good agronomic practice to introduce mycorrhizal fungi into agricultural soils.

KEYWORDS
Citrus, plant nurseries, essential oil crops, inoculation methods, soil, *Glomus intraradices*, mycorrhizae.

Une technique d'inoculation en champ pour pépinières d'agrumes, utilisant la culture préalable de plantes aromatiques mycorhizées.

RÉSUMÉ
Des espèces aromatiques, *Lavandula vera, Thymus vulgaris* et *Rosmarinus officinalis*, mycorhizées avec *Glomus intraradices*, ont été transplantées dans un terrain, utilisé 5 ans auparavant pour produire des porte-greffes de *Citrus*. Quatre traitements ont été établis : sol fumigé avec ou sans plantes aromatiques, et sol non fumigé avec ou sans plantes aromatiques. Ces plantes ont été moissonnées après 6 mois de croissance et des graines de citrange Troyer et de Cleopatra semées en place. Le nombre de propagules infectifs de champignons mycorhiziens à arbuscules a augmenté nettement avec l'introduction des plantes mycorhizées ; cela a favorisé la survie et le développement des deux porte-greffes. La rotation de cultures serait donc un bon système pour introduire les mycorhizes dans des terrains agricoles.

MOTS CLÉS
Citrus, pépinière, plantes à huiles essentielles, inoculation, sol, *Glomus intraradices*, mycorhize.

Cultivo previo con plantas aromáticas micorrizadas como sistema de introducción de micorrizas en viveros de cítricos.

RESUMEN
Plantas de *Lavandula vera, Thymus vulgaris* y *Rosmarinus officinalis*, micorrizadas con *Glomus intraradices*, se trasplantaron a un suelo utilizado como semillero de patrones de cítrico 5 años antes. Se establecieron cuatro tratamientos: semillero desinfectado con y sin plantas aromáticas, y semillero no desinfectado con y sin plantas aromáticas. Las plantas aromáticas se segaron tras seis meses de crecimiento en campo y sobre las mismas franjas de suelo se sembraron semillas de citrange Troyer y mandarino Cleopatra. El número de propágulos infectivos de hongos formadores de micorrizas arbusculares aumentó considerablemente con la introducción de especies micorrizadas, y esto favoreció netamente la supervivencia y el crecimiento de los dos patrones. Los resultados indican que la rotación de cultivos con plantas aromáticas micorrizadas es un buen sistema de incorporación de hongos formadores de micorrizas arbusculares en suelos agrícolas.

PALABRAS CLAVES
Citrus, viveros, plantas aromáticas, inoculación, suelo, *Glomus intraradices*, micorrizas.

introduction

The seedbeds used for growing citrus rootstock plants are often fumigated after removal of the previous crop to eradicate soil-borne *Phytophthora* spp and nematodes. Many authors (MENGE et al, 1978; TIMMER and LEYDEN, 1980) have shown that general biocides greatly reduce the propagule density of indigenous arbuscular mycorrhizal (AM) fungi. Due to the high mycorrhizal dependency of some citrus rootstocks (KLEINSCHMIDT and GERDEMANN, 1972; NEMEC, 1978), plants growing in fumigated soils show stunting symptoms typical of phosphorus (P) and micronutrient deficiences (MARTIN et al, 1968; KLEINSCHMIDT and GERDEMANN, 1972; SCHENCK and TUCKER, 1974). To avoid potential replant problems, nurserymen in Spain use new field sites every year that have never been planted with citrus before. These fields are usually orchards of widely spaced almond, olive or carob trees grown without irrigation. Furthermore, the orchard floor is sparsely populated with weed hosts for AM fungi. Trees are removed to prepare the field for establishment of the citrus nursery.

Some methods proposed for production and application of pot culture inoculum have limited applicability to commercial production of AM inoculum primarily because the methods are not useful in the field. FERGUSON and MENGE (1986) described various field inoculation methods with *Glomus deserticola* Trappe, Bloss et Menge, in fumigated citrus nursery soils, using different types of pot culture inoculum (soil inoculum, spores and lyophilized roots). In the present research, a field inoculation system is proposed, beginning with the cultivation of mycorrhizal aromatic plants in citrus seedbeds before sowing. Previous greenhouse studies (CAMPRUBÍ et al, 1992) have shown that the use of mycorrhizal aromatic plants, colonized with *Glomus mosseae* (Nicol et Gerd) Gerdemann et Trappe, was an effective method to obtain a high mycorrhizal root colonization level in *Pistacia* spp and to establish AM inoculum in the soil.

The purpose of this study was to evaluate the effect of a crop rotation with aromatic plants colonized with a selected isolate of *Glomus intraradices*, as a system to propagate mycorrhizal fungi in citrus nurseries and other agricultural soils.

materials and methods

Seeds of *Thymus vulgaris* L, *Lavandula vera* L and *Rosmarinus officinalis* L were surface sterilized with a 0.5% NaClO solution for 10 min, rinsed with sterile distilled water and sown in seedbed containers of sterilized sandy soil and mixed soil inoculum of a selected isolate of *G intraradices*. The fungus was isolated from a citrus nursery in Tarragona (Spain) and was found to be associated with citrus rootstocks in commercial orchards (CAMPRUBÍ and CALVET, 1996). The spores of this *Glomus* species are formed primarily inside the host roots and the mycorrhizal root fragments are the major source for inoculum. Two months after seed emergence, mixed root samples from each aromatic plant species were cleared in 10% KOH and stained with 0.05% trypan blue in lactic acid (PHILLIPS and HAYMAN, 1970; KOSKE and GEMMA, 1989), and internal mycorrhizal infection assessed using the gridline intersect method (GIOVANNETTI and MOSSE, 1980). The percentage of root colonization in these plants was 88, 78 and 79% for *T vulgaris*, *L vera* and *R officinalis*, respectively. The aromatic mycorrhizal plants were transferred to 20 l containers filled with a mixture of sphagnum peat and sand (1:2; v/v).

After 5 months growth under greenhouse conditions (July 1993), the mycorrhizal aromatic plants were transplanted to a citrus nursery soil that had been fumigated 5 years before and not used since for citriculture, or any other crop. One section (8 × 10 m) of the nursery bed was fumigated with metham-sodium (Vapam, ICI Zeltia) and the other was left nonfumigated. In each section two spaces were delimited, and, in one of them, the mycorrhizal aromatic plants were transplanted in rows at random; each plant was spaced 20 cm apart from the rest.

Six months after the introduction of aromatic plants in the field, plant tops were cut off leaving the colonized roots into the soil. The most probable number of AM propagules was calculated (PORTER, 1979; POWELL, 1980). Seeds of Troyer citrange [*Citrus sinensis* (L) Obs x *Poncirus trifoliata* (L) Raf] and Cleopatra mandarin (*Citrus reshni* Hort ex Tan) were sown in each section of the same field in rows 8 m long and 60 cm apart.

After 6 months growth of citrus rootstocks (1 year after the introduction of aromatic plants in the soil), the shoot length of the plants was measured and their survival evaluated (in three 1 m length sections of the seedbed). Root samples were collected and stained with 0.05% trypan blue in lactic acid (PHILLIPS and HAYMAN, 1970; KOSKE and GEMMA, 1989) and the presence of AM colonization in citrus roots was assessed. To estimate the number of infective AM propagules present in the soil, the most probable number (MPN) was determined using tenfold series of soil dilutions with autoclaved soil as diluent (PORTER, 1979; POWELL, 1980).

results

The results of the quantitative analysis (MPN) of AM propagules are shown in table I.

The number of AM propagules measured without introduction of aromatic plants was undetectable at the beginning and very low at the end of the experiment.

The introduction of mycorrhizal aromatic plants increased the number of AM propagules in the soil, especially in the nonfumigated nursery bed. The subsequent planting of Troyer citrange in the soil inoculated with mycorrhizal aromatic plants further improved the mycorrhizal inoculum status of the soil.

Seed emergence of Cleopatra mandarin was lower than that of Troyer citrange and less Cleopatra mandarin plants survived in the seedbed (table II).

Density of Cleopatra mandarin plants increased when mycorrhizal aromatic plants had been grown previously in the soil. Prior cropping with mycorrhizal aromatic plants in the seedbed significantly increased growth of Troyer citrange and Cleopatra mandarin (photo 1) due to the AM colonization in roots.

The plants from the inoculated beds were heavily mycorrhizal after 6 months growth with extensive internal mycelium growth, arbuscules and internal spores. Plants grown in noninoculated beds had negligible or no AM colonization at all. Troyer citrange achieved the highest shoot length in fumigated soil with the introduction of aromatic plants whereas Cleopatra mandarin grew better in nonfumigated soil with the introduction of mycorrhizal aromatic plants.

discussion

Pre-cropping with mycorrhizal aromatic plants is an effective approach to increase AM propagule density and subsequent inoculation of citrus seedlings in the field. When the soil has few or no native AM propagules, the introduction of mycorrhizal aromatic plants can increase the number of infective AM propagules. The mycorrhizal fungus *G intraradices* was established in the roots and in the adjacent soil prior to planting with the mycorrhizal-dependent citrus rootstocks.

Table I
The most probable number of arbuscular mycorrhizal propagules in 100 cm^3 of soil at the introduction of mycorrhizal aromatic plants, after 6 months growth of mycorrhizal aromatic plants before sowing citrus seeds, and after 6 months growth of Troyer citrange and Cleopatra mandarin in a soil used as citrus nursery 5 years earlier.

	Sampling period			
	Initial	*6 months*	*12 months*	
			Troyer	*Cleopatra*
Fumigated seedbed				
With mycorrhizal aromatic plants	0	17	74	11
Without mycorrhizal aromatic plants	0	0	1	1
Nonfumigated seedbed				
With mycorrhizal aromatic plants	0	27	173	45
Without mycorrhizal aromatic plants	0	3	4	1

Table II
Shoot length (cm) and density (no of plants/1 m seedbed section) of Troyer citrange and Cleopatra mandarin after 6 months growth in the seedbed.

	Troyer citrange		*Cleopatra mandarin*	
	Shoot length[1]	*Density*[2]	*Shoot length*[1]	*Density*[2]
Fumigated seedbed				
With mycorrhizal aromatic plants	33.4c	243b	21.9b	78b
Without mycorrhizal aromatic plants	11.6a	149a	12.6a	10a
Nonfumigated seedbed				
With mycorrhizal aromatic plants	26.1b	258b	27.2c	69b
Without mycorrhizal aromatic plants	12.8a	132a	15.4a	9a

(1) Data are means of 200 plants; (2) data are means of three replicates of 1 m seedbed section.
a, b, c : Means in columns followed by the same letter do not differ according to Tukey's test ($P < 0.05$).

Plants inoculated in the seedbed grew better and could be transplanted earlier. Mycorrhizal inoculation increases seedling survival and inoculated plants withstand the shock of transplanting better than noninoculated plants (GRAHAM, 1986).

The native populations of AM fungi were relatively low or nondetectable because weed species in the orchards prior to nursery establishment were in the Fumaraceae, Chenopodiaceae and Cruciferae families, which normally do not form mycorrhizal associations (GERDEMANN, 1968). This could explain the difficulty of obtaining a natural presence of AM propagules in these soils.

The results suggest that prior cropping with aromatic plants is an effective field inoculation technique that is much more useful than adding pot culture inoculum or other inoculum formulations. Other field experiments with mixed cropping or intercropping showed that inoculating beans cropped together with cassava can simplify

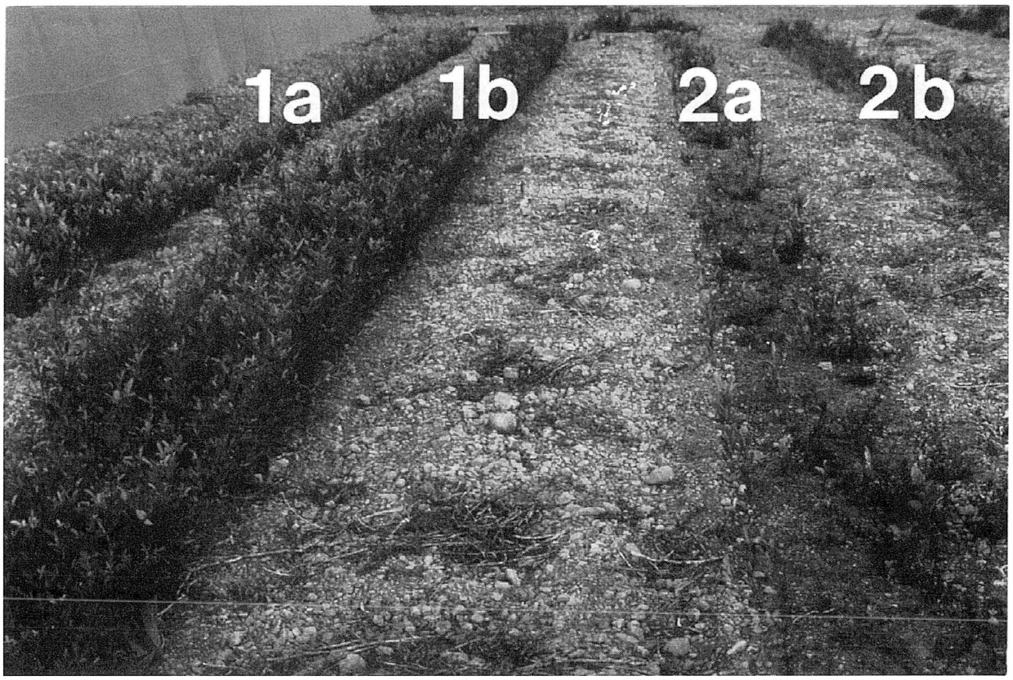

Photo 1
1) Citrus nursery bed after pre-cropping with mycorrhizal aromatic plants;
2) noninoculated nursery bed of (a) Cleopatra mandarin and (b) Troyer citrange.

the inoculation procedure (SIEVERDING, 1991). The mycorrhizal plants extend their roots throughout the soil and the inoculum spreads from the roots at the same time.

The fungus used in this study was *G intraradices*, a species that forms most of the spores within the roots (SCHENCK and SMITH, 1982). Thus, the major source of inoculum of this vesicular-arbuscular mycorrhizal fungus were the colonized roots. For this reason, when nurserymen do not fumigate soils prior to planting citrus seedbeds or nursery rows, introduction of mycorrhizal plants in the field could be a more feasible approach to extend the inoculum through the soil than large-scale introduction of soil inoculum or colonized root fragments into nursery beds (NEMEC, 1983; POWELL, 1984; FERGUSON and MENGE, 1986). In addition, the amount of inoculum required to inoculate seedlings of aromatic plants prior to their establishment is minimal. The aromatic plant species selected are adapted to Mediterranean conditions and the horticultural requirements in terms of water and nutrients are low. Moreover, the aromatic plants used, *T vulgaris, L vera* and *R officinalis,* are commercially viable crops in their own right for rotation with citrus rootstock production.

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