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Arbuscular mycorrhizal colonization of lime in different agroecosystems of the dry tropics

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Abstract Tecomán, in the Mexican state of Colima, had the world's greatest production of lime *Citrus aurantifolia* Swingle. Typical farming systems in the area include: (a) high-input monoculture, (b) a high-input system in which lime trees grow together with coconut palms, (c) a low-input system called "Family Farms" or "Family Gardens". In the Family Gardens, cultural practices are minimal and other fruit trees (about 16 species) coexist with the lime trees. This traditional minimal input system makes use of locally available resources and they are structurally very diverse. Arbuscular mycorrhizae may be crucial for sustainable production in Family Gardens. Root colonization and spore populations of fungi were scored at 2-week intervals in the three agroecosystems during a 6-month period. First samples were taken after the application of chemical fertilizer and irrigation in the high-input systems. Root colonization of lime was much higher and consistent in the low-input plots than in conventionally farmed plots, with colonization levels of 50–62% that remained the same throughout the sampling time; the high-input systems showed a high variation and lower level of colonization, 36% and 27% in associated and monoculture systems, respectively. Spore abundance was higher in the high-input systems but showed constant variation. The results suggest a strong effect of agroecosystem on mycorrhizal colonization of lime roots.

Key words Arbuscular mycorrhiza · *Citrus aurantifolia* · Cultural practices · Agroecosystems

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Introduction

Lime (*Citrus aurantifolia* Swingle) is a commercially important tree species in Mexico, which is the leading producer in the world. The area with the highest production is the state of Colima, located in the west . The most common farming systems of lime trees in this area are: (a) high-input monoculture, (b) high-input in association with coconut palms, and (c) a low-input system known in the region as "Family Farms" or "Family Gardens".

The arbuscular mycorrhizal (AM) fungi are known to improve the growth of many crop plants under low fertility conditions and thus have potential use in agriculture. However, both the AM community and colonization may vary greatly in different agrosystems with different cropping practices (Abbott and Robson 1991; Johnson et al. 1991). Most authors accept that the level of mycorrhizal colonization decreases with high-input of phosphorus and nitrogen (Hayman 1975), and this can be of great importance in a low external input system such as the Family Gardens system for lime production. Many plants whose growth is enhanced by AM, like lime, show acceptable growth if high rates of soil fertility are maintained. Thus, in the high-input ecosystem lime yields are high. On the other hand, lime is considered highly AM dependent, and without AM rootstock seedlings do not grow well (Nemec 1978). Thus AM may be crucial for sustainable production in low-input Family Gardens.

The interaction of cultural practices and AM has not been clarified for indigenous AM fungi in *C. aurantifolia*. We examined the hypothesis that the plant diversity and minimal practices present in the Family Gardens may play an important role in the maintenance and colonization by AM propagules.

Materials and methods

Study sites

The lime fields are located in an area 15–60 m above sea level surrounding the city of Tecomán, in the state of Colima, Mexico. This region lies between 18° 55′ and 18° 56′ N and the meridians 103° 53′ and 103° 57′. Mean annual precipitation is 760 mm, occurring during the summer from July to October, with a mean annual temperature of 26 °C. The vegetation of the region is that of the dry tropics.

The three most widely found farming systems of *C. aurantifolia* were selected for this study: a low-input system (so-called Family Gardens), and two high-input systems, one associated with coconut palm (*Cocos nucifera*) and a monoculture. The high-input agroecosystems were irrigated every 22–30 days and fumigated 2–4 times a year with Citruline. They were fertilized every year in amounts ranging from 60–30–00 to 120–60–00. In contrast, the cultural practices at the Family Gardens were minimal, and other fruit trees coexisted with the lime trees, as well as ornamental and medicinal plants; domestic animals were also an integral factor. The Family Gardens were structurally very diverse, with an overstory of trees (about 16 different fruit tree species) and an understory of small trees, shrubs and herbs. The harvest of food products, spices, medicinal and ornamental plants continues throughout the year. The fruit trees included *Anona muricata, Carica papaya, Mangifera indica, Calocarpum mammosum,* and *Tamarindus indica.*

Rhizosphere soil and root sampling

The number of spores and the AM colonization of 6- to 7-year old lime trees were measured in three different agroecosystems every 2 weeks during 6 months. Soil and roots were sampled at five differents sites in each of the agroecosystems. At each site, a total of three subsamples were taken, each containing approximately 200 g fresh weight of soil, which were mixed for analysis. Soil samples were taken at depths of 0–25 cm at the root zone and were transported to the laboratory and kept at 4 °C until use. Sampling from each location was repeated every 2 weeks, from December to May. The first samples were taken just after irrigation and application of chemical fertilizer in the high-input systems. Feeder roots samples were taken from five trees at five sites in each agroecosystem. The rootlets were directly preserved in formyl acetic alcohol until analysis. Lime rootlet samples were taken every 2 weeks.

Soil physical and chemical analysis

Soil texture was determined by the Bouyoucos hydrometer method and pH was measured in water using a glass electrode. Humidity was determined by the gravimetric method and organic matter content by the Walkley-Black oxidation method. Extractable P and K were determined colorimetrically (Olsen's) and total N by Kjeldahl's procedure. Water-holding capacity was measured using a pressure membrane apparatus. Foliar diagnosis of nutrient level was done according to the Hach Co. method.

Analysis of mycorrhizal fungi

Spores of AM fungi were extracted from five 100-g soil replicates from each site using the floating and decanting procedure (Gerdemann and Nicolson 1963). Those spores retained on the 38- to 350-μm pore sieves were sedimented and recovered by sucrose gradient centrifugation. Only visually intact spores were counted. The roots of all field grown plants were processed in the same manner. Roots were separated from soil, washed in water and cut into 1-cm long pieces. The pieces were mixed and a subsample cleared and stained for AM fungi using a modification of the Phillips and Hayman technique (Kormanick et al. 1980), in which phenol was omitted from the lacto-glycerin solution. One hundred root pieces of each tree were mounted between glass slides and examined on a microscope for AM hyphae, vesicles and arbuscules. The assessment of colonization was done using the +/– slide method in which 20 1-cm root segments were randomly selected from each root sample, and AM colonization expressed as the percent of root segments colonized for each root sample. The mean percent colonization of the five root samples from each sampling site was used in subsequent analyses.

Analysis of data

One-way analysis of variance (ANOVA) was performed using a random experimental design with three treatments (the three agroecosystems) and five replicates (the sampled trees). Data for percent AM colonization collected from the field were subjected to arcsin transformations and were analyzed using the repeated measures analyses of the General Linear Model procedure (SAS Institute 1982). Data on the number of spores in the rhizosphere soil were subjected to logarithm transformation. Differences between treatments were confirmed using Duncan's test (5%).

Results

Soil data

There were highly significant differences ($P<0.01$) between the edaphic variables and between mycorrhization in different agroecosystems. Significant differences were also found in root harvest time and in the interaction agroecosystem-root harvest time ($P<0.05$) (Table 1). The studied agroecosystems differed in various edaphic factors. Although all soils are sandy loams, those from the Family Gardens had less sand and clay. All soils were approximately neutral. The Family Gard-

Table 1 F values from one-way ANOVA of soil physical and chemical characteristics, mycorrhizal colonization and spore numbers, using as factors (a) agroecosystem, (b) root harvest time, and (c) interaction agroecosystem-root harvest time (*A–H*)

Source of	DF	Moisture	pH	Organic matter	Spore number	Root colonization
Agroecosystem	2	14.56**	73.06**	51.45**	15.51**	520.46**
Harvest time	11	5.64*	1.27 ns	0.09 ns	9.90*	74.50*
A-H	22	1.25*	0.85 ns	0.31 ns	3.21*	3.56*
MS	144	0.204	2992.478	0.004	14.044	0.306
C		37.077	62.798	28.247	9.287	11.533

* $P<0.05$
** $P<0.01$

Table 2 Soil physical and chemical characteristics of lime ecosystems. Values are means of five different sites of each system. Different letters indicate a significant difference between treatment means ($P<0.01$)

Agroecosystem	Soil moisture (%)	pH	Organic matter (%)	P (ppm)	Sand (%)	Silt (%)	Clay (%)
Family Gardens	12.2a	7.4b	2.43a	3.5a	85.2	10.8	2.6
Associated	8.7b	7.8a	1.43c	1.8b	87.0	8.8	3.0
Monoculture	9.5b	7.9a	1.83b	1.0c	89.6	7.6	2.3

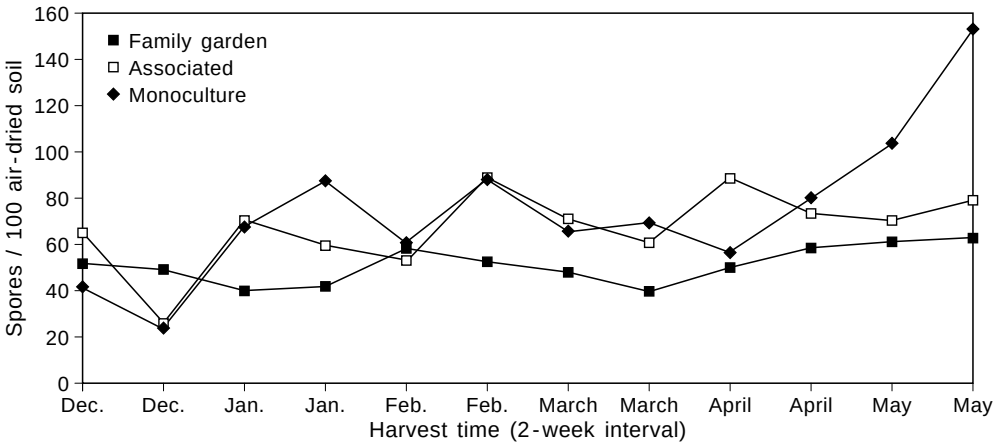

Fig. 1 Fluctuation through a 6-month period in the population of AM fungi from the rhizosphere of lime trees growing in three different agroecosystems, as measured by number of spores

ens had the highest amounts of organic matter. The monoculture soils showed very similar pH values as well as organic matter contents to the associated cultures (Table 2).

Number of spores

Spore abundance of AM fungi was higher in the high-input systems, ranging from 26 to 154/100 g soil versus 39 to 63/100 g soil in the Family Gardens (Fig. 1). The Duncan's multiple range test separated the ecosystems according to the spore numbers into the high-input and the low-input systems (Table 3). Spores of *Glomus* spp. were the most abundant at all the sampling times in all studied soils. Spores resembling *Gigaspora* spp. were also observed. Prevalent colors among the indigenous AM spores were dark and honey.

AM colonization in field-grown citrus

Percent root colonization differed with agrosystem type. The lime plants grown in the Family Gardens had significantly higher ($P<0.01$) percent AM colonization than plants grown in the associated or monoculture systems (Table 2). More than 50% of the roots of lime trees from the Family Gardens were colonized by AM fungi. Harvest time variation in the mycorrhizal colonization of plants was observed for the high-input sites, but not for the low-input sites. Lime plants grown in the Family Gardens showed high percent colonization (48–64%) through all the sampling times (Fig 2).

Levels of N-P-K in lime leaves

Leaf P concentrations of lime trees from the two high-input systems were very similar (Fig. 3). Trees from the Family Gardens had the highest P concentrations. For total N, the lowest concentration was found among trees in the monoculture system and the highest in the Family Gardens. In contrast, leaf K concentrations were the same in trees from all three agroecosystems.

Discussion

Agroecosystem variation in the percent colonization and spore production by AM fungi was observed among lime trees growing at different production sites under different management systems. Mycorrhizal spore numbers associated with lime at Colima were higher in soils with high pH, very low P and low organic

Table 3 Duncan's multiple range test of spore numbers and root colonization by AM fungi of 6- to 7-year-old lime trees growing in three different agroecosystems. Data are means of total values from each ecosystem. Different letters whithin a column indicate a significant difference between treatment means ($P<0.01$)

Agroecosystem	Spore number (per 100 g soil)	Root colonization (%)
Family Gardens	26b	52a
Associated	33a	37b
Monoculture	37a	27c

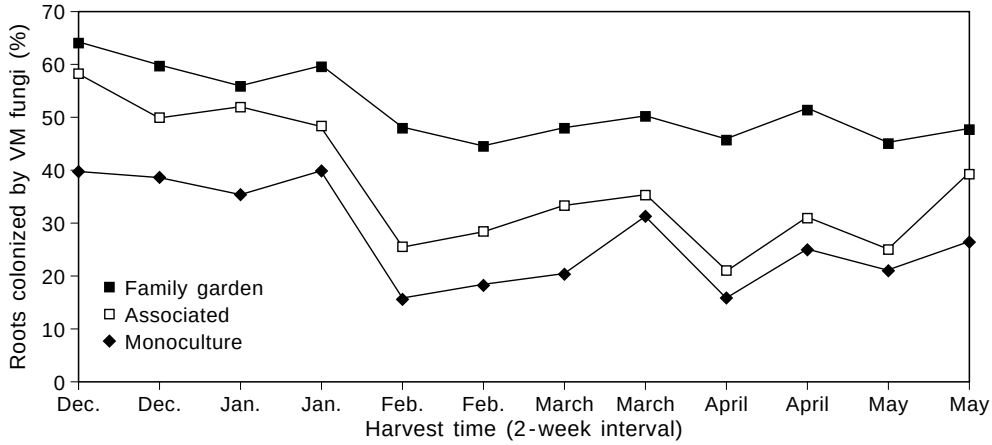

Fig. 2 Fluctuation through a 6-month period in the AM colonization level of lime roots growing in three different agroecosystems

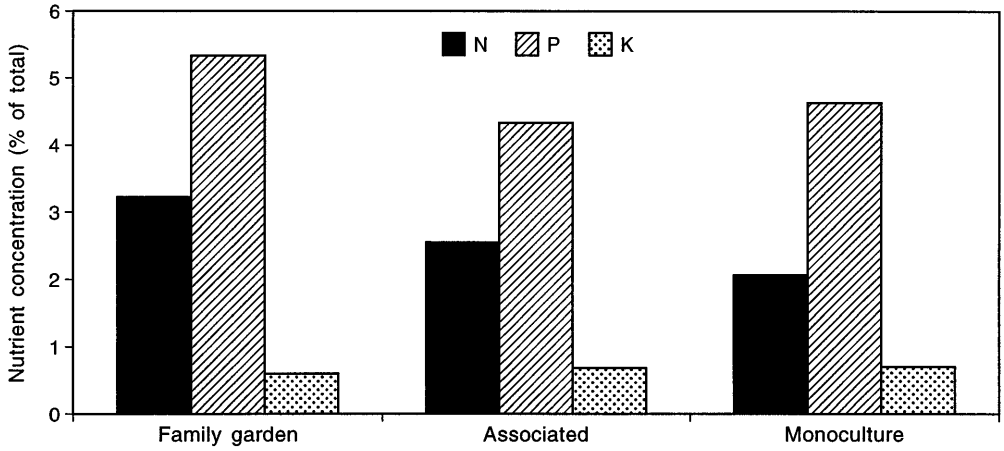

Fig. 3 Leaf concentrations of N, P and K in 6- to 7-year old lime trees growing in three different agroecosystems

matter content. This conclusion should be interpreted with care because these three variables are closely interrelated at Colima. In fact, all the studied sites had sandy soils which were low in P and are supposed to favor mycotrophy (El-Giahmi et al. 1976); however, this type of texture also favors lixiviation. This may explain why the high-input systems were lower in P (1–1.8 ppm) despite P fertilization.

The organic matter was low in the high-input systems. The high organic matter content of the Family Gardens arises from applications of manure and from the domestic animals which form an integral part of the gardens. These soils showed lower numbers of spores and a higher, though still low, P content (3.5 ppm). P may reduce mycorrhizal spore production at some sites but not at others (Abbott and Robson 1977; Powell 1977). Similar disagreements about the spore production or colonization by mycorrhizal fungi have arisen on the effect of other edaphic factors. Nemec et al. (1981) found that spore production by some species of mycorrhizal fungi associated with lime was favored by high soil P, B, Ca + Mg, or salinity, while production by other species was not. Thus the total count of spores in soils can differ with many physical, chemical and biological factors.

The levels of AM root colonization also varied with agroecosystem type. These differences might be related to environmental conditions such as soil moisture, pH or nutrient levels (Talukdar and Germida 1993). The general consensus is that P fertilization reduces the colonization of plant roots by AM fungi (Abbott and Robson 1984). In relation to N fertilization, Johnson (1984) found inhibition and Furlan and Bernier-Cardou (1989) showed stimulation of root colonization. Fertilization practices are also causative of some of these variations (Daft and Nicholson 1969). In this study, lime trees grown in the low-input plots showed much higher and more consistent root colonization than those grown in high-input plots. The Family Gardens showed a constant moisture content throughout the study time. Humidity and other soil characteristics related to the presence of many other AM plants may contribute to the germination of spores, leading to higher rates of colonization of trees from the Family Gardens.

Stöppler et al. (1990) state that AM colonization of plants in low-input systems is greater than those cultivated conventionally and that with this type of agriculture rather low concentrations of plant-available phosphate are often found. The results of this present study show that the levels of N and P in the leaves of the lime plants growing in the Family Gardens were higher than in plants from the high-input systems. Since AM fungi are an important component of the ecosystems supporting lime tree growth, a better knowledge of condi-

tions required for an optimal balance between tree growth and symbiotic development would contribute to improving the production of AM dependent plants using low amounts of fertilizer.

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