AEM Accepts, published online ahead of print on 7 October 2011
Appl. Environ. Microbiol. doi:10.1128/AEM.05577-11

Evidence of differences in the communities of AM fungi colonising galls and roots of *Prunus persica* infected by the root-knot nematode *Meloidogyne incognita*

Maria del Mar Alguacil,[1,]* Emma Torrecillas[1], Zenaida Lozano[2], and Antonio Roldán[1]

[1]CSIC-Centro de Edafología y Biología Aplicada del Segura. Department of Soil and Water Conservation. P.O. Box 164, Campus de Espinardo 30100-Murcia (Spain).

[2]Universidad Central de Venezuela (UCV), Facultad de Agronomía, Instituto de Edafología, Av. Universidad, El Limón, Campus Universitario, 2101- Maracay (Venezuela). Apdo. 4579.

Running title: Communities of AMF colonising galls of nematodes

*Corresponding author: M.M. Alguacil, Tel.: +34-968-396337; Fax: +34-968-396213; E-mail: mmalguacil@cebas.csic.es

ABSTRACT

Arbuscular mycorrhizal fungi (AMF) play important roles as plant-protection agents, reducing or suppressing nematode colonisation. However, it has never been investigated whether the galls produced in roots by nematode infection are colonised by AMF. This study tested whether galls produced by *Meloidogine incognita* infection in *Prunus persica* roots are colonised by AMF. We also determined the changes in AMF composition and biodiversity mediated by the infection with this root-knot nematode.

DNA from galls and roots of infected and roots of non-infected plants by *M. incognita* was extracted, amplified, cloned and sequenced using AMF-specific primers. Phylogenetic analysis using the SSU rDNA data set revealed twenty-two different AMF sequence types (17 *Glomus*, three *Paraglomus*, one *Scutellospora* and one *Acaulospora)*. The highest AMF diversity was found in uninfected roots followed by infected roots and galls. This study indicates that the galls produced in *P. persica* roots due to infection with *M. incognita* were colonised extensively by a community of AMF belonging to the families Paraglomeraceae and Glomeraceae, which was different from the community detected in roots. Although the function of the AMF in the galls is still unknown, we hypothesise that they could act as protection agents against opportunistic pathogens.

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are obligate, symbiotic fungi which form mutualistic associations with the roots of 80% of terrestrial plant species. Apart from an improvement in plant nutrition, AMF play important roles in the reduction of pathogen infections (7, 25). The protective effect of AMF against a broad range of soil-borne fungi and bacteria (31, 35) as well as root-feeding nematodes (14, 17) has been well-documented. In some crops, it has been shown that mycorrhizal associations have a suppressive effect on endoparasitic nematodes (12, 32, 39), so that the AMF could be considered biological control agents (24).

Previous molecular study, using PCR-DGGE, indicated that the presence of nematodes alters the composition of the AMF communities inside *Ammophila arenaria* roots (27). However, when this methodology is used without subsequent cloning procedures, it is not possible to determine the identity of the AMF, since this technique is based on the observation of the composition of the fungal or bacterial communities using DGGE profiles (3).

Earlier reports showed that AMF can colonise nitrogen-fixing legume root nodules (29) and old, senescent nodules after nitrogen fixation has ceased (30). Also, mycorrhizal nodules belonging to the genus *Glomus* have been found in the root systems of angiosperms such as *Gymnostoma deplancheanum* and *G. nodiflorum* (13). In spite of the ecological and economic relevance of the interactions between AMF and root-knot nematodes, it has never been investigated whether the galls produced in roots by nematode infection are colonised by AMF.

Meloidogyne incognita is the most-widespread root-knot nematode and probably the most-serious plant parasitic nematode pest of tropical and sub-tropical regions throughout the world (41). *M. incognita* has been found to be associated with *Prunus*

persica (L.) Batsch in Venezuela where causes severe decreases in the productivity of this important fruit crop (9, 10).

In the present study, we intended to elucidate whether galls produced by *M. incognita* infection in *P. persica* roots are colonised by AMF, as well as the changes in AMF composition and biodiversity mediated by the infection with this root-knot nematode.

MATERIAL AND METHODS

Study site and Sampling

The study was conducted in a commercial orchard located at the "Colonia Tovar", Aragua State, in the north of Venezuela (latitude 10º 29' N, longitude 67º 07' W, 1790 msl). Its climate is temperate with a mean annual temperature of 16.8 ºC, and an annual average rainfall of 1271 mm (mostly concentrated in a rainy season between June and October). The soil in the experimental area was a sandy loam Inceptisol in the USDA soil classification system (33). The soil characteristics were: pH of 5.18, 5.75% clay, 40.5% silt, 53.75% sand, 6.46 cmol kg^{-1} of cationic exchange capacity, Total N 2.7 g Kg^{-1}, Available P 32 $\mu g.g^{-1}$, 5.9% organic matter and bulk density 1.29 $g.cm^{-3}$

The plants used in this survey were 13-year old peach (*Prunus persica* (L). Batsch cv. Criollo Amarillo). The experimental sampling was designed as a randomized factorial design with six replication blocks ($100m^2$ each) in an experimental area of approximately 1800 m^2. Each block consisted of 20 trees, some of them naturally infected by the root-knot nematode. The sampling was conducted during fruiting. Six plants infected and six plants uninfected belonging to each of the six replication blocks were sampled. The roots were sampled using three soil cores from three points/single

100 tree/block. Roots and galls of *M. incognita* infected plants and roots of uninfected plants
101 were selected.
102 The root samples (secondary and tertiary) were washed with distilled water,
103 nematode galls were separated and thoroughly surface washed. The material was frozen
104 until processing.
105
106 **Nematodes identification**
107 *Meloidogyne incognita* was isolated from the infected roots and the identity was
108 checked by morphology and sequencing of the ITS region according to de la Peña *et al*.,
109 (11). The uninfected roots were also checked for the absence of nematodes by the same
110 method.
111
112 **Roots and galls DNA extraction and PCR**
113 All PCR experiments were run using DNA preparations consisting of 100 mg of
114 pooled roots and galls extracts for each plant and replication block separately. Thus,
115 eighteen DNA extractions and PCRs from 12 root samples (6 infected plants and 6
116 uninfected plants) and 6 galls samples were carried out. For each sample, total DNA
117 was extracted from frozen material, (an average of 18 cm root length and 13 nematode
118 galls in each case) using a DNeasy plant mini Kit following the manufacturer's
119 recommendations (Qiagen). The roots or galls samples were placed into a 2-ml screw-
120 cap propylene tube and the DNA extracts were obtained by disrupting roots or galls
121 with a sterile disposable micro-pestle in liquid nitrogen. The DNA was resuspended in
122 20 µl of water.
123 Several dilutions of extracted DNA (1/10, 1/50, 1/100) were prepared and 2 µl
124 were used as template. Partial small subunit (SSU) ribosomal RNA gene fragments

were amplified using nested PCR with the universal eukaryotic primers NS1 and NS4 (40). PCR was carried out in a final volume of 25 µl using the "ready to go" PCR beads (Amershan Pharmacia Biotech), 0.2 µM dNTPs and 0.5 µM of each primer (PCR conditions: 94 °C for 3 min, then 30 cycles at 94 °C for 30 s, 40 °C for 1 min, 72 °C for 1 min, followed by a final extension period at 72 °C for 10 min).

Two µl of several dilutions (1/10, 1/20, 1/50 and 1/100) from the first PCR were used as template DNA in a second PCR reaction performed using the specific primers AML1 and AML2 (20). PCR reactions were carried out in a final volume of 25 µl using the "ready to go" PCR beads (Amershan Pharmacia Biotech), 0.2 µM dNTPs and 0.5 µM of each primer (PCR conditions: 94 °C for 3 min, then 30 cycles of 1 min denaturation at 94 °C, 1 min primer annealing at 50 °C and 1 min extension at 72 °C, followed by a final extension period of 10 min at 72 °C). Positive and negative controls using PCR positive products and sterile water respectively were also included in all amplifications. All the PCR reactions were run on a Perkin Elmer Cetus DNA Thermal Cycler. Reactions yields were estimated by using a 1.2 % agarose gel containing ethidium bromide.

Molecular analysis

The PCR products were purified using a Gel extraction Kit (Qiagen) cloned into pGEM-T Easy (Promega) and transformed into *Escherichia coli* (Xl1 blue). Forty putative positive transformants were screened in each resulting SSU rRNA gene library, using 0.7 unit of RedTaq DNA polymerase (Sigma) and a re-amplification with AML1 and AML2 primers with the same conditions described above. Product quality and size were checked in agarose gels as described above. All clones having inserts of the correct size in each library were sequenced.

Clones were grown in liquid culture and the plasmid extracted using the QIAprep Spin Miniprep Kit (Qiagen). The sequencing was done by Laboratory of Sistemas Genómicos (Valencia, Spain) using the universal primers SP6 and T7. Sequence editing was done using the program Sequencher version 4.1.4 (Gene Codes Corporation). 102 representatives sequences of the 263 clones generated in this study have been deposited at the National Centre for Biotechnology Information (NCBI) GenBank (http://www.ncbi.nlm.nih.gov) under the accession numbers FR847980-FR848081.

Sequence similarities were determined using the Basic Local Alignment Search Tool (BLASTn) sequence similarity search tool (2) provided by GenBank. Phylogenetic analysis was carried out on the sequences obtained in this study and those corresponding to the closest matches from GenBank. Sequences were aligned with other published glomeralean sequences using the program ClustalX (38) and the alignment was adjusted manually in GeneDoc (22). Neighbour-joining (NJ) (28) and maximum likelihood (ML) phylogenetic analyses were performed with the programs PAUP4.08b (36) and RAxML v.7.0.4 (34), respectively. Distances for the NJ tree were computed using the default parameters. For the ML analysis, a GTR-GAMMA model of evolution was used. A total of 200 independent bootstrap analyses were performed to provide nodal support. The ML bootstrap values were calculated with 1000 replicates using the same substitution model. *Endogone pisiformis* Link and *Mortierella polycephala* Coem, were used as the out-groups.

Different AMF sequence types or phylotypes, were defined as groups of closely related sequences, usually with a high level of bootstrap support in the phylogenetic analyses (higher than 80%) and sequence similarity ≥ 97%. The pairwise analysis within clusters was carried out using MEGA software version 4 (37).

175 The presence or absence of AMF phylotypes in each root and gall sample was
176 used to construct the sampling effort curves (with 95 % confidence intervals) using the
177 software EstimateS 8.00 (8). The sample order was randomized by 100 replications.
178
179 **Statistical analysis**
180 The Shannon-Weaver (H') index was calculated as an additional measure of diversity,
181 as it combines two components of diversity, i.e., species richness and evenness. It is
182 calculated from the equation $H' = -\sum p_i(\ln p_i)$, where p_i is the proportion of individuals
183 found in the ith species (in a sample, the true value of p_i is unknown but is estimated as
184 n_i/N, [here and throughout, n_i is the number of individuals in the ith species]).
185 We applied a general log-linear analysis (SPSS, version 19.0) to test whether the
186 composition of the AMF communities differed between the three experimental cases. A
187 correspondence analysis (CA) with presence and/or absence data for all AMF sequence
188 types at three experimental cases was performed, and the results were summarized in an
189 ordination diagram. CA is a multivariate statistical method that allows comparisons of
190 AM fungal community compositions between all experimental cases.
191
192 **RESULTS**
193 **Phylogeny**
194 From the 18 clone libraries, a total of 720 clones were screened by PCR; out of
195 these, 263 contained the small-subunit rRNA gene fragment and subsequently all were
196 sequenced. All 263 clones resulted to correspond to AM fungi sequences. The
197 phylogenetic tree constructed using homologous sequences of AMF species from
198 GenBank and our sequences made possible the recognition of 22 different AMF
199 sequence types or phylotypes (Fig. 1), 17 of which belonged to the genus *Glomus*, three

to the genus *Paraglomus*, one to the genus *Scutellospora* and one to the genus *Acaulospora*. Since identical sequences were detected, the clones producing the same sequence for each experimental case were represented once in the alignment for clarity (see supplemental material for a detailed description of the number of clones of each AMF sequence type that were recovered from each experimental case).

Eight sequence types corresponded to morphologically-defined species (Pa1 to *Paraglomus laccatum*, Pa2 to *Paraglomus brasilianum*, Glo G1 to *Glomus intraradices/irregulare* group, Glo G4 to *Glomus sinuosum*, Glo G13 to *Glomus mosseae*, Glo G10 to *Glomus indicum*, Scu1 to *Scutellospora cerradensis* and Aca1 to *Acaulospora scrobiculata/laevis* group) and one sequence type (Glo G15) was not related to any sequences found in the database. The remaining sequence types were related to uncultured glomalean species sequences in GenBank (Fig. 1).

The sampling effort curves (Fig. 2) showed stabilisation considering the AMF sequence types found and the number of samples analysed.

AMF community composition

Uninfected plant roots harboured a mean number of AMF sequence types per root sample similar to that of infected roots (5.8±0.4 and 5.5±0.3, respectively). The number of AMF sequence types per gall sample showed the lowest mean value (3.7±0.3). The compositions of the AMF communities from roots and galls were determined based on the number of clones of the 22 AMF sequence types detected (Fig. 3). A comparative analysis of AMF diversity did not show significant differences between blocks ($F=0.643$; $P=0.540$).

The Shannon diversity index and the total number of AMF sequence types were slightly higher for uninfected than for infected plants roots ($H'= 2.22$ and 17 AMF

sequence types for uninfected plants, compared with H'= 2.05 and 16 AMF sequence types for infected plants). However, their AMF communities were clearly different (P<0.05) (Fig. 3). Also, the composition of the AMF communities colonising the galls differed significantly with respect to both infected and uninfected roots (P<0.001). The galls had both the lowest number of AMF sequence types (10) and the lowest diversity index (H'= 1.55).

Eight AMF sequence types (Pa 1, Glo G1, Glo G2, Glo G6, Glo G9, Glo G12, Glo G14 and Glo G15) occurred in all three experimental cases (Fig. 3). Of these, five (Pa 1, Glo G1, Glo G9, Glo G12 and Glo G14) represented the highest percentages, accounting for 68.30 % of the AMF clones analysed. Also, it is noteworthy that, the highest number of clones for Pa 1, Glo G1, Glo G9, Glo G14 and Glo G15 occurred in the galls. Some AMF sequence types were found exclusively in particular experimental cases analysed: thus, Pa2 appeared exclusively in galls, while Pa3, Glo G3, Glo G8, Glo G16 and Aca1 were found only in uninfected roots, Glo G4, Glo G5 and Glo G7 were specific to infected plant roots and Glo G11 appeared only in the galls and roots of infected plants (Fig. 4).

DISCUSSION

This study shows, for the first time using a molecular approach, that the galls produced in *P. persica* roots by *M. incognita* infection are colonised by a characteristic AMF community that differs clearly from both the root AMF community where the galls were collected and the community of uninfected roots.

The AMF communities associated with the roots of infected and uninfected plants were also different (Fig. 3). Rodríguez-Echevarría *et al*. (27) also found that nematodes altered the composition of the AMF communities inside *Ammmophila*

arenaria roots, although they did not identify the AMF species. We found similar numbers of AMF sequence types in infected and uninfected roots (16 and 17, respectively); however, the compositions of the two AMF communities clearly differed, 12 fungal sequence types being shared. Although the reason for this association is not known, it could be due to the fact that some AMF species are more sensitive to nematodes than others (19). Changes in root physiology after the nematode infection might have altered root exudation or chemistry (5, 6). In fact, it has been reported that root exudates are fundamental in stimulating the growth of microorganisms, due to the release of organic compounds, such as carboxylic acids, and enzymes, such as acid phosphatases (15, 26). Thus, these processes also could affect in some way the ability of certain AMF to colonise infected roots.

The galls produced on the infected roots were colonised by two families of glomalean fungi (Paraglomeraceae and Glomeraceae). Between them, 10 AMF sequence types were found; the two most-abundant types were Glo G1 and Glo G14. They were also the most-abundant sequence types in the infected plant roots where galls were collected. Glo G1 is related to the *G. intraradices/irregulare* group, which are the most-generalistic AMF found in the molecular diversity studies conducted so far (16, 23). Glo G14 matched with database sequences belonging to uncultured *Glomus*, which had been reported previously from *Phytolacca americana* and *Perilla frutescens* roots (21). The second-most-abundant sequence type in galls, appearing also in infected and uninfected roots, was Pa1, which corresponded to *Paraglomus laccatum.* This fungal type was not related to any sequence type in the database obtained from environmental samples. Only the fungal type Pa2, related to *Paraglomus brasilianum* and reported previously from *Panax japonicus* roots (20), was found exclusively in galls and was represented by two clones, each clone belonging to a different plant.

The galls showed a lower Shannon diversity index (H'=1.55) than both infected and uninfected roots (H'=2.05 and H'=2.22, respectively). Although the results are not directly comparable, Sheublin *et al*. (29) also found that legume root nodules had less AMF diversity than the roots from which they were collected, corroborating the idea that AMF communities may vary among the different parts of a root system (29). The AMF diversity found in the galls is surprisingly high if we suppose that a greater surface area should favour greater colonisation by different AMF sequence types, since the gall samples analysed had a surface area that was around 12-times lower than that of the roots for the same weight (71 mm^2 per 100 mg for galls and 850 mm^2 per 100 mg for roots).

The AMF colonisation of the galls of nematode-infected roots has not been studied previously. The ecological and physiological roles of these symbionts in the root knots is not clear. Root-feeding nematodes stimulate the production of galls (root knots) on the roots of their host plants. The galls disturb the root´s ability to absorb water and nutrients and also can serve as entry points for pathogens, such as fungi and bacteria, which cause plant diseases (4, 5, 26). This could have been the mechanism which enabled the AMF to colonise the galls to a high degree. The high diversity of AMF could compete successfully for the space and nutrient sources with other endophytes, which also can use the openings in the galls produced by the nematode infection; thus, the AMF might protect the galls from opportunistic attack by pathogens.

In conclusion, the galls produced in *P. persica* roots by *M. incognita* infection were colonised by an AMF community belonging to the families Paraglomeraceae and Glomeraceae, which was different from the AMF community detected in roots. Although the function of the AMF in the galls is still unknown, we hypothesise that the AMF could act as protective agents against opportunistic pathogens. This study was

carried out only with *P. persica* roots; therefore, more research has to be done to test whether the galls produced in other plant species are colonised by AMF, since the outcomes of AMF-nematode interactions are influenced by many factors, including the physical, physiological and temporal factors (17) and functional differences between different AMF taxa (18).

ACKNOWLEDGMENTS

MM Alguacil was supported by the Ramón and Cajal programme (Ministerio de Educación y Ciencia, Spain).

REFERENCES

1. **Alguacil, M.M., M.P. Torres, E. Torrecillas, G. Díaz, and A. Roldán.** 2011. Plant type differently promotes the arbuscular mycorrhizal fungi biodiversity in their rhizospheres after revegetation of a degraded, semiarid land. Soil Biol. Biochem. **43:**167-173.

2. **Altschul, S.F., T.L. Madden, A.A. Schäffer, J. Zhang, Z. Zhang, W. Miller, and D.J. Lipman.** 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. **25:** 3389-3402.

3. **Anderson, I.C., and J.W.G. Cairney.** 2004. Diversity and ecology of soil fungal communities: Increased understanding through the application of molecular techniques. Environ. Microbiol. **6:** 769–779.

4. **Back, M.A., P.P.J. Haydock, and P. Jenkinson.** 2002. Disease complexes involving plant parasitic nematodes and soilborne pathogens. Plant Pathol**. 51:** 683–697.

5. **Back, M., P. Jenkinson, T. Deliopoulos, and P. Haydock.** 2010. Modifications in the potato rhizosphere during infestations of *Globodera rostochiensis* and

325 subsequent effects on the growth of *Rhizoctonia solani.* Eur. J. Plant Pathol. **128:**
326 459-471.
327 6. **Bardgett, R.D., R. Cook, G.W. Yeates, and C.S. Denton.** 1999. The influence of
328 nematodes on below-ground processes in grassland ecosystems. Plant Soil **212:** 23-
329 33.
330 7. **Borowicz, V.A.** 2001. Do arbuscular mycorrhizal fungi alter plant–pathogen
331 relations?. Ecology **82**: 3057–3068.
332 8. **Colwell, R.** 2005. EstimateS: statistical estimation of species richness and shared
333 species from samples, version 8.0. User´s guide. University of Connecticut, Storrs.
334 http://viceroy.eeb.uconn.edu/estimates.
335 9. **Crozzoli, R., and G. Vargas.** 1989. Reacción de trece patrones de durazno a
336 infestaciones de *Meloidogyne javanica*. Fitopatol. Venez. 2: 16-18.
337 10. **Crozzoli, R.** 2002. Especies de nematodos fitoparasíticos en Venezuela. Interciencia
338 **27:** 354-364.
339 11. **de la Peña, E., G. Karssen, and M. Moens.** 2007. Diversity and distribution of
340 root-lesion nematodes (*Pratytlenchus* spp.) associated with *Ammophila arenaria* L.
341 (Link) in coastal dunes of Western Europe. Nematology **9**: 881–901.
342 12. **de la Peña, E., S. Rodriguez-Echeverria, W.H. van der Putten, H. Freitas, and**
343 **M. Moens.** 2006. Mechanism of control of root-feeding nematodes by mycorrhizal
344 fungi in the dune grass *Ammophila arenaria.* New Phytol**. 169**: 829–840.
345 13. **Duhoux, E., G. Rinaudo, H.G. Diem, F. Auguy, D. Fernandez, D. Bogusz, C.**
346 **Franche, Y. Dommergues, and B. Huguenin.** 2001. Angiosperm *Gymnostoma*
347 trees produce root nodules colonized by arbuscular mycorrhizal fungi related to
348 *Glomus*. New Phytol**. 149**: 115–125.

14. **Elsen, A., D. Gervacio, R. Swennen, and D. De Waele.** 2008. AMF-induced biocontrol against plant parasitic nematodes in *Musa* sp.: a systemic effect. Mycorrhiza **18:** 251-256.

15. **Grayston, S.J., D. Vaughan, and D. Jones.** 1997. Rhizosphere carbon flow in trees, in comparison with annual plants: the importance of root exudation and its impact on microbial activity and nutrient availability. Appl. Soil Ecol. **5:** 29–56.

16. **Helgason, T., J.W. Merryweather, J.P.W. Young, and A.H. Fitter.** 2007. Specificity and resilience in the arbuscular mycorrhizal fungi of a natural woodland community. J. Ecol. **95:** 623-630.

17. **Hol, W.H.G., and R. Cook.** 2005. An overview of arbuscular mycorrhizal fungi-nematode interactions. Basic Appl. Ecol. **6:** 489-503.

18. **Klironomos, J.N.** 2003. Variation in plant response to native and exotic arbuscular mycorrhizal fungi. Ecology **84:** 2292-2301.

19. **Klironomos, J.N., J. McCune, and P. Moutoglis.** 2004. Species of arbuscular mycorrhizal fungi affect mycorrhizal responses to simulated herbivory. Appl. Soil Ecol. **26:** 133-141.

20. **Lee, J., S. Lee, and J.P.W. Young.** 2008. Improved PCR primers for the detection and identification of arbuscular mycorrhizal fungi. Fems Microbiol. Ecol. **65:** 339-349.

21. **Long, L.K., Q. Yao, J. Guo, R.H. Yang, Y.H. Huang, and H.H. Zhu.** 2010. Molecular community analysis of arbuscular mycorrhizal fungi associated with five selected plant species from heavy metal polluted soils. Eur. J. Soil Biol. **46:** 288-294.

22. **Nicholas, K.B., and H.B.Jr. Nicholas.** 1997. GeneDoc: a tool for editing and annotating multiple sequence alignments. Distributed by the authors.

374 23. **Öpik, M., M. Moora, J. Liira, and M. Zobel.** 2006. Composition of root-
375 colonizing arbuscular mycorrhizal fungal communities in different ecosystems
376 around the globe. J. Ecol. **94:** 778–790.
377 24. **Pinochet, J., C. Calvet, A. Camprubi, and C. Fernández.** 1996. Interactions
378 between migratory endoparasitic nematodes and arbuscular mycorrhizal fungi in
379 perennial crops: A review. Plant Soil **185:** 183-190.
380 25. **Pozo, M.J., and C. Azcon-Aguilar.** 2007. Unraveling mycorrhiza-induced
381 resistance. Curr. Opin. Plant Biol. **10:** 393-398.
382 26. **Raaijmakers, J.M., T.C. Paulitz, C. Steinberg, C. Alabouvette, and Y. Moënne-**
383 **Loccoz.** 2009. The rhizosphere: a playground and battlefield for soilborne
384 pathogens and beneficial microorganisms. Plant Soil **321:** 341-361.
385 27. **Rodriguez-Echeverria, S., E. de la Peña, M. Monees, H. Freitas, and W.H. van**
386 **der Putten.** 2009. Can root-feeders alter the composition of AMF communities?
387 Experimental evidence from the dune grass *Ammophila arenaria*. Basic Appl. Ecol.
388 **10:** 131-140.
389 28**. Saitou, N., and M. Nei.** 1987. The Neighbor-Joining method: a new method for
390 reconstructing phylogenetic trees. Mol. Biol. Evol. **4:**406-425.
391 29. **Scheublin, T.R., K.P. Ridgway, J.P.W. Young, and M.G.A. van der Heijden.**
392 2004. Nonlegumes, legumes, and root nodules harbor different arbuscular
393 mycorrhizal fungal communities. Appl. Environ. Microbiol. **70:** 6240–6246.
394 30. **Scheublin, T.R., and M.G.A. van der Heijden.** 2006. Arbuscular mycorrhizal
395 fungi colonize nonfixing root nodules of several legume species. New Phytol**. 172**:
396 732–738.

31. **Singh, R., A. Adholeya, and K.G. Mukerij.** 2000. Mycorrhiza in control of soil-borne pathogens. In: Mukerij KG, Chamalo BP, Singh J (eds) Mycorrhizal biology. Academic-Plenum Publishers, New York, pp 173–196

32. **Smith, G.S., and D.T. Kaplan.** 1988. Influence of mycorrhizal fungus, phosphorus, and burrowing nematode interactions on growth of rough lemon citrus seedlings. J. Nematol. **20:** 539-544.

33. **Soil Survey Staff.** 2006. Soil Taxonomy. A basic systemof soil classification for making and interpreting soil surveys. 2, Washington: US Government Printing Office, DC, USA, Agriculture Hadbook No.436. 332 pp.

34. **Stamatakis, A.** 2006. RaxML-VI-HPC: maximum likelihoodbased phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics **22**:2688–2690.

35. **St-Arnaud, M., and A. Elsen.** 2005. Interaction or arbuscular-mycorrhizal fungi with soil-borne pathogens and non-pathogenic rhizosphere micro-organisms. In: S. Declerck, D.-G. Strullu and J. A. Fortin (eds). Vitro culture of mycorrhizas. Berlin Heidelberg, Springer-Verlag, pp. 217-231.

36**. Swofford, D. L.** 2002. PAUP: Phylogenetic Analysis Using Parsimony (and other methods), (version 4.08b10 for Machintosh). Sinauer Associates Inc., Sunderland, MA.

37. **Tamura, K., J. Dudley, M. Nei, and S. Kumar.** 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol. **24**:1596-1599.

38. **Thompson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin, and D. G. Higgins.** 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. **24:**4876-4882.

421 39. **Vaast, P., E.P. Cashwell-Chen, and R.J. Zasoski.** 1998. Influences of a root-
422 lesion nematode, *Pratylenchus coffeae* and two arbuscular mycorrhizal fungi,
423 *Acaulaspora mellea* and *Glomus clarum* on coffee (*Coffea arabica* L.). Biol. Fert.
424 Soils **26:** 130-135.
425 40. **White, T.J., T. Bruns, S. Lee, and J. Taylor.** 1990. Amplification and direct
426 sequencing of fungal ribosomal RNA genes for phylogen-etics. In *PCR protocols. A*
427 *guide to methods and applications*. Innis, M.A., Gelfand, D.H., Sninsky, J.J., and
428 White, T.J. (eds). San Diego: Academic, pp 315–322.
429 41. **Zarina, B., and F. Shahina.** 2010. Research work carried out on the management
430 of root-knot nematode diseases in Pakistan. Pakistan J. Nematol. **28:** 153-239.
431
432
433
434
435
436
437
438
439
440
441
442
443
444
445

446 **FIGURE LEGENDS**

447

448 **Figure 1.** Neighbour-Joining (NJ) phylogenetic tree showing AM fungal sequences
449 isolated from roots and galls of the *M. incognita*-infected plants and roots of the
450 uninfected plants as well as reference sequences from GeneBank. All bootstrap values >
451 80% are shown (1000 replicates). Numbers above branches indicate the bootstrap values
452 of the NJ analysis; numbers below branches indicate the bootstrap values of the
453 maximum likelihood analysis. Sequences obtained in the present study are shown in
454 bold type. They are identified by the different tissues from which they were obtained (G
455 = Galls, IP = Infected roots and NIP = Uninfected roots) and the clone identity number.
456 Group identifiers (for example Glo G1) are AM fungal sequence types found in our
457 study. See the supplemental material for a detailed description of the all clones obtained
458 in the present study for each group. *Endogone pisiformis* and *Mortierella polycephala*
459 were used as out-groups.

460

461 **Figure 2.** Sampling effort curves for galls, *M. incognita*-infected roots and uninfected
462 roots. The sample order was randomised by 100 replications in EstimateS, version 8.0
463 (Colwell, 2005).

464

465 **Figure 3.** Proportional distribution of the total number of clones detected for each AMF
466 sequence type in the galls, roots of *M. incognita*-infected plants and roots of uninfected
467 plants.

468

469 **Figure 4.** Correspondence analysis of the AM fungal community composition found in
470 the galls, roots of *M. incognita*-infected plants and roots of uninfected plants. The

eigenvalues of the 1st and 2nd axes in the two-dimensional ordination diagrams are as follows: dimension 1=0.51 and dimension 2= 0.35. Circles represent the respective AMF sequence types and the triangles the experimental cases. Ovals with full, dotted, and dashed lines represent the distribution of the AMF diversity in the galls (G), roots of uninfected plants (NIP) and roots of *M. incognita*-infected plants (IP), respectively.