Original article

Effect of the combined inoculation of arbuscular mycorrhizal fungi and plant growth-promoting rhizobacteria on papaya (*Carica papaya* L.) infected with the root-knot nematode *Meloidogyne incognita*

María del Carmen JAIZME-VEGA, Ana Sue RODRÍGUEZ-ROMERO*, Luis Antonio BARROSO NÚÑEZ

Dpto. Protección Vegetal, Instituto Canario Investigaciones Agrarias, Apdo. 60, 38200 La Laguna, Tenerife, España

arguezr@icia.es

Effect of the combined inoculation of arbuscular mycorrhizal fungi and plant growth-promoting rhizobacteria on papaya (*Carica papaya* L.) infected with the root-knot nematode *Meloidogyne incognita.*

Abstract — Introduction. Arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) can be considered important rhizospheric beneficial microorganisms. Their use as biocontrol strategies against soilborne pathogens such as nematodes should be taken into account. However, optimal management of soil microbiota communities is not easy because of the high specificity involved in these types of interactions. The aim of our study was to determine whether the combined inoculation of two AMF species and a *Bacillus* consortium based on three strains previously described as PGPR in other crops were able to reduce nematode infection and damage on papaya. **Materials and methods**. Papaya seedlings were inoculated with two AMF isolates (*Glomus mosseae* or *G. manihotis*) at the beginning of the nursery phase. Once the mycorrhizal symbiosis was established, a *Bacillus* consortium was applied. Nematode inoculum was applied 20 d after transplanting to individual pots. Plants were harvested 160 d after nematode inoculation. **Results**. In terms of plant development and nutrition, benefits due to AMF inoculation persisted in the presence of PGPR. However, the effect of dual inoculation was different, depending on the *Glomus* species. This positive effect was also evident in plants with nematode. *Meloidogyne* infection was significantly reduced in mycorrhizal plants. However, the addition of PGPR does not seem to improve the results of AMF single treatments in terms of nematode infection. **Conclusion**. Dual application of AMF and PGPR must be considered for papaya threatened by the root-knot nematode, although a previous screening should be done in order to select the best microbe combination to optimise results.

Spain / *Carica papaya* / arbuscular mycorrhizae / *Glomus* / rhizobacteria / *Bacillus* / symbiosis / growth / plant nutrition / biological control

Effet de l'inoculation combinée de champignons mycorhiziens à arbuscules et de rhizobactéries favorisant la croissance des plantes, sur le papayer (*Carica papaya* L.) infecté par *Meloidogyne incognita*, nématode des racines.

Résumé — Introduction. Les champignons mycorhiziens à arbuscules (CMA) et les rhizobactéries favorisant la croissance des plantes (RFCP) peuvent être considérés comme d'importants micro-organismes bénéfiques à la rhizosphère. Leur utilisation dans une stratégie de lutte biologique contre les pathogènes du sol, contre les nématodes par exemple, devrait être envisagée. Cependant, la gestion optimale des populations de micro-organismes du sol n'est pas facile en raison de la grande spécificité de ces types d'interactions. Le but de notre étude a été de déterminer si l'inoculation combinée de deux espèces de CMA et d'un consortium de rhizobactéries *Bacillus* spp., basé sur trois souches précédemment décrites comme RFCP pour d'autres cultures, pouvaient limiter l'infection des nématodes et leurs dommages sur papayer. **Matériel et méthodes**. De jeunes plants de papayers ont été inoculés avec deux isolats de CMA (*Glomus mosseae* ou *G. manihotis*) en début de croissance en pépinière. Une fois la symbiose mycorhizienne établie, un consortium de *Bacillus* spp. a été appliqué. Une inoculation par le nématode *Meloidogyne incognita* a été effectuée 20 j après transplantation des plants en pots individuels. Les jeunes papayers ont été étudiés 160 j après l'inoculation du nématode. **Résultats**. L'effet bénéfique de l'inoculation de CMA sur le développement et la nutrition des plants a persisté en présence de RFCP. Cependant, l'effet de la double inoculation a été différent selon l'espèce de *Glomus* considérée. Cet effet positif a également été évident pour les plants infectés par des nématodes. L'infection par *Meloidogyne* a été sensiblement réduite dans les plants mycorhizés. Cependant, pour cette infection par le nématode, l'addition de RFCP n'a pas semblé améliorer les résultats imputables à l'utilisation de CMA seuls. **Conclusion**. Une double application de CMA et de RFCP pourrait être envisagée pour protéger le papayer menacé par le nématode de la racine, mais un criblage préliminaire devra être fait afin de choisir la meilleure combinaison de micro-organismes apte à optimiser des résultats.

Espagne / *Carica papaya* / mycorhize à arbuscule / *Glomus* / rhizobactérie / *Bacillus* / symbiose / croissance / nutrition des plantes / lutte biologique

* *Correspondence and reprints*

Received 3 January 2005
Accepted 18 November 2005

Fruits, 2006, vol. 61, p. 151–162

DOI: 10.1051/fruits:2006013
www.edpsciences.org

***RESUMEN ESPAÑOL**, p. 162*

1. Introduction

Soil microbiota communities have demonstrated their crucial role in maintaining the soil ecological balance and, therefore, the sustainability of both natural ecosystems and agroecosystems [1]. Particularly important from the point of view of plant surface microbiology are the interactions at the root-soil interface, where microorganisms, plant roots and soil constituents interact [2]. Hiltner defines what is known as the rhizosphere [3]: the most dynamic environment of microbe-plant interaction, since it is the zone of influence of plant roots on the soil microbiota. Two main groups of microorganisms can be distinguished: saprophytes and symbionts. Both of them comprise detrimental, neutral and beneficial bacteria and fungi. Beneficial rhizospheric microbe-plant interactions have a great influence on plant health and soil quality [4]. Among these beneficial rhizospheric microbes, arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) can be considered.

Arbuscular mycorrhiza fungi are obligate symbionts that colonise the roots of most cultivated plant species. Mycorrhizal symbiosis can be found in nearly all types of ecological situations and most plant species are able to form this symbiosis naturally [5]. These associations occur naturally when plantlets are transplanted into the field, favouring plant development by increasing nutrient uptake, growth rates and hormonal activities [5, 6]. Mycorrhizae may also increase plant tolerance to stress conditions such as salinity [7], drought [8], heavy metals [9], root soilborne pathogens [10] and the improvement of soil structure [11].

Plant growth-promoting rhizobacteria are able to colonise the root surface, survive and multiply in microhabitats associated with the root surface, in competition with native microbiota; at least to express their plant-promotion activities [12]. Their positive effects on plant development and establishment of seedlings have been described for different crops; either herbaceous such as potato [13] and soybean [14], or woody ones such as apple [15] and citrus [16]. Several mechanisms, which involve phytohormone production [17], mineral solubilisation and availability [18] or biological control of soil-borne pathogens [19], have been proposed to explain bacterial activity. Authors have frequently described as PGPRs certain strains of *Pseudomonas*, *Bacillus*, *Azospirillum*, *Azotobacter, Enterobacter* and *Serratia* [19].

Since they share common habitats, i.e., the root surface, and common functions, the AMF and PGPR have to interact during their processes of root colonisation or functioning as root-associated microorganisms. Soil microorganisms, particularly PGPR, can influence AM formation and function and consequently, mycorrhizae can affect PGPR populations in the rhizosphere [20]. Relationships between both types of microbes are under high specificity rules [21].

Several species of the root-knot nematode *Meloidogyne* are widespread in the Canary Islands, Spain [22]. Papaya is susceptible to the *Meloidogyne* species and so this nematode can become an important limitation in papaya production in dry subtropical conditions [23]. Apart from the typical symptoms in root tissues (gall formation), nematode infection of papaya leads to a deficient plant development, higher susceptibility to different stresses, significant growth suppression and reduction in fruit yield [24].

Work on the application of AMF and/or PGPR in tropical and subtropical crops of ecological and economic importance for the Canary Islands such as papaya is not very extensive. However, the results obtained by authors provide evidence that papaya growth can be improved when it is inoculated with AMF [25–27]. In other tropical crops such as banana, early mycorrhizal inoculation has been shown to increase tolerance to nematode by enhancing the plant and/or by exerting a suppressive effect over nematode reproduction [28]. In the same way, PGPR single inoculation or in combination with AMF favours papaya growth during the nursery phase [29]. The same positive effects have been demonstrated in other tropical crops inoculated with PGPR [30].

The aim of our study was to determine whether the combined inoculation of two AMF species and a *Bacillus* consortium based on three strains previously described as PGPR in other crops were able to reduce nematode infection and damage on papaya.

2. Materials and methods

2.1. Mycorrhizal inoculum and procedure

Two AMF isolates were used:

– *Glomus mosseae* (isolated from ecological farm Pome banana, *Musa* AAB, in the North of Tenerife) cultured under Sudan grass [*Sorghum bicolor* (L.) Moench] with a percentage of 83% of root colonisation,

– *Glomus manihotis* (a collection isolate from Colombia) cultured under tomato (*Lycopersicon esculentum* Mill.) with 74% of root colonisation.

In both cases, the AMF inoculum consisted of rhizospheric soil containing pieces of mycorrhizal roots, hyphae and spores. Two kilograms of inoculum were applied per seed tray for both AMF isolates.

2.2. Plant material

The papaya (*Carica papaya* L.) cv. 'Baixinho Santa Amalia' seedlings came from Brazil. Seeds were germinated in 24-L seed trays filled with a water steam-sterilised substrate mixture (1:1:1= soil:volcanic ash:peat TKS®1-Instant Sphagnum-Torf Klasmann Deilmann GmbH, Germany).

2.3. Bacterial inoculum material

A *Bacillus* consortium containing strains INR7, T4 and IN 937b isolated and identified by Dr. Kloepper (Alabama, USA) was kept in TSB (Trytone Soy Broth) with 20% glycerol at the *Institut de Recerca i Tecnologia Agroalimentàries*, IRTA (Spain).

The bacterial inoculum was prepared after culturing the strains on Petri dishes with TSA (Tryptone Soy Agar) for 2 weeks. For each culture session, plates were incubated for 48 h at 25 °C. The bacterial inoculum consisted of a sterilised NaCl (0.85%) suspension containing approximately an equal amount of the three *Bacillus* strains. The inoculum concentration was approximately 10^8 CFU (colony-forming units)·mL^{-1}; it was determined by using a viable versus absorbance at 600 nm curve for each *Bacillus* strain. Bacterial inoculation was carried out twice during the trial. A first dose (5 mL·plant^{-1}) was applied 25 days after seed germination and a second one (50 mL·plant^{-1}) 10 days after transplanting to individual pots.

The nursery phase lasted for 50 days (25 days after application of the first dose of bacterial suspension) during which plantlets were grown under an acclimatisation tunnel with an ambient temperature of 27–32 °C and a relative humidity of 80%. Plantlets were irrigated with distilled water (50 to 75) mL according to hydric requirements. Then, plants were transplanted to individual 6-L pots filled with a water steam-sterilised substrate mixture (2:2:1= soil:volcanic ash:peat TKS®1-Instant Sphagnum-Torf Klasmann Deilmann GmbH, Germany). The substrate surface of each plant was covered by a volcanic ash layer in order to keep the substrate humid.

2.4. Nematode inoculation procedure

The nematode inoculum consisted of a population of *Meloidogyne incognita* isolated from the same papaya cultivar ('Baixinho Sta. Amalia') originally collected in the North-East of Tenerife. Nematode identification was made by perineal patterns (20 females per population). The nematode inoculum was prepared by macerating infected roots in a blender for 15 s at 14 500 rpm in a 0.12–0.15% NaClO solution [31]. Eggs and juveniles (J2) were collected using a 25-µm-pore sieve (500 mesh) and rinsed with tap water. The inoculum was adjusted to deliver a suspension of 5 780 nematodes per plant through four 2-cm deep holes located at a 3-cm distance from the base of the plant in nematode treatments.

2.5. Experimental design and culture conditions

An experiment with 12 treatments lasting 160 days after nematode inoculation was established: 3 (2 AMF + 1 control) × 2 (1 PGPR + 1 control) × 2 (1 *M. incognita* + 1 control) with 12 replicates per treatment (144 plants). Plants were disposed in the greenhouse in a completely randomised design.

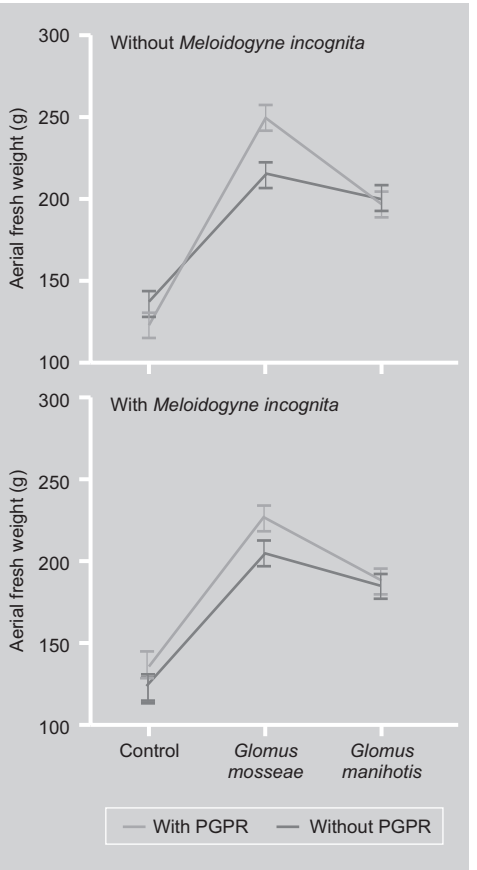

Figure 1.
Effect of the interaction between AMF (*Glomus mosseae* and *G. manihotis*) and PGPR (*Bacillus* spp.) on aerial fresh weight of papaya plants infected with *Meloidogyne incognita*.

In this period, plants were grown under greenhouse conditions in a tunnel with an ambient temperature of 25–30 °C and a relative humidity of 70%, and they were irrigated with distilled water according to hydric requirements. During the first month after transplanting, plants were fertilised twice a week with a nutritive solution low in P content [32]. Then 3 g·plant^{-1} of a soluble fertiliser with low P content (Nitrofoska 20+5+10s+3, Suprem Campo® Basf, Germany) was applied twice a month. Once a month, plants were fertilised with Wuxal-Ca® (Argos Shering, Agrevo, S.A. Valencia) using a dose of the product of 3% (foliar application).

2.6. Assessment of variables

At harvest, 160 days after nematode inoculation, plant and nematode parameters were assessed. The following physical parameters were measured: total fresh weight (aerial and root), plant length, root length and foliar surface. Foliar surface was determined by using the surface measurer Li-COR, Inc., Lincoln, Nebraska, USA. Mod. Li-3100.

Macroelements, i.e., nitrogen, phosphorus and potassium, were determined on shoots. The stem and leaves of the papaya plants were thoroughly washed in mild detergent, rinsed three times in distilled water avoiding senescent or necrotic tissue and prepared for foliar analysis. Samples were then dehydrated in a controlled-temperature fan-ventilated oven at 60 °C for 24 h, ground in a ball mill and digested in wet acid [33] using nitric and perchloric acid. Analysis for all elements except nitrogen was done with a F586-587 Varian Liberty 220 inductively coupled plasma (ICP) emission spectrometer. Two readings were made per sample. Nitrogen content was determined according to the Kjeldahl procedure [34].

For nematode parameter assessment, the percentage of galled root system was determined [35], as well as the number of nematodes per gram of root and the reproduction rate (final population/initial population). The nematode extraction method from roots was similar to that used for inoculum preparation. Nematodes were concentrated using 150-, 74- and 25-µm-pore sieves (100, 200 and 500 mesh, respectively). The suspension on the 25-µm sieve was collected and concentrated in order to determine the number of nematodes per mL by using a Hawksley slide under a light microscope.

To assess mycorrhizal infection, a small root sample (5% in fresh weight) of the whole root system was used to estimate the percentage of AM root infection. Samples were stained with 0.05% trypan blue in lactic acid [36] modified by the procedure described by Koske and Gemma [37]. The percentage of root colonisation was determined using the grid-line intersect method [38]. Mycorrhizal root samples, inoculated or non-inoculated with *M. incognita*, were excised after clarifying and staining the root, mounted on millimetric slides and observed under a light microscope.

2.7. Statistical analysis

All data were analysed by ANOVA. Data on nematode reproduction were $\log_{10}(x+1)$ transformed for analyses. Means were compared by Tukey's multiple range test ($P \leq 0.05$). The analysis was performed by using Systat® 7.0.1. (SPSS. Inc.© 1997).

3. Results

3.1. Plant development

Benefits due to mycorrhizal fungi inoculation persisted in the presence of PGPR (*figures 1 to 4*) although two different trends could be observed depending on *Meloidogyne* presence. In the absence of the nematode, plants co-inoculated with both types of beneficial microorganisms did not show in general a significantly better development than those treated just with one microbe (AMF). On the other hand, in the presence of *Meloidogyne* the combined application of AMF and PGPR seems to increase benefits due to single mycorrhization. Nematode infection seems to stimulate the positive effect of the combination AMF-PGPR in terms of plant development: a generally significant improvement could be registered due to the double inoculation. Also, those papayas single-inoculated with *Bacillus* spp. did not show significant differences from non-inoculated control plants in terms of plant development.

3.1.1. Aerial fresh weight

In the absence of *Meloidogyne incognita*, the combined inoculation of AMF and PGPR significantly increased aerial fresh weight in those plants treated with *G. mosseae* (*figure 1*). Values registered in plants treated with the combination of *G. manihotis*-PGPR were identical to those of *G. manihotis* single inoculation. In the presence of the pathogen, *Bacillus* spp. inoculation significantly improved aerial fresh weight in those papayas inoculated with *G. mosseae*.

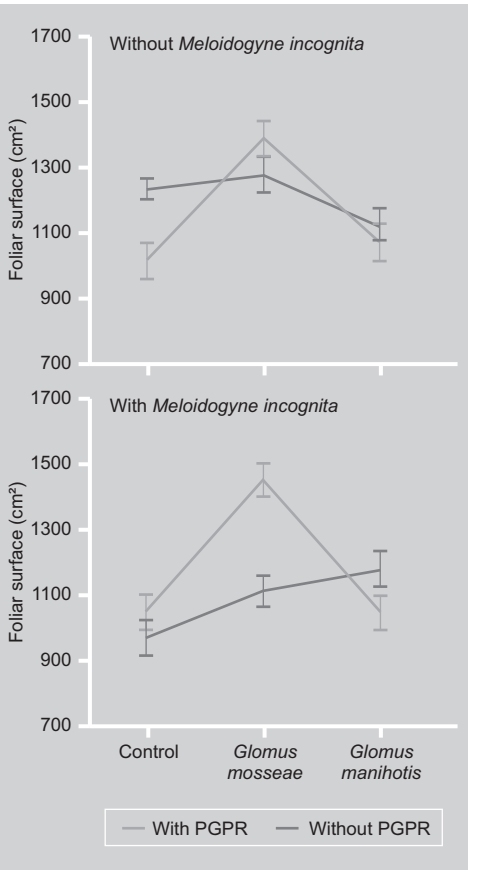

Figure 2.
Effect of the interaction between AMF (*Glomus mosseae* and *G. manihotis*) and PGPR (*Bacillus* spp.) on foliar surface of papaya plants infected with *Meloidogyne incognita*.

3.1.2. Foliar surface

In the absence of *Meloidogyne incognita*, single inoculation of any AMF did not significantly increase foliar surface (*figure 2*). However, combined application of both beneficial microorganisms did this in the case of *G. mosseae*-PGPR. In plants infected with the pathogen, a negative effect due to *Meloidogyne* could be detected in the absence of PGPR. Bacterial inoculation significantly improved foliar surface values in those plants treated with *G. mosseae*. On the other hand, the *G. manihotis*-PGPR combination did not favour this parameter.

3.1.3. Shoot length

The results registered in nematode non-infected plants show significant differences due to AMF single inoculation (*figure 3*). However, significant increases due to AMF were not improved by adding PGPR. On the

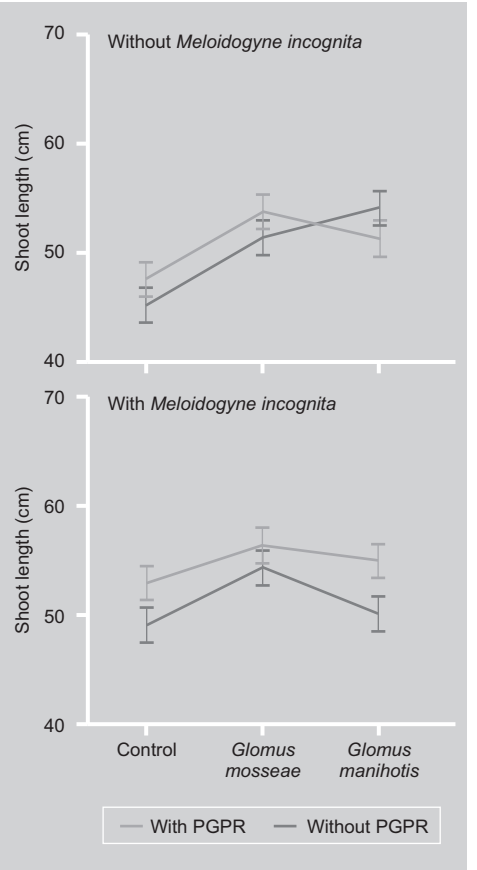

Figure 3.
Effect of the interaction between AMF (*Glomus mosseae* and *G. manihotis*) and PGPR (*Bacillus* spp.) on shoot length of papaya plants infected with *Meloidogyne incognita*.

other hand, bacterial inoculation significantly increased shoot length in the presence of the pathogen (non-mycorrhizal plants and those with *G. manihotis*).

3.1.4. Root length

A significant root shortening due to *Meloidogyne* infection could be detected (*figure 4*). The roots of infected plants treated with both AMF and PGPR were significantly longer than those just treated with AMF. Again, in the absence of nematode, combined application of both microbes did not improve results from AMF single inoculation.

3.2. Macroelement content

Concerning mineral content, the studied macroelements varied in a similar way depending on treatments.

3.2.1. Nitrogen

A positive significant effect due to AMF inoculation could be detected both in the presence of nematode and not, although higher increases were detected for *G. mosseae* (*table I*). In the absence of *M. incognita* and for *G. mosseae*, the combined inoculation of this fungal isolate and PGPR increased nitrogen content compared with *G. mosseae* single treatment, although this improvement was not significant. On the other hand, the presence of the nematode led to a significant decrease in N content compared with *G. mosseae*-PGPR treatment.

3.2.2. Phosphorus

As was observed in the case of nitrogen, a positive significant effect due to AMF inoculation could be detected in all cases, although higher increases were detected for *G. mosseae*, both in the presence of *M. incognita* and not (*table I*). However, the combined inoculation of *G. mosseae* and PGPR also significantly increased phosphorus content compared with *G. mosseae* single inoculation, in the absence of *Meloidogyne*.

3.2.3. Potassium

Both AMF isolates were able to increase K levels in the absence of *Meloidogyne*, but not significantly (*table I*). On the other hand, only *G. manihotis* inoculation increased K levels in nematode-infected plants. The combined inoculation of both AMF and *Bacillus* spp. did not improve the results registered in AMF single treatments.

3.3. Nematode reproduction

The presence of nematode significantly affected plant root length, although different behaviours could be detected depending on the treatment. In general, although more especially in *G. manihotis* treatments, the presence of AMF could increase tolerance to *Meloidogyne*. Nematode reproduction was significantly reduced in the presence of either any of the AMF isolates or any of the AMF-PGPR combinations (*table II*).

Compared with non-mycorrhizal plants, percentages of galled root decreased

3.6 times in mycorrhizal ones, treated or not with PGPR. Significant differences due to AMF isolate (either alone or combined with *Bacillus* spp.) could be observed in other nematode parameters. Although *G. mosseae* treatments showed significant reduction in nematode population levels, *G. manihotis* alone or combined with PGPR seems to be more effective at promoting reduction of nematode levels (only 8 nematodes per gram of root and a reproduction rate of nearly 0.12). No significant improvement due to the presence of *Bacillus* spp. could be observed from these results.

3.4. Mycorrhizal colonisation

Mycorrhizal colonisation levels were relatively low (values between 15% and 31%) at the end of the trial (*figure 5*). Each AMF isolate showed a different behaviour and, in general, plants treated with *G. manihotis* registered a mycorrhizal infection index slightly higher than *G. mosseae* plants. For *G. manihotis* treatments a significant decrease could be detected in those plants infected with *M. incognita* and also inoculated with PGPR. This phenomenon contrasts with the identical treatment for *G. mosseae* which shows the highest mycorrhizal colonisation index in the *G. mosseae* series.

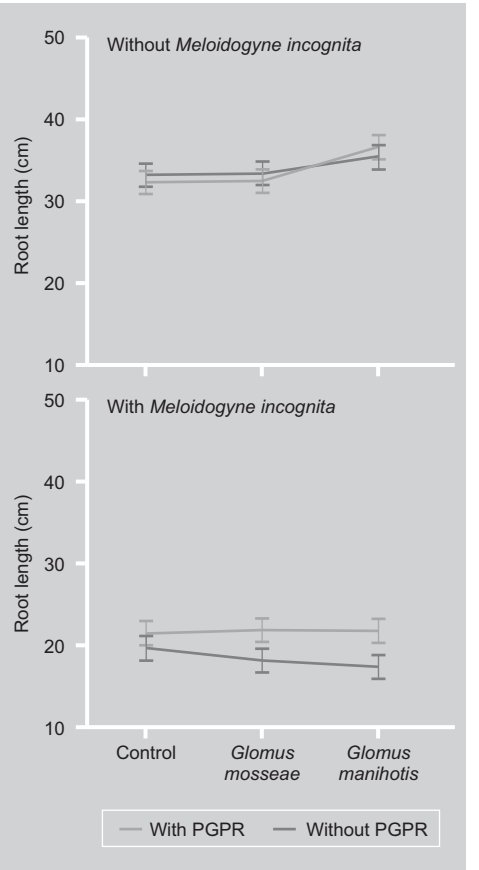

Figure 4.
Effect of the interaction between AMF (*Glomus mosseae* and *G. manihotis*) and PGPR (*Bacillus* spp.) on root length of papaya plants infected with *Meloidogyne incognita*.

4. Conclusion

The interaction between AMF and *Bacillus* spp. led to benefits in terms of plant development in papaya infected with *Meloidogyne incognita*. In the absence of the pathogen, PGPR seems to enhance the mycorrhizal effect, especially in those plants treated with *G. mosseae*. However, the most evident effects were registered in the presence of the nematode. As available data about the mycorrhizal symbiosis in papaya is lacking, discussion is required, comparing our results with other vegetal species. Some authors have reported the synergistic beneficial effect in plant development promoted by the AMF-PGPR association [39, 40]. Dual inoculation with both soil microorganisms also induced higher biomass and yield [39, 40] and even higher nutrient uptake [40]. However, diversity in the response depending on the microbial combination has also been described [41]. In our experiment, this diversity could be detected since each AMF isolate showed a singular response to the combination with *Bacillus* spp. In general, in the absence of the pathogen, a greater vegetative development was registered in plants inoculated with the combination *G. mosseae-Bacillus* spp. This diversity in the response agrees with the high specificity in the rhizosphere microbial interactions previously described by other work [21].

In this experiment, no negative effect of PGPR on mycorrhizal symbiosis establishment could be detected, since the colonisation index in the roots was statistically similar both with *Bacillus* spp. and without them. Our results are in agreement with other references where no negative effect of PGPR on mycorrhizal symbiosis has been

Table I.
Effect of the interaction between AMF (*Glomus mosseae* and *G. manihotis*) and *Bacillus* spp. in the presence of the root-knot nematode *Meloidogyne incognita* on leaf mineral content of papaya (means of 12 replicates).

Treatments	N	P	K
		(mg·plant^{-1})	
Control	554.6 de	30.1 cd	456.0 ab
Control + *Bacillus* spp.	503.6 e	28.0 de	404.4 b
Control + *Meloidogyne incognita*	441.4 e	22.3 e	411.0 b
Control + *Bacillus* spp. + *M. incognita*	527.0 e	22.6 e	460.7 ab
Glomus mosseae	995.2 ab	40.3 b	601.1 ab
G. mosseae + *Bacillus* spp.	1145. 5 a	52.8 a	610.6 ab
G. mosseae + *M. incognita*	925.5 abc	40.6 b	464.4 ab
G. mosseae + *Bacillus* spp. + *M. incognita*	890.0 bc	39.0 bc	513.5 ab
Glomus manihotis	926.2 abc	40.5 b	648. 6 ab
G. manihotis + *Bacillus* spp.	863.6 c	37.0 bcd	506.0 ab
G. manihotis + *M. incognita*	871.5 bc	33.5 bcd	683.4 a
G. manihotis + *Bacillus* spp. + *M. incognita*	784.2 cd	33.7 bcd	559.7 ab

Within the same column, values followed by the same letter are statistically identical according to Tukey's test ($P \leq 0.05$).

Table II.
Effect of the interaction between AMF (*Glomus mosseae* and *G. manihotis*) and *Bacillus* spp. on nematode reproduction (means of 12 replicates) studied with papaya seedlings.

Treatments	Galled root (%)	Nematodes per g root	Nematodes per root	Reproduction rate[1]
Meloidogyne incognita	89 a	1 961 ab[2]	133 543 ab[2]	24 a
Bacillus spp. + *M. incognita*	91 a	3 359 a[2]	276 527 a[2]	46 a
G. mosseae + *M. incognita*	28 b	305 b	25 832 b	4 b
G. mosseae + *Bacillus* spp. + *M. incognita*	25 b	108 b	10 236 b	2 b
G. manihotis + *M. incognita*	25 b	8 c	649 c	0.11 c
G. manihotis + *Bacillus* spp. + *M. incognita*	25 b	8 c	672 c	0.12 c

[1] Nematode reproduction rate = [final population / initial population].

[2] Data have been transformed to $\log_{10}(x+1)$ to ease the analysis.

Within the same column, values followed by the same letter are statistically identical according to Tukey's test ($P \leq 0.05$).

reported [42], while the opposite situation has also been described [43]. Reasons to explain this phenomenon must again be observed under the high specificity of microbial interactions. Under some conditions, PGPR are able to promote fungal spore germination and germinative tube elongation [44] or even to enhance mycorrhizal hypha density [41].

Concerning the bacteria-pathogen interactions, our results show that single inoculation of *Bacillus* spp. has no evident effect on papaya or nematode. Increases in tolerance against nematode were detected in

plants treated with the AMF-bacteria combination. Double application of beneficial microorganisms led to an improvement in plant development and nutrition in the presence of the pathogen. Also, nematode reproduction was significantly reduced in the presence either of any AMF isolate or the combination of AMF-PGPR. References concerning the use of PGPR as biocontrol agents against nematodes are not very extensive. However, some authors have demonstrated in several crops that the inoculation of certain PGPR strains was able to increase plant development and reduce nematode damage and infection [45, 46]. In our conditions, *Bacillus* single inoculation does not affect nematode infection; moreover, absolute values were even higher than those registered in control plants. The specificity of microbial interactions which has been already mentioned [21] must be taken into account again to explain these results. On the other hand, several studies confirm the AMF role in reducing nematode reproduction or promoting nematode tolerance in other tropical crops such as banana [47]. Our experiment verified this positive effect of AMF: single inoculation of both *Glomus* species significantly reduced nematode infection, as well as increasing plant development. However, a difference in intensity of the effect due to the AMF isolate could be detected. The combined inoculation of AMF and PGPR seems to describe again a singular behaviour for each combination. Although the literature on this subject is not very extensive, a few works have described the reduction of *Meloidogyne* infection due to AMF-PGPR inoculation on tomato [48].

In conclusion, our results confirm the suitability of AMF inoculation to improve plant health of papaya infected with root-knot nematode. The management of these symbionts represents a suitable biocontrol strategy against *Meloidogyne* in this crop. The supplementary addition of other beneficial microorganisms such as PGPR can also be taken into account as a method of enhancing the AMF effect. However, due to the high specificity involved in these types of interactions, a previous screening to select the best microbe-host plant combination should be done in order to optimise results. The absence of references concerning this triple interaction in papaya allows us to propose this method as a hopeful one.

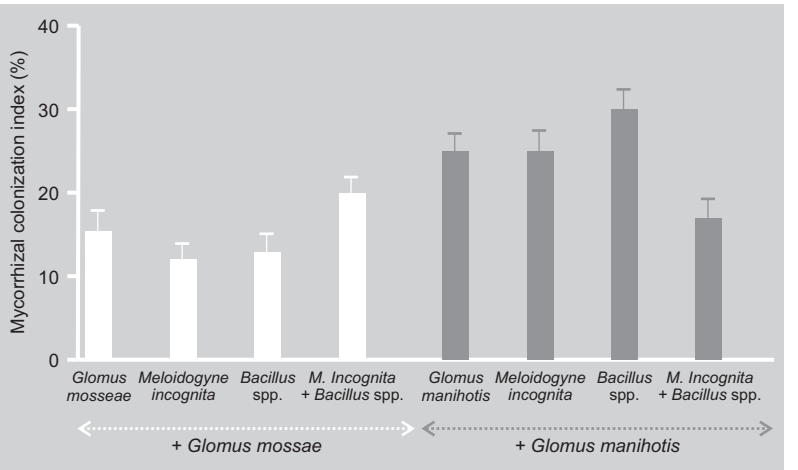

Figure 5.
Effect of the interaction between AMF (*Glomus mosseae* and *G. manihotis*) and PGPR (*Bacillus* spp.) on papaya plants infected with *Meloidogyne incognita*: mycorrhizal colonisation index at the end of the experiment, i.e., 160 days after nematode inoculation.

Acknowledgements

The authors would like to thank A.R. Socorro Monzón from ICIA (Dpto. Suelos y Riegos) for foliar mineral analysis.

References

[1] Kennedy A.C., Smith K.L., Soil microbial diversity and the sustainability of agricultural soil, Plant Soil 170 (1995) 75–86.

[2] Bowen G.D., Rovira A.D., The rhizosphere and its management to improve plant growth, Adv. Agron. 66 (1999) 1–102.

[3] Hiltner L., Über neuere Erfahrungen und Probleme auf dem Gebiet der Bodenbakteriologie und unter besonderer Berücksichtigung der Gründüngung und Brache, Arb. Dtsch. Landwirtsch. Ges. 98 (1904) 59–78.

[4] Lynch J.M., The rhizosphere, John Wiley, New York, USA, 1990.

[5] Smith S.E., Read D.J., Mycorrhizal symbiosis, Acad. Press, London, UK, 1997.

[6] Linderman R.G., Vesicular-arbuscular mycorrhizae and soil microbial interactions, in: Bethlenfalvay G.J., Lindeman R.G. (Eds.), Mycorrhizae in sustainable agriculture, ASA Spec. Publ., Madison, Wisconsin, USA, 1992, pp. 45–70.

[7] Safir G.R., Involvement of cropping systems, plant produced compounds and inoculum production in the functioning of VAM fungi, in: Pfleger F.L., Linderman R.G. (Eds.), Mycorrhizae and Plant health, APS Press, Minnesota, USA, 1994, pp. 239–259.

[8] Requena N., Pérez-Solís E., Azcón-Aguilar C., Jeffries P., Barea J.M., Management of indigenous plant-microbe symbiosis aids restoration of desertified ecosystems, Appl. Environ. Microbiol. 67 (2001) 495–498.

[9] Hetrick B.A.D., Wilson G.W.T., Figge D.A.H., The influence of mycorrhizal symbiosis and fertilizer amendments on establishment of vegetation in heavy metal mine spoil, Environ. Pollut. 86 (1994) 171–179.

[10] Azcón-Aguilar C., Jaizme-Vega M.C., Calvet C., The contribution of arbuscular mycorrhizal fungi to the control of soil-borne plant pathogens, in: Gianinazzi S., Schüepp H., Barea J.M., Haselwandter K. (Eds.), Mycorrhizal technology in agriculture: from genes to bioproducts, Birkhäuser Verlag, Switzerland, 2002, pp. 187–197.

[11] Barea J.M., Azcón-Aguilar C., Azcón R., Interactions between mycorrhizal fungi and rhizosphere microorganisms within the context of sustainable soil-plant systems, in: Gange A.C., Brown V.K., (Eds.), Multitrophic interactions in terrestrial systems, Blackwell Science, Oxford, UK, 1997, pp. 65–77.

[12] Kloepper J.W., Plant growth-promoting rhizobacteria (other systems), in: Okon Y. (Ed.), *Azospirillum* / plant associations, CRC Press, Boca Ratón, USA, 1994, pp. 111–118.

[13] Burr T.J., Schroth M.N., Suslow T.V., Increased potato yields by treatments of seed pieces with specific strains of *Pseudomonas fluorescens* and *P. putida*, Phytopathol. 68 (1978) 1377–1383.

[14] Polonenko D.R., Scher F.M., Kloepper J.W., Singleton C.A., Laliberté M., Zaleska I., Effects of root colonizing bacteria on inoculation of soybean roots by *Bradyrhizobium japonicum*, Can. J. Microbiol. 33 (1987) 498–503.

[15] Caesar A.J., Burr T.J., Growth promotion of apple seedlings and rootstocks by specific strains of bacteria, Phytopathol. 77 (1987) 1583–1588.

[16] Gardner J.M., Chandler J.L., Feldman A.W., Growth promotion and inhibition by antibiotic-producing fluorescent *Pseudomonads* on *Citrus* roots, Plant Soil 77 (1984) 103–113.

[17] Dobbelaere S., Croonenborghs A., Thys A., Vande Browk A., Vanderleyden J., Phytostimulatory effect of *Azospirillum brasilense* strains and auxins on wheat, Plant Soil 212 (1999) 155–164.

[18] Ompal S., Panwar J.D.S., Effect of nitrogen fixing and phosphorus solubilizing bacteria on nutrient uptake and yield of wheat, Indian J. Plant Physiol. 2 (1997) 211–213.

[19] Kloepper J.W., Plant growth-promoting rhizobacteria as biological control agents, in: Metting F.B., Dekker M. (Eds.), Soil microbial ecology, applications in agriculture, forestry and environmental management, Dekker M., Inc., New York, USA, 1992, pp. 255–274.

[20] Barea J.M., Rhizosphere and mycorrhiza of field crops, in: Toutant J.P., Balazs E., Galante E., Lynch J.M., Schepers J.S., Werner D., Werry P.A. (Eds.), Biological resource management: connecting science and policy (OECD), INRA éditions and Springer, Paris, France, 2000, pp. 110–125.

[21] Azcón R., Selective interactions between free-living rhizospheric bacteria and vesicular-arbuscular mycorrhizal fungi, Soil Biol. Biochem. 21 (1989) 639–644.

[22] Rodríguez R., Los nematodos de la platanera (*Musa acuminata* AAA, sub grupo Cavendish Enana) en Canarias (1963–1984), XOBA-Monografía 4, Caja Insul. Ahorr. Canar., Las Palmas de Gran Canaria, Spain, 1990, 58 pp.

[23] Singh V.S., Nath R.P., Pathogenicity of root-knot nematode *Meloidogyne incognita* on papaya, Indian J. Nematol. 26 (1996) 115–116.

[24] Ramakrishnan S., Rajendran G., Influence *of Meloidogyne incognita* on yield components and physiological functions of papaya, Nematol. Med. 26 (1998) 225–228.

[25] Jaizme-Vega M.C., Azcón R., Response of some tropical and subtropical cultures to endomycorrhizal fungi, Mycorrhiza 5 (1995) 213–217.

[26] Balakrishna R., Bararaj D.J., Mallesha B.C., Selection of efficient VA mycorrhizal fungi for papaya, Biol. Agric. Hortic. 13 (1996) 1–6.

[27] Trindade A.V., Siqueira J.O., Almeida F.P., Mycorrhizal dependency of papaya commercial varieties, Pesqui. Agropecu. Bras. 36 (2001) 1485–1494.

[28] Jaizme-Vega M.C., Tenoury P., Pinochet J., Jaumot M., Interactions between the root-knot nematode *Meloidogyne incognita* and

the mycorrhizal association *Glomus mosseae* and Grande Naine banana, Plant Soil 196 (1997) 27–35.

[29] Alarcón A., Davies F.T. Jr., Egilla J.N., Fox T.C., Estrada- Luna A.A., Ferrera-Cerrato R., Short term effects of *Glomus claroideum* and *Azospirillum brasilense* on growth and root acid phosphatase activity of *Carica papaya* L. under phosphorus stress, Rev. Latinoam. Microbiol. 44 (2002) 31–37.

[30] Jaizme-Vega M.C., Rodríguez-Romero A.S., Piñero Guerra M.S., Potential use of rhizobacteria from the *Bacillus* genus to stimulate the plant growth of micropropagated banana, Fruits 59 (2004) 83–90.

[31] Hussey R.S., Barker K.R., A comparison of method of collecting inocula of *Meloidogyne* spp. including a new technique, Plant Dis. Rep. 57 (1973) 1025–1028.

[32] Hewitt E.J., Sand and water culture method used in the study of plant nutrition, Techn. Comm. 22, Farnham R. Commonw. Agric. Burlaux, Bucks, UK, 1952.

[33] Jones J.B., Benjamin B., Mills H.A., Plant analysis handbook. 1. Methods of plant analysis and interpretation, Micro-Macro Publ., Athens, GA, USA, 1991, 213 p.

[34] Rund R.C., Fertilizers, in: Williams S. (Ed.), Official Methods of Analysis of the Association of Official Analytic Chemist, 14th Edition, AOAC, Ed. Sidney Williams, Arlington, Virginia, USA, 1984, pp. 8–37.

[35] Baker K.R., Nematode extraction and bioassays, in: Barker K.R., Carter C.C., Sasser J.N. (Eds.), An advanced treatise on *Meloidogyne*, Vol. II. Methodology, North Carol. State Univ., Graphics Raleigh, NC, USA, 1985.

[36] Phillips J.M., Hayman D.S., Improved procedures for cleaning roots and stain parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection, Trans. Brit. Mycol. Soc. 55 (1970) 158–161.

[37] Koske R.E., Gemma J.H., A modified procedure for staining root to detect VA mycorrhizas, Mycol. Res. 92 (1989) 486–505.

[38] Giovanetti M., Mosse B., An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots, New Phytol., 84 (1980) 489–500.

[39] Dhillion S.S., Dual inoculation of pretransplant stage *Oryza sativa* L. plants with indigenous vesicular-arbuscular mycorrhizal fungi and fluorescent *Pseudomonas* spp., Biol. Fertil. Soils 13 (1992) 147–151.

[40] Singh S., Kapoor K.K., Effects of inoculation of phosphate-solubilizing microorganisms and an arbuscular mycorrhizal fungus on mungbean grown under natural soil conditions, Mycorrhiza 7 (1998) 249–253.

[41] Ravnskov S., Jakobsen I., Effects of *Pseudomonas fluorescens* DF57 on growth and P uptake of two arbuscular mycorrhizal fungi in symbiosis with cucumber, Mycorrhiza 8 (1999) 329–334.

[42] Attia M., The efficiency improvements of mineral fertilizers used and maize yield by arbuscular mycorrhizal fungus and plant growth-promoting rhizobacteria, Ann. Agr. Sci. Cairo 44 (1999) 41–53.

[43] Germida J.J., Walley F.L., Plant growth-promoting rhizobacteria alters rooting patterns and arbuscular mycorrhizal fungi colonization of field-grown spring wheat, Biol. Fertil. Soils 23 (1997) 113–120.

[44] Andreucci F., Fusconi A., Gamalero E., Piras R., Repetto O., Sampó S., Trotta A., Martinotti M.G., Berta G., Reduction of the chemical inputs in a vegetable crop by the use of beneficial rhizospheric microorganisms, INCO-DC, Second Ann. Rep., INCO, 1999.

[45] Siddiqui I.A., Ehteshamul-Haque S., Shaukat S.S., Use of *Pseudomonas aeruginosa* in the control of root-knot disease complex in tomato: the effects of different inoculum of *Meloidogyne javanica* and *Rhizoctonia solani*, Acta Agrobot. 54 (2001) 45–54.

[46] Khan M.R., Kounsar K., Hamid A., Effect of certain rhizobacteria and antagonistic fungi on root-nodulation and root-knot nematode disease of green gram, Nematol. Med. 30 (2002) 85–89.

[47] Jaizme-Vega M.C., Pinochet J., Growth response of banana to three mycorrhizal fungi in *Pratylenchus goodeyi* infested soil, Nematropica 27 (1997) 69–76.

[48] Siddiqui I.A., Mahmood I., Effect of a plant growth-promoting bacterium, an AM fungus and soil types on the morphometrics and reproduction of *Meloidogyne javanica* on tomato, Appl. Soil Ecol. 8 (1998) 77–84.

Efecto de la inoculación conjunta de hongos formadores de micorrizas arbusculares y rizobacterias promotoras del crecimiento vegetal en papaya (*Carica papaya* L.) infectada con el nematodo agallador *Meloidogyne incognita*.

Resumen — Introducción. Los hongos formadores de micorrizas arbusculares (MA) y las rizobacterias promotoras del crecimiento vegetal (PGPR) están considerados como importantes microorganismos rizosféricos benéficos, pudiendo ser utilizados como estrategia de control biológico frente a determinados patógenos de la raíz como los nematodos. El empleo de estos microorganismos, no es siempre fácil, debido al alto grado de especificidad que regula este tipo de interacciones. El objetivo del presente trabajo fue determinar si la inoculación conjunta de dos especies de hongos MA y un cóctel de *Bacillus* que contenía tres cepas ya descritas como PGPR en otros cultivos era capaz de reducir la infección y el daño ocasionado por *M. incognita* en papaya. **Material y métodos**. Las semillas de papaya fueron inoculadas con dos aislados micorrícicos (*Glomus mosseae* y *G. manihotis*) al inicio de la fase de enraizamiento. Una vez establecida la simbiosis micorrícica, se procedió a añadir el cóctel de *Bacillus*. Veinte días después del transplante a maceta, se aplicó el inóculo que contenía el nematodo agallador. El ensayo se dio por finalizado 160 días después de la inoculación con *M. incognita*, momento en el que se analizaron las plantas. **Resultados**. Los beneficios en términos de desarrollo vegetal y nutrición debidos a la micorrización persistieron en presencia del cóctel de *Bacillus*. El efecto positivo de la doble inoculación microbiana fue sin embargo diferente en función de la especie de *Glomus* presente. Este efecto positivo fue también evidente en aquellas plantas infectadas con el nematodo. Así, en las plantas micorrizadas, los niveles de infección de *M. incognita* fueron significativamente inferiores. La adición de las cepas PGPR no mejoró sin embargo el efecto positivo de la micorrización frente al nematodo. **Conclusión**. Estos resultados aconsejan la realización de ensayos previos que permitan seleccionar la combinación microbiana más adecuada a cada situación. De este modo, la aplicación conjunta de de hongos micorrícicos y rizobacterias promotoras del crecimiento vegetal podría ser utilizada como alternativa en el control de nematodos agalladores de raíz, con ciertas garantías de éxito.

España / *Carica papaya* / micorrizas arbusculares / *Glomus* / rizobacterias / *Bacillus* / simbiosis / crecimiento / nutrición de las plantas / control biológico