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Effects of rhizobacteria on parasitism by *Meloidogyne ethiopica* on grapevines

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Abstract *Meloidogyne ethiopica* is one of the most important plant-parasitic nematodes affecting vines in Chile and is very aggressive and difficult to control. This study evaluated 16 strains of rhizobacteria, originally isolated from roots of grapevines, for their effects on parasitism and nematode damage to potted vine plants. The antagonistic effect of rhizobacteria was assessed by treating 2-month-old plants of cv. Chardonnay in 3-l pots with a suspension containing 1×10^6 cfu ml^{-1} of the bacteria and 1,000 nematode eggs. After 6 months of growth, the plants were cut and root and canopy weights, nematode populations and root damage determined. The effect of rhizobacterial culture filtrate on hatching of nematode eggs was also assessed in vitro. Seven strains of rhizobacteria proved effective in inhibiting damage or reproduction of the nematode. These were strains of *Serratia marcescens*, *Comamonas acidovorans*, *Pantoea agglomerans*, *Sphingobacterium spiritivorum*, *Bacillus mycoides*, *Alcaligenes piechaudii* and *Serratia plymuthica*. A further three strains, of *Bacillus megaterium*, *P. agglomerans* and *Pseudomonas savastanoi*, significantly increased root weight, but did not decrease nematode damage or population density. The supernatant of all strains significantly decreased hatching of juvenile nematodes after 24 h of immersion, with isolates of *P. putida* and *B. megaterium* being the most effective.

Keywords Biological control · PGPR · Plant-parasitic nematodes

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Introduction

The presence of plant-parasitic nematodes continues to be one of the main problems affecting the root system of grapevines (*Vitis vinifera* L.), with damage normally reflected in lower production and in some cases total crop loss. Smiley (2005) estimates that plant-parasitic nematodes cause losses of US$78 billion in agriculture world-wide; however these losses have not been clearly estimated for grapevines in Chile, since damage caused by nematodes varies depending on many factors, such as soil type, vine cultivar, climate, crop management among others (Ferris and McKenry 1975).

In Chile several genera and species of plant-parasitic nematodes have been reported to cause economic

damage and to be present in the whole area under cultivation (Aballay et al. 2009). Among them, *Meloidogyne* species are frequently found in association with the roots of grape plants and are especially harmful in the wine grape cultivars Chardonnay, Cabernet Sauvignon, Merlot and Shiraz. Some table grape cultivars, such as Red Globe and Flame Seedless, are also very sensitive to root-knot nematodes, which are frequently associated with the fungi responsible for black-foot disease and grapevine decline (Scheck et al. 1998; Montealegre et al. 2009). One of the most frequently found species is *M. ethiopica,* present alone or in combination with other *Meloidogyne* species. *M. ethiopica* was for many years confused with *M. incognita*, *M. arenaria* or *M. javanica* due to its morphological characteristics and the differential host test (Carneiro et al. 2004, 2007). Over 99 % of the vineyards infested with this genus show the presence of *M. ethiopica*. This species has been proven to be more aggressive than other *Meloidogyne* species present in Chile, as evidenced by the larger size of galls on the roots, the number of eggs per gram of roots and the premature decline of plants. The high population density of this species is a perennial problem for farmers. Multiple classical control methods and strategies are employed for infested soils including the use of fallow, organic amendments, non-fumigant nematicides and others.

Under replanting conditions, soil fumigants and rootstocks are alternatives, although these are not extensively used by most farmers because chemicals are fairly expensive and dangerous and because rootstocks are not tolerant to all nematodes, e.g. *Xiphinema index* or *Mesocriconema xenoplax*, at the same time. Chemical nematicides continue to be the most frequently employed tool, but in recent years many restrictions have been imposed on the use of agrochemicals by the market and thus the need for new methods is increasing. In the past 10 years, the interest in biological control as an alternative to chemical control of nematodes in vineyards affected by *M. ethiopica* and others has increased and some research is being conducted by private companies and government institutions.

The potential use of rhizobacteria as a control agent has been evaluated for different pathogens, including the endoparasitic nematodes *M. incognita* and *M. javanica* (Ali et al. 2002; Siddiqui et al. 2007) and some ectoparasitic nematodes, e.g. *Criconemella xenoplax* (Kluepfel et al. 1993), *Paratrichodorus pachydermus* and *Trichodorus primitivus* (Insunza et al. 2002).

Rhizobacteria isolated from Chilean vineyards have recently been assessed to determine their effect on parasitism by the grapevine nematode *X. index*. Bacteria isolated from the roots and rhizosphere of grape plants grown in four different vineyards were chosen for assessments using in vitro plants. The results showed that species from four bacterial genera, *Bacillus*, *Pseudomonas*, *Stenotrophomonas* and *Serratia*, reduced root damage and also suppressed *X. index* populations (Aballay et al. 2011).

The starting hypothesis in the present study was that rhizosphere bacteria showing suppressive effect against the ectoparasitic nematode *X. index* also have a suppressive effect on the development of, and parasitism by, the endoparasitic nematode *M. ethiopica*. This hypothesis was examined in pot experiments on a very sensitive grape cultivar.

Materials and methods

A set of 16 rhizobacteria isolates previously used in experiments to assess their effect on parasitism by *X. index* was used to inoculate new grapevines infected with *M. ethiopica*. These isolates were originally isolated from the roots of grape plants and were stored at −70 °C in a medium composed of 1:1 Nutrient Broth (Difco, USA) and glycerol prior to use (Aballay et al. 2011).

Preparation of bacterial inocula

The 16 selected bacterial isolates were grown in the dark for 48 h at 22 °C on tryptic soy broth agar (TSBA; Becton Dickinson & Co., USA) medium to verify their purity. One loop-full of pure culture was suspended in liquid medium (half-strength TSB) and allowed to multiply for 48 h on a rotary shaker at 160 rpm at the same temperature. The multiplied bacterial suspension was centrifuged at 4,000 rpm for 20 min and the bacterial pellet was re-suspended in an isotonic solution (0.01 M) of $MgSO_4$ and adjusted to a final concentration of 10^6 colony forming units (cfu) per ml according to Kluepfel et al. (1993). The

bacterial suspensions obtained were used for inoculation of plant roots and substrates.

Plant material and bacterial inoculation

Plants were obtained from grapevine cuttings, rooted in steamed substrate. Two-month-old plants, with two leaves, were removed, washed with sterile water and the roots soaked in bacterial suspension for 20 min. Inoculated plants were planted in 3-l pots, filled with a sterile substrate composed of sand:agricultural soil in proportions of 2:1, pH8.0, 0.72 % organic matter, 23, 9 and 125 ppm of N, K and P, respectively. An additional volume of 100 ml per pot of the bacterial suspension was added to the surface of the substrate and allowed to soak in.

Egg extraction and pot inoculation

Fifteen days after bacterial inoculation, nematode eggs were extracted from grapevine roots infested with *M. ethiopica* according to the method described by Hussey and Barker (1973) and 1,000 eggs per pot were distributed into four holes, each 3 cm deep.

Once inoculated, plants were incubated in a shaded 10 m×20 m greenhouse covered by a protection mesh, which intercepted 30 % of sunlight and prevented overheating of plants and pots. Plants were watered with unsterilised well water once or twice per week, depending on temperature. The maximum and minimum temperature outside the greenhouse in mid-summer was approx. 34 °C and 15 °C, respectively, and within the greenhouse 28 °C and 15 °C, respectively.

Experiments were performed with the 16 rhizobacteria isolates plus three controls. The first control consisted of a suspension with the chemical nematicide fenamiphos (NEMACUR 240 CS, Bayer CropScience), at a rate of 0.5 ml per pot; the second was a suspension containing only nematodes and the third was the isotonic solution 0.01 M of $MgSO_4$.

The treatments were evaluated 6 months after inoculation, once plants entered dormancy. The plants were removed from the pots, the roots were washed with tap water and the plants were stored in a cool room at 7 °C until analysis. Numbers of galls, eggs and second-stage juveniles (J2) were recorded, as well as fresh weight of aerial parts and roots. Eggs were extracted with the Hussey and Barker (1973) method. Soil was processed according to the soil sieving and Baermann funnel method, using 250 cm^3 of the substrate for J2 extraction (Christie and Perry 1951). Identification and counting were carried out under a dissecting microscope (Carl Zeiss, Stemi 2000 C) at 50–90X magnification.

Effect of bacterial culture filtrate on hatching of *M. ethiopica* eggs

To determine the effect of the 16 bacterial culture filtrates on hatching, culture filtrates collected after centrifugation of bacteria were used. Fresh eggs were extracted from tomato roots previously inoculated with nematode eggs and cultivated in sterile substrate for two months. The eggs were superficially sterilised by immersion for 12 h in 0.001 % chlorhexidine digluconate, washed three times and maintained in sterile tap water for 24 h at 4 °C before further use (Huettel and Rebois 1985).

The 16 isolates of the rhizobacteria were cultivated on half-strength TSBA (15 g l^{-1}), with one loopful of pure 24-h culture inoculated into an Erlenmeyer flask containing 50 ml sterile half-strength TSB, and grown at 22 °C with rotary shaking at 180 rpm for 48 h. The cell suspension concentration of all isolates was then adjusted to 10^6 cfu ml^{-1}.

After two centrifugations of bacterial suspensions at 4,000 rpm for 20 min, the culture filtrate was collected. Working under sterile conditions, 500-µl aliquots of nematode suspension in sterile tap water containing approximately 50 eggs, with about 30 % containing second-stage juveniles and 70 % different embryonic stages, were placed in 35-mm diameter sterile glass Petri dishes. A volume of 2 ml culture filtrate was then added to each plate, in the presence of the antibiotic streptomycin sulphate (200 µg ml^{-1} final concentration). The plates were kept at 26 °C for 24 h (Siddiqui et al. 2007) and hatching was determined by counting the second stage juveniles hatched in a Baermann funnel over a period of 10 days, with counts every 2 days. Two control treatments were used, eggs in TSB and eggs in the chemical organo-phosphate nematicide fenamiphos (1.5 µl ml^{-1} water).

Experimental design and statistical analysis

For assays in the greenhouse, the 19 treatments were replicated 10 times per treatment, distributed according to a completely randomised design. The data obtained were subjected to one-way analysis of variance (ANOVA) and treatment means were compared with the control plants infested with eggs, according to the Dunnet's test at $P<0.05$. The experiment was performed twice.

For in vitro culture filtrates, 18 treatments were performed twice, with six replicates per treatment. Data from both tests were compared statistically and as they were not different ($p<0.05$), the averages of both tests are presented.

Prior to Dunnet's test at $p<0.05$, data were transformed to arcsine of percentage data and MINITAB for Windows, Release 13 software (Minitab Inc., Pennsylvania, USA), was used for the analysis of results.

Results

The effects of rhizobacteria on parasitism by *M. ethiopica* on grapevine roots and its reproduction in inoculated plants were evaluated after 6 months of incubation. In most of the parameters evaluated, plants treated with rhizobacteria had a lower degree of damage or fewer eggs and juveniles compared with the nematodes-only control treatment. All the bacterial isolates gave a lower number of galls per g root than the control plants infected with nematodes and no bacterial treatment (10.1) and eight were significantly different ($p<0.05$) (Table 1). However, this was not reflected in the number of eggs present in the roots or juveniles in soil, since of the eight more effective rhizobacteria isolates, only *S. marcescens* 6 also gave a significantly different number of eggs and juveniles compared with control plants.

Seven of the bacterial isolates tested were considered effective, as they were effective in at least two of the three parameters assessed (Table 1). Of these isolates, *S. marcescens* 6 was the most effective, showing good activity as regards number of galls, eggs and juveniles. The other effective isolates were *C. acidovorans* 49, *P. agglomerans* 54, *S. spiritivorum* 64, *B. mycoides* 83, *A. piechaudii* 97 and *S. plymuthica* 213.

The effect of the isolates in decreasing parasitism by the nematodes was not directly associated with positive effects on plant growth. Only two isolates, *P. agglomerans* 54 and *P. savastanoi* 176, with significant effect on root galling or population of *M. ethiopica*, gave plants that had significantly greater root weight than plants inoculated with nematodes only (Table 2). The effect of the *P. agglomerans* 54 strain could be related to its effect on decreasing the population of the nematode, while the *P. savastanoi* 176 strain gave fewer galls than the control (Table 1). None of the bacteria-treated plants had lower foliar weight than the isotonic solution control or damage attributable to the bacteria ($p<0.05$).

The culture filtrates of all rhizobacteria isolates significantly reduced hatching of *M. ethiopica* eggs after 24 h of immersion compared with TSB (Table 3). A large proportion of eggs (92.1 %) hatched in TSB and only a small proportion (7.9 %) in fenamiphos. Of the rhizobacterial treatments, the least hatching was observed for eggs in culture filtrates of isolates of *Pseudomonas putida* 188 (14.3 %), *Bacillus megaterium* 69 (16.9 %), *B. pumilus* 72 (20.6 %) and *P. fluorescens* 144 (25.4), all of which were as effective as fenamiphos ($p<0.05$). Hatching in culture filtrates of the remaining rhizobacteria ranged from 33.9 to 57.1 %. The nematode eggs showed different kinds of damage and most of them looked dark, with the cuticle eroded or with small punctures. Some of the emerged juveniles were dead, but most of them had normal appearance.

The chemical nematicide fenamiphos was shown to be effective and worked well as a control, since it was able to decrease damage and reproduction of the nematodes. The parameters assessed were different from the control plants inoculated with nematodes in all cases.

Discussion

The effect of rhizobacteria on populations of nematodes from the genus *Meloidogyne* and their impact in limiting nematode damage and improving growth of different crops has been studied previously. However such studies have mainly focused on interactions with *M. incognita* (Becker et al. 1988; Kokalis-Burelle et al. 2002; Huang et al. 2010), *M. javanica* (Ali et al. 2002; Siddiqui et al. 2007) and a few others, such as

Table 1 Effects of rhizobacterial isolates on root galling and population of *M. ethiopica* in potted plants

Bacteria species	Isolate	Galls, number per g root	Eggs, number per g root	Juveniles (J2) per 250 cm^3 soil
Alcaligenes piechaudii	97	3.8[a]	531.4	15.2[a]
Bacillus megaterium	69	6.6	672.4	35.9
Bacillus megaterium	185	6.3	675.6	47.3
Bacillus mycoides	83	6.5	329.5[a]	27.0[a]
Bacillus pumilus	72	7.1	551.7	30.3[a]
Comamonas (Pseudomonas) acidovorans	49	3.9[a]	1209.8	32.1[a]
Micrococcus luteus	14	5.8	808.0	31.6[a]
Pantoea agglomerans	54	6.1	317.3[a]	21.9[a]
Pseudomonas fluorescens	144	4.1[a]	1376.4	69.1
Pseudomonas putida	188	6.9	868.2	29.7[a]
Pseudomonas savastanoi pv. Oleae	176	3.4[a]	914.6	35.5
Rahnella aquatilis	203	4.2[a]	891.9	50.2
Serratia marcescens	6	2.7[a]	352.9[a]	18.9[a]
Serratia plymuthica	213	2.6[a]	833.0	19.5[a]
Sphingobacterium spiritivorum	64	4.4[a]	842.9	29.7[a]
Stenotrophomonas sp.	158	6.3	393.4[a]	44.1
Nematodes (*M. ethiopica*) only		10.1	804.7	66.1
Fenamiphos (0.5 ml pot^{-1})		0.1[a]	47.4[a]	0.0[a]
Isotonic solution		0.0[a]	0.0[a]	0.0[a]

[a] Significantly different at $P<0.05$ according to Dunnet's test compared with control with nematodes only. Values represent means of 10 replicates

M. exigua (Oliveira et al. 2007). To our knowledge, this is the first assessment of the effects of rhizobacteria on *M. ethiopica*. The results obtained here indicate that the effects of rhizobacteria treatment on *M. ethiopica* were similar to those on other species.

The culture filtrates from the 16 bacterial strains studied here can be considered effective against the nematode, since all filtrates decreased juvenile hatching after 24 h of immersion, by 14.3 to 57.1 % compared with the TSB control. Similar hatching results have been obtained in other studies with 2 or 3 days of immersion (Ali et al. 2002; Mendoza et al. 2008).

In most earlier studies, the results obtained in vitro with eggs or juveniles of *Meloidogyne* spp. showed that the number of rhizobacterial strains able to kill nematodes is much larger than the number of strains with similar activity when inoculated into pots or in field assays (Becker et al. 1988; Oliveira et al. 2007; Huang et al. 2010). In this study, the 16 bacterial isolates tested were all significantly different from the control in the in vitro test on hatching rates, and seven showed a significant effect in decreasing the number of galls or nematode reproduction rate. This means that about 44 % of the strains maintain their nematicidal activity when applied to potted plants, a high proportion by previous standards (Mekete et al. 2009). The reason for this higher proportion may be that the strains used in this study were isolated from grapevine roots growing in soils with low nematode densities, cultivated using normal agronomic practices. Thus some of these soils may have been exerting some degree of control over this or other nematodes by some means. Most of the rhizobacteria used in other studies on nematodes have been isolated from different cultivated plants and assessed in greenhouse tests in tomato plants, which may affect root colonisation.

The inhibition of nematode hatching observed in this study may be caused by secondary metabolites produced by the rhizobacteria, which may result in egg lysis and affect egg viability (Westcott and Kluepfel 1993; Neipp and Becker 1999; Mendoza et al. 2008; Abo-Elyours et al. 2010). Siddiqui and

Table 2 Effect of rhizobacterial strains on the growth of potted grape plants parasitised by M. *ethiopica*

Bacteria species	Isolate	Foliar weight (g)	Cane weight (g)	Root weight (g)
Alcaligenes piechaudii	97	0.6	10.0	9.6
Bacillus megaterium	69	0.6	8.8	12.5[a]
Bacillus megaterium	185	0.4	10.6	5.9
Bacillus mycoides	83	0.3	9.5	4.8
Bacillus pumilus	72	0.4	8.3	5.6
Comamonas (Pseudomonas) acidovorans	49	0.5	11.8	7.8
Micrococcus luteus	14	0.5	11.0	8.3
Pantoea agglomerans	54	0.4	10.5	13.8[a]
Pseudomonas fluorescens	144	0.6	13.9	11.0
Pseudomonas putida	188	0.5	9.0	8.7
Pseudomonas savastanoi pv. Oleae	176	0.5	10.5	13.5[a]
Rahnella aquatilis	203	0.7	9.7	10.6
Serratia marcescens	6	0.4	10.3	5.9
Serratia plymuthica	213	0.6	10.8	10.5
Sphingobacterium spiritivorum	64	0.4	8.8	6.1
Stenotrophomonas sp.	158	0.5	9.9	12.0
Nematodes (*M. ethiopica*)		0.4	11.5	8.6
Fenamiphos (0.5 ml pot^{-1})		0.5	10.5	12.8[a]
Isotonic solution		0.4	10.7	7.6

[a]Significantly different at $P<0.05$ according to Dunnet's test compared with control with nematodes only. Values represent means of 10 replicates

Shaukat (2003) and Siddiqui et al. (2005) also reported that some metabolites such as 2,4 diacetylphloroglucinol (DAPG) and cyanhydric acid produced by *Pseudomonas spp*. inhibit hatching of *M. javanica* and *M. incognita*.

The low number of eggs of *M. ethiopica* compared with the untreated control observed in potted grape plants inoculated with strains of *Pantoea agglomerans* and *Bacillus mycoides* was also reported by Munif et al. (2001) and Mekete et al. (2009) for *M. incognita*. Those authors concluded that these endophytic bacteria are able to decrease root penetration by juveniles and inhibit their growth into the roots through induction of systemic resistance.

The inhibitory effect of the rhizobacteria on hatching of nematode eggs was not related to inhibition of parasitism in plants, even with the two most effective strains *P. putida* 188 and *B. megaterium* 69 or the least effective *S. marcescens* 6, which showed completely different results when inoculated onto roots of vines. This means that the effects observed in vitro do not necessarily reflect the effectiveness of a bioantagonist and that in a long-term assay (such as 6 months in this study), many other factors influence the biocontrol activity.

Evaluation of the strains in terms of vine growth showed that plants inoculated with *P. agglomerans* 54, *P. savastanoi* 176 and *B. megaterium* 69 had greater root weight (Table 2), despite these strains not showing good antagonistic activity in potted plants. Rhizobacteria are known to promote the growth of plants through several mechanisms, including fixing atmospheric nitrogen, synthesis of hormones (IAA) and antibiotic production (Glick et al. 1998; Asghar et al. 2002; Rives et al. 2007; Rokhzadi et al. 2008), which may have occurred in our test.

The species *B. megaterium* has been reported to produce metabolites and potentially to be a good candidate for biological control of nematodes (Neipp and Becker 1999; Oliveira et al. 2007). Huang et al. (2010) reported several nematicidal volatiles and an antagonistic effect on *M. incognita* infection, especially with inoculum concentrations between 1×10^7 and 1×10^9 cfu ml^{-1}, much higher than

Table 3 Hatching rate (%) of *M. ethiopica* eggs after immersion in the culture filtrates of the rhizobacteria for 24 h at 26 °C

Bacteria species	Isolate	Hatching (%)
Alcaligenes piechaudii	97	33.9[a,b]
Bacillus megaterium	69	16.9[a]
Bacillus megaterium	185	56.9[a,b]
Bacillus mycoides	83	38.1[a,b]
Bacillus pumilus	72	20.6[a]
Comamonas (Pseudomonas) acidovorans	49	52.9[a,b]
Micrococcus luteus	14	48.7[a,b]
Pantoea agglomerans	54	43.9[a,b]
Pseudomonas fluorescens	144	25.4[a]
Pseudomonas putida	188	14.3[a]
Pseudomonas savastanoi pv. Oleae	176	43.9[a,b]
Rahnella aquatilis	203	55.6[a,b]
Serratia marcescens	6	57.1[a,b]
Serratia plymuthica	213	47.6[a,b]
Sphingobacterium spiritivorum	64	44.4[a,b]
Stenotrophomonas sp.	158	43.4[a,b]
Fenamiphos (3.0 µl plate^{-1})		7.9[a]
TSB		92.1[b]

[a] Significantly different at $P<0.05$ according to Dunnet's test compared with control TSB

[b] Significantly different at $P<0.05$ according to Dunnet's test compared with control fenamiphos. Values represent means of 6 replicates

in our study, which could explain the difference in effect on potted plants. It has also been reported that strains of this bacteria can effectively promote plant growth by phosphate solubilisation or alter the root system through induction of auxin and ethylene formation (López-Bucio et al. 2007).

The seven rhizobacterial isolates which gave a significant effect in controlling *M. ethiopica* in this study gave different results in a previous study when assessed for the ectoparasitic nematode *X. index*. According to Aballay et al. (2011), most of these isolates showed good nematicidal activity when culture filtrates were evaluated in Petri dishes on *X. index*, but only *S. plymuthica* 213 showed a good suppressive effect when in vitro plants were inoculated with *X. index* and bacteria. The other isolates gave more variable results and *C. acidovorans* 49 did not show good nematicidal activity against *X. index*, but produced an increase in shoot and root weight of potted grape plants.

M. ethiopica is an endoparasitic nematode and majority of its life cycle occurs in the roots, while the ectoparasitic nematode *X. index* lives freely in the soil, which may mean differences in their cuticle structure or composition, e.g. type of collagens or soluble cuticle proteins associated with the outer coat (Blaxter and Robertson 1998). *M. incognita* has collagen-like proteins distributed through the entire cuticle (Spiegel et al. 1995), but in *X. index* the structure of the collagens is not known (Blaxter and Robertson 1998). There are also differences between the species in terms of other epicuticle components, such as carbohydrate recognition domains, which means that they may contain components with a different collagen sequence, no collagenous domains, or collagens masked by other components (Spiegel et al. 1995). Other studies have compared the nematicidal activity of exudates from a particular rhizobacterial isolate on two different nematodes species, *Panagrellus redivivus* and *Bursaphelenchus xylophilus*, and completely different answers have been obtained (Gu et al. 2007). This selectivity must be considered for effective use of rhizobacterial isolates in control programmes.

Most of the studies assessing the effect of rhizobacteria on parasitism, damage or death of plant-parasitic nematodes have been performed on endoparasitic nematodes such as *Meloidogyne* spp. or *Heterodera* spp., but those strains showing good effects on some parameters are generally not evaluated against other nematode genera and feeding habits. Considering that in natural soils several nematode genera normally parasitise crop roots at the same time, more studies on combinations of nematode species are needed.

The results obtained in this study demonstrate the possibility of using some strains of rhizobacteria for the control of *M. ethiopica* in grapevines. However, the mortality or control effect in treatments with rhizobacteria was not 100 % and rhizobacteria-treated plants had higher nematode populations and damage than those treated with the chemical nematicide. Therefore inoculum of the nematode is still present and it could cause serious damage. To improve the efficacy of treatment, it may need to be modified in some respects, e.g. by increasing the number of cfu ml^{-1} of carrier or the number of applications over the treatment period. In the longer term, if bacteria have successfully colonised the roots they may be superior to the chemical nematicide, since it is degraded in the soil.

Therefore further experimentation is needed to identify environmental conditions stimulating the survival and reproduction of rhizobacteria to be used for biocontrol purposes under field conditions.

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