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Assessment of rhizobacterial consortia to manage plant parasitic nematodes of grapevine

Erwin Aballay *, Simona Prodan, Pia Correa, Jorge Allende

University of Chile, Faculty of Agronomical Sciences, Department of Crop Protection, Santa Rosa 11.315, Santiago, Chile

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ABSTRACT

Two of the most damaging plant parasitic nematodes affecting vineyards in Chile are *Xiphinema index* and *Meloidogyne ethiopica*, whose control is primarily performed with chemical nematicides. The aim of this study was to assess the effect of formulations based on native rhizobacterial consortia and increasing cell concentrations on the mortality and root system damage of potted plants. The grapevine cultivar Cabernet Sauvignon grown in naturally infested soils was used. The isolates *Bacillus amyloliquefaciens* FR203A, *B. megaterium* FB133M, *B. thuringiensis* FS213P, *B. thuringiensis* FB833T, *B. weihenstephanensis* FB25M, *B. frigoritolerans* FB37BR, and *Pseudomonas fluorescens* FP805PU were initially assessed in mixtures of three, four or five bacteria in liquid, powder and isotonic solution (0.01 M $MgSO_4$) formulations. The concentrations 10^6, 10^8 and 10^9 colony-forming units per mL were tested in a second study using one of the consortia. Results showed that the three initial consortia in first assay had similar effects on parasite control, with significantly lower reproductive indices observed after 6 months compared to the control. Damages caused by *X. index* were also lower for all the treatments, with no differences observed among the formulations. In contrast, the effects of the consortia against damages caused by *M. ethiopica* were more variable and did not correspond to the decrease of the juvenile densities in soil. The second assay confirmed previous results also showing that nematode control did not increase with the increasing concentrations evaluated.

1. Introduction

Grapes (*Vitis vinifera* L.) are an economically important crop in Chile, with a cultivated area of approximately 198,000 ha at present. Chilean vineyards are free from several pests and diseases such as the grape aphid (*Daktulosphaira vitifoliae*). However plant-parasitic nematodes (PPN) are the primary problem, affecting the root systems of grapevines and causing damages that are typically reflected in lower production and, in some cases, total crop loss. The two most damaging nematodes are the ectoparasite *Xiphinema index*, present in 48% of soils cropped with grapevines, and species of *Meloidogyne*, primarily *M. ethiopica*, which are present in 20% of the planted area (Carneiro et al., 2007; Aballay et al., 2009). Other PPN frequently associated with grapevines include *Mesocriconema xenoplax* and *Tylenchulus semipenetrans* (Aballay et al., 2009). Plantations affected by PPN species, alone or in combination, show destroyed roots due to direct as well as secondary damages caused by several root-associated fungi. This situation results in the replacement of plants before they are 15 years old, corresponding to less than 50% of their potential productive life.

Multiple classical management methods have been used to deal with infested soils, including the use of fallow, organic amendments, and grafting of desired cultivars on nematode resistant rootstocks. Some chemical nematicides are registered for use, but they are active over a very short period of time, no more than 30 days, after which damages increase. Importantly, with the withdrawal of some older nematicides in the last two years, such as carbofuran and oxamyl, the need for new control strategies increased.

In the past 10 years, the interest in biological tools as an alternative or complement to other control strategies in vineyards affected by PPN increased, and some research being conducted by private companies and government institutions, with rhizobacteria being one of the most considered alternatives (cita agregar). The potential use of rhizobacteria has been evaluated under different conditions for several pathogens, including *M. incognita* and *M. javanica* (Ali et al., 2002; Siddiqui et al., 2007) and other ectoparasitic nematodes, such as *Criconemella xenoplax* (Kluepfel et al., 1993), *Paratrichodorus pachydermus* and *Trichodorus primitivus* (Insunza et al., 2002).

In Chile, rhizobacteria isolated from healthy vineyards cropped in

* Corresponding author.
E-mail address: eaballay@uchile.cl (E. Aballay).

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suppressive soils have been previously assessed to determine their control effects on *X. index*, *M. ethiopica* and other nematodes through *in vitro* and pot assays. Species from at least four genera, *Bacillus*, *Pseudomonas*, *Stenotrophomonas* and *Serratia*, were capable to reduce root damage and suppressed nematode populations (Aballay et al., 2011, 2012, 2013). These findings have promoted research to develop practical tools to exploit some strains in management programmes. However, it is necessary to develop formulations that keep the viability and efficacy of the microorganisms for an easy application (Nakkeeran et al., 2005; Viguera and Delgado, 2007). Furthermore, it is necessary to consider the use of a mix of different, rather than single isolates, to ensure that at least one of them may work under the highly variable environmental conditions encountered in the field (Nakkeeran et al., 2005; Raupach and Kloepper, 1998).

The aim of this study was to evaluate the efficacy of some liquid and powder formulations of a novel rhizobacterial consortium to control PPN affecting grapevines, compared with an unformulated mix, and to determine its potential under field conditions.

2. Materials and methods

2.1. Assays

Two assays were performed to investigate the biocontrol potential of several rhizobacteria strains, using naturally infested soils exhibiting a high level of PPN infestation. The bacteria were originally isolated from healthy feeding roots of grapevines (*V. vinifera*) as described by Aballay et al. (2011).

The assays were designed to determine the effectiveness of different strains mixtures (assay 1), and to assess the PPN control levels in relation to their concentrations, expressed as colony-forming units (cfu) mL^{-1} (assay 2).

2.2. Rhizobacterial inocula

The selected bacterial isolates were grown in dark for 48 h at 22 °C on tryptic soy broth agar (TSBA, Becton Dickinson & Co., USA) to verify their purity. A loop-full of cells was used to inoculate liquid medium (half-strength TSB) for culturing. After incubation for 48 h on a rotary shaker (160 rpm), the liquid medium was centrifuged at 4000 rpm for 15 min. The bacterial pellet was then resuspended in an isotonic solution (0.01 M) of $MgSO_4$ (Johansson et al., 2003) and adjusted to a final concentration of 10^6 cfu mL^{-1} according to Kluepfel et al. (1993).

The rhizobacterial species and isolates selected for use in the assays were as follows:

Bacillus amyloliquefaciens FR203A, *B. megaterium* FB133M, *B. thuringiensis* FS213P, *B. thuringiensis* FB833T, *B. weihenstephanensis* FB25M, *Brevibacterium frigoritolerans* FB37BR, *Pseudomonas fluorescens* FP805PU.

These isolates were previously assessed under *in vitro* and glasshouses conditions as unformulated organisms, showing effectiveness towards *Xiphinema* and *Meloidogyne* spp. (Aballay et al., 2012, 2013; Castañeda, 2014).

Prior to use, the rhizobacteria used to inoculates roots and substrates, were prepared in both liquid and powder formulations and mixed in different combinations for comparisons with unformulated suspensions, which were prepared as previously described in point 2.2.

The liquid formulation was prepared using a mix of trehalose, xanthan gum and glycerol which have a pseudoplastic rheology, i.e. more viscous, useful for cellular microencapsulation allowing stability during storage (Bashan et al., 2014).

Powder formulations were prepared using a mix of diatomaceous earth, trehalose and yeast extract (Bashan et al., 2014). The drying times used are suitable for line production, lasting 72–96 h, resulting in a final moisture content around 3–5%.

2.3. Soil

PPN naturally infested soil was obtained from an ungrafted 10-year-old vineyard located in Casablanca Valley, Chile, cultivated with the cv. Chardonnay. The soil was mixed and introduced into 5-L pots for assays 1 and 2. The soil had median densities of 168 and 100 specimens of *X. index* and *M. ethiopica* per 250 cm^3 of soil, respectively.

The physicochemical characterization of the substrate showed a sandy loam texture, a pH of 6.6, an electrical conductivity of 6.1 dSm^{-1}, 1.86% organic matter, and NPK levels of 12, 16 and 649 mg kg^{-1}, respectively.

2.4. Plants

Plants used in both assays were obtained by propagating cuttings from virus-free cv. Cabernet Sauvignon plants rooted in growth medium consisting of perlite that had been autoclaved at 121 °C for 30 min. Irrigation with distilled water was performed every two days until new roots appeared, with Hoagland fertilizer (Hoagland and Arnon, 1950) added once a week.

2.5. Treatments

2.5.1. Assay 1

Formulated and unformulated bacteria were assessed at the same final concentration of 2×10^6 cfu mL^{-1} per isolate, which were distributed in 11 treatments, including a chemical control, as described in Table 1. After being formulated, the bacteria were then mixed to obtain a final concentration of 1×10^8 cfu and diluted with water to obtain the final needed concentration.

Prior to transplanting, the roots were immersed for 30 min in the bacterial suspensions for each treatment and planted in 5-L pots filled with the PPN naturally infested soil. Each pot was treated with 600 mL of the same bacterial suspension in which the plants had been submerged, since the use of different delivery systems for application of rhizobacteria increases their population load in the sites of activity (Nakkeeran et al., 2005).

As a chemical control, Rugby® 200 CS (a.i. cadusafos) was applied at 0.5 mL L^{-1}, 50 mL per pot.

Once inoculated, the plants were incubated in a shaded 10 m × 20 m greenhouse covered with a Rashel mesh, which intercepted 30% of the sunlight and prevented plants and pots overheating. The pots were watered with unsterilized well water once or twice per week, depending on temperature for five months. The maximum and minimum

Table 1
Mixture of rhizobacterial isolates under different formulations for the control of plant parasitic nematodes.

SET	Rhizobacteria	Type of formulation (Treatment)
A	*B. frigoritolerans* FB37BR, *B. megaterium* FB133M, *B. thuringiensis* FB833T *B. weihenstephanensis* FB25M	Liquid (T1) Powder (T2) Unformulated (T3)
B	*B. amyloliquefaciens* FR203A, *P. fluorescens* FP805PU *B. thuringiensis* FS213P	Liquid (T4) Powder (T5) Unformulated (T6)
C	*P. fluorescens* FP805PU *B. frigoritolerans* FR37BR, *B. thuringiensis* FS213P, *B. weihenstephanensis* FB25M	Liquid (T7) Powder (T8) Unformulated (T9)
Rugby 200 CS (a.i. cadusafos)	0.5 mL L^{-1} water	T10
Control	Isotonic solution 0.01 M $MgSO_4$	T11

temperatures outside of the greenhouse in mid-summer were approximately 34 and 18 °C, respectively; inside the greenhouse they were 28 and 18 °C, respectively.

2.5.2. Assay 2

A novel combination of isolates was used in a second assay since no differences were detected in assay 1 between the three specific consortia, on PPN control. Five isolates were used in the liquid and powder formulations as described for assay 1, modifying the number of cfu mL^{-1}, assessing three concentrations, 2×10^6, 2×10^8 and 2×10^9 cfu mL^{-1} (Table 2). Plants were inoculated as described in assay 1.

Once inoculated, the plants were cultivated in a glasshouse with a temperature of 24 °C for five months during the winter, making it necessary to temper the room.

2.6. Compatibility test

An *in vitro* test was performed to rule out incompatibility among the bacteria tested. For this assay, the isolates were cultivated in Petri dishes with agar medium and 75% TSB. Bacterial growth was assessed every 24 h for 10 days.

2.7. Assessments

After 5 months of growth, the plants were uprooted and the roots were washed, weighed and stored at 7 °C for damage evaluation. To determine the *X. index* densities, 250 cm^3 of soil from each pot was processed according to the Brown and Boag method (1988) for optimal recovery of adult and juvenile stages.

For *Meloidogyne* juveniles (J2), the same amount of soil was processed according to the soil sieving and Baermann funnel method (Hooper and Evans, 1993). Identification and counting were performed under a dissecting microscope (Carl Zeiss, Stemi 2000 C) at 50–90 × magnifications.

The effect of the isolates on the parasitism by PPN was evaluated by determining the specific damage associated with nematode feeding. For *X. index*, the total number of root tip galls in the root system was recorded, while for *M. ethiopica*, the number of galls and eggs was recorded, as well as the gall index on a 1–10 scale, where 1 = 0–10% roots showing galls and 10 = 90–100% (Hussey and Barker, 1973; Bridge and Page, 1980). Fresh weight of roots was also recorded.

2.8. Experimental design and statistical analysis

For both assays, a completely randomized design was performed, with 11 and 7 treatments performed using 6 and 8 replicates, respectively.

To evaluate the effect of the different treatments, the reproductive index (R) was calculated, which relates the final population (Pf) to the initial population (Pi) (Oostenbrink, 1966), where the final population corresponds to that observed at the end of the season of study. Prior to calculating the R value and performing an analysis of variance (ANOVA), the nematode population density data were transformed as log (x + 1) for normalization, as suggested for nematode counts that are skewed, with a normally negative binomial distribution (Noe, 1985).

When significance at $P \leq 0.05$ was detected, the treatment means were compared according to LSD Fisher's Test. The gall index values were analysed with the Kruskal-Wallis nonparametric test.

Table 2
Treatments in assay 2, mixtures of rhizobacteria, three bacterial concentrations (cfu mL^{-1}), formulated as a liquid and powder.

SET	Rhizobacteria mixture	Type of formulation (Treatment)
D	*B. frigoritolerans* FB37BR	Powder 2×10^6 cfu mL^{-1} (T1)
	B. weihenstephanensis FB25M	Liquid 2×10^6 cfu mL^{-1} (T2)
	B. thuringiensis FS213P	Powder 2×10^8 cfu mL^{-1} (T3)
	B. thuringiensis FB833T	Liquid 2×10^8 cfu mL^{-1} (T4)
	P. fluorescens FP805PU	Powder 2×10^9 cfu mL^{-1} (T5)
		Liquid 2×10^9 cfu mL^{-1} (T6)
Control	Isotonic solution 0.01 M $MgSO_4$	(T7)

3. Results

3.1. Assay 1

No signs of incompatibility or inhibition among the rhizobacteria were observed, with all strains showing good growth and strain *B. weihenstephanensis* FB25M colonizing the medium faster than the other strains.

Most of the treatments based on formulations and bacterial broths were able to reduce *X. index* populations compared to the control (Table 3), with significantly lower R values observed for the three liquid formulations (T1, T4 and T7).

Results of treatments with powder formulations (T2, T5, and T8) were more variable, with T2 (R = 0.65) being the most effective and similar to the chemical control (R = 0.5). Treatments 5 and 8 did not achieve significant differences compared to the negative control. Two treatments (T6 and T9) of unformulated bacteria exhibited a significantly positive efficacy compared to control, and only one isolate from set A was not different from control (T3).

The effect of the different sets and formulations on the presence of *X. index* was also reflected in the damages produced, which primarily included swelling of the root apex. All treatments, independent of the bacterial set, reduced damages, and four of them were similar to the chemical control ($p < 0.05$). Reductions in damage varied between 40.3 and 77%, while the nematicide decreased damage by approximately 72%. There were no clear differences among the formulations (Table 4).

Considering *M. ethiopica*, all formulated and unformulated mixtures of rhizobacteria, induced a significant decrease in number of J2 in the soil. Interestingly, six of the treatments produced the same results as the control nematicide(Table 3). The performances of the formulated bacteria was not different from those in the isotonic solution, except for Set A, where the results obtained using the liquid formulation was different from the other two, confirming that bacteria found good niches in the two carriers used ($p < 0.05$).

No effect, on knots per root, was observed for the formulations based on rhizobacteria since most of the treatments did not achieve significant differences with respect to the absolute control (Table 4). Only treatment 6 (bacteria in an isotonic solution) achieved a result similar to that of the nematicide ($p < 0.05$) significantly different from control, with a 49.2% reduction in damage.

The gall index showed lower values than control ($p < 0.05$) for some

Table 3
Reproductive indices of *X. index* and *M. ethiopica* after 6 months of growth on vines of cv Cabernet Sauvignon (assay 1).

Treatments	Reproductive Index	
	Xiphinema index	*Meloidogyne* spp.
T1: Set A, Liquid	1.08 bc[a]	0.56 a
T2: Set A, Powder	0.65 ab	2.26 bcd
T3: Set A, Unformulated	2.0 cd	3.29 cd
T4: Set B, Liquid	1.13 bc	1.23 abcd
T5: Set B, Powder	3.40 e	2.09 bcd
T6: Set B, Unformulated	1.13 bc	1.14 abcd
T7: Set C, Liquid	1.51 c	0.98 abcd
T8: Set C, Powder	1.62 cd	0.70 a
T9: Set C, Unformulated	1.59 c	0.82 ab
Rugby® 200 CS (a.i. cadusafos)	0.50 a	0.70 a
Control - Isotonic solution 0.01 M $MgSO_4$	2.83 de	6.60 e

[a] Means within columns followed by the same letter are not significantly different according to Fisher's Least Significant Difference (LSD) test ($p < 0.05$). The values represent the means of 6 replicates.

Table 4
Damages to grapevines per gram of root caused by *X. index* and *M. ethiopica* (assay 1).

Treatments	*X. index*	*M. ethiopica*	
	Lesions g root^{-1}	Galls g root^{-1}	Gall index (1–10)
T1: Set A, Liquid	13.9 cd[a]	8.1 bc	6.8 cde
T2: Set A, Powder	6.1 a	6.4 b	5.1 ef
T3: Set A, Unformulated	9.8 abc	9.7 cd	8.7 ab
T4: Set B, Liquid	7.9 ab	8.3 bc	6.7 cde
T5: Set B, Powder	15.7 d	7.4 bc	7.5 cd
T6: Set B, Unformulated	9.9 abc	3.8 a	6.0 de
T7: Set C, Liquid	13.5 cd	8.3 bc	6.1 de
T8: Set C, Powder	7.6 ab	9.8 cd	8.5 abc
T9: Set C, Unformulated	10.9 bc	12.8 de	9.2 ab
Rugby® 200 CS	7.4 ab	4.6 a	2.8 f
Control - Isotonic solution 0.01 M $MgSO_4$	26.3 e	7.4 bc	9.9 a

[a] Means within columns followed by the same letter are not significantly different according to Fisher's Least Significant Difference (LSD) test ($p < 0.05$). The values represent the means of 6 replicates.

treatments, including T5, T1, T4, T7, T6, and T2. The latter exhibited the lowest value and did not differ from the chemical treatment, which had the lowest score (2.8).

For both nematode species, the chemical treatment significantly reduced the root damage.

3.2. Assay 2

For the assay comparing formulations with different cfu concentrations, concentrations 2×10^6 through 2×10^9 cfu mL^{-1} showed few differences, related to the amount of inoculum (Table 5). The reproductive index (Pf/Pi) had values lower than 1, for most of the treatments, showing that two and four were different from the control in *X. index* and *M. ethiopica* respectively ($p < 0.05$).

These data confirm the capability of the mixtures of rhizobacteria to decrease PPN densities compared to the untreated plants. The observed increase in PPN in most of the observed treatments, including the control, was less than in the previous assay, likely due to lower temperatures in the greenhouse conditions. An assessment of the damages for both PPN confirm a higher efficacy of the rhizobacteria towards *X. index* compared to *M. ethiopica*, despite the significant decrease in J2. Highest cfu concentrations showed a lower incidence for endoparasite.

4. Discussion

Although the effectiveness of using rhizobacteria to control PPN has been demonstrated in many studies (Almaghrabi et al., 2013; Burkett-Cadena et al., 2008; Castaneda-Alvarez and Aballay, 2016; Fernandes et al., 2013; Jonathan et al., 2000; Radwan et al., 2012), many factors are able to affect their efficacy (Castro-Sowinski et al., 2007).

Table 5
Effect of the concentration of bacterial inocula on the reproduction of *X. index* and *M. ethiopica* and damages to root systems (assay 2).

Treatments	Reproductive Index		Swellings or Galls per g of root	
	X. index	*M. ethiopica*	*X. index*	*M. ethiopica*
1. Liquid 2×10^6 cfu	0.80 b[a]	0.72 b	7.2 b	8.4 a
2. Powder 2×10^6 cfu	0.90 a	0.90 ab	7.5 ab	8.5 a
3. Liquid 2×10^8 cfu	0.96 ab	0.74 b	7.0 b	6.6 b
4. Powder 2×10^8 cfu	0.98 ab	0.66 b	7.1 b	8,7 ab
5. Liquid 2×10^9 cfu	0.97 ab	0.82 b	6.8 b	6.2 b
6. Powder 2×10^9 cfu	0.66 b	1.2 ab	6.5 b	7.5 ab
7. Control - Isotonic solution 0.01 M $MgSO_4$	1.33 a	1.46 a	9.3 a	11.8 a

[a] Means within columns followed by the same letter are not significantly different according to Fisher's Least Significant Difference (LSD) test ($p < 0.05$). The values represent the means of 6 replicates.

The primary function of using formulations is to maintain the viability of the microorganisms, providing them with the necessary nutrients and protection in which the rhizobacteria can physiologically adjust to the new environment so that they can multiply, spread along the root system and compete with other microorganisms to properly colonize roots (Stirling, 2014).

Duffy et al. (1996) showed that formulations based on mixtures of microorganisms with different control mechanisms could ensure the desired effects under varying conditions.

The results obtained in this study showed that the formulations based on the different mixtures of rhizobacteria were able to reduce PPN densities and their damage to the roots of *V. vinifera*. Most formulations produced similar or better results than the unformulated bacterial suspensions of the same rhizobacteria mixture, and a number of treatments exhibited performances that were similar to that of the nematicide used as control, the organophosphate cadusafos.

For *Meloidogyne*, several studies have described its control using different biological agents, including *Pseudomonas* spp. (Akhtar and Panwar, 2012) and other rhizobacterial species (Siddiqui et al., 2002, 2007). Commercial formulations based on *Bacillus* strains have also been evaluated, with the results showing that it is possible to decrease *Meloidogyne* densities and damages (Burkett-Cadena et al., 2008), with effects equal to that of commercial nematicides (Terefe et al., 2009; Xiong et al., 2015).

Insunza et al. (2002) reported a reduction between 56.7 and 74.4% of *Paratrichodorus pachydermus* and *Trichodorus primitivus* on potatoes in naturally infested soil, without negative effects on plant growth. Kluepfel et al. (1993) showed more than a 50% reduction of a *Criconemella xenoplax* with strains of *P. fluorescens*.

No differences observed between liquids and powders nor with unformulated consortia (in an isotonic solution). In first assay, an analysis of root damages showed that all rhizobacteria-based treatments decreased the number of lesions caused by *X. index*, by more than 50%. However, the results for *M. ethiopica* were different, since although PPN juvenile control was effective, only T6, unformulated bacteria, showed a reduction in the number of galls (49.2%), similar to that of the chemical control. Despite the high degree of juveniles control in soil, this effect was not translated into fewer galls per gram of root, which may be due to the unaffected larvae being able to enter the roots and complete its lifecycle without being affected by the bacterial exudates once within the roots. However, decrease in root damage was observed for several treatments, which was related to the control of juveniles in the soil.

The gall index data also was not consistent with the number of gall per g of root, differences may be due to biological aspects. It may be related to the reproduction of the nematodes that escaped the bacterial activity, during the five months period following treatments. This may indicate that the efficacy of the bacteria tested may vary in time, due to other rhizosphere effects or microorganisms.

The results of the second assay showed no differences between lowest and highest bacterial concentrations tested or in the type of formulation used with respect to both *X. index* control and root damage. For *M. ethiopica*, practically all doses of both formulations were sufficient to differentiate from the control. These results were consistent with those obtained in previous studies, where even concentrations of 1×10^6 cfu mL^{-1} were effective for PPN control (Burkett-Cadena et al., 2008; Castañeda, 2014; Aballay et al., 2013; Reyes et al., 2008; Radwan et al., 2012).

Burges (1998) noted that the greatest advantage in the use of bacterial broths is that microorganisms are active and able to immediately compete for root colonization with other microorganisms present, unlike those in formulations, where are typically in a dormant state. However, the use of a bacterial broth in the field has some

disadvantages, such as the lack of protection against temperature fluctuations during storage, transport and application, or from the variable field conditions during the adaptation process (Burges, 1998; Stirling, 2014). In this study, the formulated organisms behaved similarly or were superior to the unformulated bacterial suspensions, which would greatly facilitate their use under field conditions.

Few studies have been performed to determine optimum bacterial concentrations for control of PPN, with 1×10^6 cfu mL^{-1} being one of the most frequently used concentrations (Reyes et al., 2008; Terefe et al., 2009). These data are useful, as *in vitro* studies carried out by Khan et al. (2008), showed that higher bacterial concentrations longer exposure times increased the mortality of nematodes.

No differences were detected in the weights of the aerial parts and roots for the two assays, likely because the experimental time was not sufficient to reveal any variation (Siddiqui and Akhtar, 2009).

The assays performed in this study were consistent with previous results from *in vitro* and controlled conditions showing the potential of individual isolates to control *X. index* (Aballay et al., 2011, 2012), and *Meloidogyne* species (Castañeda, 2014; Aballay et al., 2013). However, in these previous studies, the observed effects varied depending on the crop conditions, which is why the construction and testing of consortia is justified. On the other hand, Castañeda (2014) also determined that strains FB25M, FB37BR, FR203A and FS213P succeeded in decreasing the hatching of *M. ethiopica* eggs.

In conclusions, the results of this study showed that the use of formulations based on mixtures of the assayed rhizobacteria is able to suppress populations of PPN, exhibiting effects similar to those of a nematicide, confirming the proposed hypothesis.

It was also noted that differences in bacterial cfu mL^{-1} were not reflected in higher degrees of PPN control, which may validate the use of lower concentrations in management programmes.

Finally, it is important to note that the use of these products should be considered in conjunction with an integrated pest management plan that combines cultural practices, including the use of clean plant material and resistant rootstocks (Perry and Moens, 2006).

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Declaration of competing interest

All authors declare that they have no conflict of interest.

CRediT authorship contribution statement

Erwin Aballay: Writing - original draft, Supervision. **Simona Prodan:** Methodology. **Pia Correa:** Investigation. **Jorge Allende:** Investigation.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cropro.2020.105103.

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