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SHORT COMMUNICATION

Effect of entomopathogenic nematodes on the plant-parasitic nematode *Nacobbus aberrans*

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Abstract *Nacobbus aberrans* is a sedentary endoparasite nematode that forms galls in the roots of infected plants and produces important economic losses in some countries of the American continent. It has a wide host range, attacking mainly potato, tomato, sugar beet, and pepper crops. A reduction in the plant-parasitic nematode populations in the presence of entomopathogenic nematodes (EPNs) has been frequently reported. In the present work, the effect of the application of two native EPN isolates (*Steinernema rarum* and *Heterorhabditis bacteriophora*) on a *N. aberrans* population was evaluated in tomato plants under greenhouse conditions. Sixty days after inoculation, the number of galls and egg masses and the reproduction factor (RF) of *N. aberrans* were calculated. Of the variables analyzed, only the RF was significantly lower in both EPN treatments than in control. *N. aberrans* reproduction decreased by 57 and 53 % in plants inoculated with *S. rarum* and *H. bacteriophora*, respectively. These results showed that EPNs and their bacterial symbionts affected the reproductive potential of the *N. aberrans* population. This is the first study addressing the use of EPNs in the control of this important plant-parasitic nematode.

Keywords Biological control · *Heterorhabditis bacteriophora* · *Steinernema rarum* · *Nacobbus aberrans* · Plant-parasitic nematode · Entomopathogenic nematodes

Introduction

Nacobbus aberrans causes serious losses to agriculture in the American continent. It is a sedentary endoparasite that forms galls on the roots of infected plants. Because these galls are similar to those caused by species of the genus *Meloidogyne*, *N. aberrans* is also known as "false root-knot nematode". The species is distributed in Argentina, Bolivia, Chile, Ecuador, USA, Mexico, and Peru (Reid et al. 2003). It has a wide host range that comprises 18 families with approximately 84 species, including crops and weeds (Manzanilla-López et al. 2002). *N. aberrans* affects mainly potato (*Solanum tuberosum*), tomato (*Lycopersicon esculentum*), sugar beet (*Beta vulgaris*), pepper (*Capsicum annuum*), and bean (*Phaseolus vulgaris*). Because of the serious impact it causes to agriculture in general, with losses that range between 35–90 %, depending on the crop (Manzanilla-López et al. 2008) and geographical location, it is considered an A1 quarantine pest (EPPO 1984). In Argentina, *N. aberrans* is widely distributed. In the province of Córdoba, it causes severe damage to greenhouse tomato and pepper crops. The infected plants may show poor development and signs of wilting; at high densities, the pest can even cause plant death (Lax et al. 2011a).

Due to the growing concern for the environmental impact caused by chemical nematicides, as well as the difficulties to develop resistant cultivars (Lewis and Grewal 2005), there is an increasing interest in developing management strategies compatible with production systems, such as the use of organic amendments (Tabarant et al. 2011), arbuscular mycorrhizal fungi (Affokpon et al. 2011), and plant growth-promoting rhizobacteria (Burkett-Cadena et al. 2008). The use of natural antagonists as a biological control method is an alternative tool that can play an important role for the management of plant-parasitic nematodes (PPNs) (Ashoub and Amara 2010).

Entomopathogenic nematodes (EPNs) belonging to the families Steinernematidae and Heterorhabditidae are used as biological control agents of pest insects (Shapiro-Ilan and Gaugler 2002). The third-stage infective juvenile (IJ)

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penetrates the insect haemocel. Shortly after entry, IJs release symbiotic bacteria (*Xenorhabdus* spp. in Steinernematidae and *Photorhabdus* spp. in Heterorhabditidae), which multiply there and serve as food source for the nematodes. Bacteria generate metabolites that cause septicemia and kill the host within 48–72 h after infection (Fallon et al. 2004). Different EPN species have been found to have a suppressive effect on PPNs under different conditions, both in the field and in greenhouse (Grewal et al. 1997; Somasekhar et al. 2000; Jagdale et al. 2002; Molina et al. 2007). In soybean (*Glycine max*) roots inoculated with IJs of *Steinernema feltiae*, a reduction in *Meloidogyne javanica* penetration was reported (Fallon et al. 2002). This reduction was also detected with *Meloidogyne hapla* in peanut (*Arachis hypogaea*) in the presence of *S. feltiae* and *Steinernema riobrave* (Pérez and Lewis 2004). The application of IJs of *S. feltiae* on tomato plants reduced the number of eggs of *Meloidogyne incognita* and the number of galls in treated roots with respect to control (Lewis et al. 2001).

The suppressive effect of EPNs on PPNs has been attributed to diverse factors mostly related to symbiotic bacteria. In studies conducted in vitro, metabolites of *Photorhabdus luminescens* (obtained from *Heterorhabditis* sp.) reduced egg hatching of *M. incognita* and produced paralysis of second-stage juveniles (J2); these effects would be induced by indole and stilbene isolated from bacteria (Hu et al. 1999). On the other hand, cell-free filtrates of *Xenorhabdus nematophila* (associated with *Steinernema carpocapsae*) and of *Xenorhabdus bovienii* (symbiont of *S. feltiae*) caused 98–100 % mortality of J2 of *M. incognita* and a delay in egg hatching (Grewal et al. 1999). These authors attributed the nematicidal action to the ammonia produced by *Xenorhabdus* spp. However, the use of EPNs does not always reduce PPN populations, and the outcomes of their interactions vary with the EPN and PPN species, the host crop, and the method used to evaluate the impact on PPNs (Lewis and Grewal 2005).

The present study is part of a program aimed at evaluating different potential antagonists of *N. aberrans* (Lax et al. 2011a,b), which might be useful for integrated management of this pest. In this work, the suppressive effect of two native isolates of EPNs on a local population of *N. aberrans* on tomato roots is postulated. Up to the present, this effect has not been analyzed in this species.

Materials and methods

Nematode inoculum

A population of *N. aberrans* from the locality of Río Cuarto (department of Río Cuarto, province of Córdoba, Argentina) was employed. The nematodes were maintained on plants of tomato cv Platense under laboratory conditions. Egg masses were extracted from infected roots and placed in Petri dishes containing distilled water; they were left to hatch at room temperature, and J2 were extracted with a pipette.

Two native isolates of EPNs were used: *Steinernema rarum* from the locality of Arroyo Cabral (ACAB), department of General San Martín (province of Córdoba, Argentina) and *Heterorhabditis bacteriophora* from Rama Caída (RACA), department of San Rafael (province of Mendoza, Argentina). These isolates were multiplied on larvae of *Galleria mellonella* (Lepidoptera: Pyralidae), following the procedure described by Kaya and Stock (1997). Infective juveniles were collected using White traps (White 1927) and kept in water at 25±1 °C until use for 21 days (Pérez and Lewis 2004).

Plant material and treatments

Seeds of tomato cv Platense were placed to germinate in trays with sterile soil and vermiculite (1:1). After 4 weeks, four-leaf seedlings were selected and individually put in plastic pots (3.8 cm in diameter×20 cm in height) containing a mixture of soil and sterile sand (1:1). Three treatments with six replications each were performed: (1) J2 of *N. aberrans* (control); (2) J2 of *N. aberrans*+IJs of *H. bacteriophora* RACA; and (3) J2 of *N. aberrans*+ IJs of *S. rarum* ACAB. The experiment was conducted twice in 2011 (trial 1, January–March; trial 2, May–July) under the same conditions. Roots were inoculated with 100 J2 of *N. aberrans* (initial population) present in 1.5-mL water (Lax et al. 2011b) and covered with the substrate. In EPN treatments, the inoculum (25 IJ/cm^2 in 4 mL of water) (Pérez and Lewis 2002; 2004; Molina et al. 2007) was immediately applied to the soil surface of each pot. The EPN dose applied is the one commonly used for insect control in the field (2.5×10^9 IJ/ha) (Georgis and Hague 1991). The experiment design was completely randomized. The plants were maintained under greenhouse conditions at 24±1 °C with a 14-h photoperiod; they were watered as needed, maintaining soil moisture at field capacity. After 60 days, the plants were uprooted, and the roots were carefully washed free of adhered soil particles. Dry weight of shoot and root was measured. The soil of each pot was processed using the centrifugal flotation technique (Jenkins 1964) for extraction of filiform individuals. Roots were analyzed under stereoscopic microscope, and galls and egg masses were counted. To count the eggs, egg masses were extracted and immersed in 1 % NaClO for 4 min (Hussey and Barker 1973). For each replicate, the final *N. aberrans* population was calculated by adding the total number of eggs plus the nematodes extracted from soil. That value was used to estimate the reproduction factor (RF=final population/initial population).

Calculations and data analysis

The effect of each EPN isolate on *N. aberrans* (Nematode response) was calculated, as indicated by Hol and Cook (2005): the difference between nematode number in the control and nematode number in the treatment was divided by the nematode number in the control and multiplied by one hundred. A positive value of "nematode response" indicates fewer nematodes in the plants treated with EPNs than in the control.

Before performing statistical analyses, assumptions of normality (Shapiro–Wilk's test) and homogeneity were tested for all the parameters obtained. Only the parameter "number of egg masses" did not meet the assumptions and was transformed to $\log_{10}(x+1)$. The effect of the different treatments on the variables was evaluated with an analysis of variance, and means were separated with a Tukey's test ($P \leq 0.05$). All analyses were made with InfoStat program (InfoStat 2002).

Results and discussion

Galls and egg masses produced by *N. aberrans* were observed in all the treatments (Fig. 1). No differences in the number of galls were observed among plants treated with EPNs with respect to control (Table 1). A similar situation was observed for number of egg masses. The RF was significantly reduced by the application of IJs of *S. rarum* ACAB and of *H. bacteriophora* RACA. No differences between RF values obtained in both EPN treatments were detected. Regarding "nematode response," the final population of *N. aberrans* decreased by 57 and 53 % in plants inoculated with *S. rarum* ACAB and *H. bacteriophora* RACA, respectively. No significant differences in dry weight of shoot or root were detected between treatments (data not shown), which is in agreement with previous experiments using *Meloidogyne* spp. (Fallon et al. 2004; Shapiro-Ilan et al. 2006).

Table 1 Effect of two isolates of entomopathogenic nematodes on *N. aberrans* in tomato plants

Treatment	Number of galls	Number of egg masses	Reproduction factor
Control	9.6±4.6a	5.9±3.7a	44.2±36.5a
S. rarum ACAB	8.1±6.2a	3.7±2.8a	19.2±15.9b
H. bacteriophora RACA	6.5±3.7a	3.7±2.4a	20.6±17.9b

Data are means of 12 replicates. The *N. aberrans* inoculum was 100 J2 per 3.8×20-cm pot; all counts were on a per pot basis. Means in the same column followed by the same letter did not differ according to Tukey's test ($P \leq 0.05$)

The IJ dose of *H. bacteriophora* and *S. rarum* (25 IJ/cm^2) used did not reduce the number of galls formed by *N. aberrans* in tomato roots. These results are in disagreement with findings on the effect of *Heterorhabditis baujardi* and *S. feltiae* on *Meloidogyne mayaguensis* reported by Molina et al. (2007). Using the same IJ dose and the same host, those authors observed a gall reduction of 56 and 37 %, respectively. Likewise, the application of a higher dose (125 IJ/cm^2) of *H. bacteriophora* and of *S. carpocapsae* on roots of eggplant (*Solanum melongena*) reduced the number of galls formed by *M. incognita* (by 86.4 and 89.6 %, respectively) and of egg masses (93 and 91 %, respectively) (Abd-Elgawad and Mohamed 2006). On the other hand, the application of a

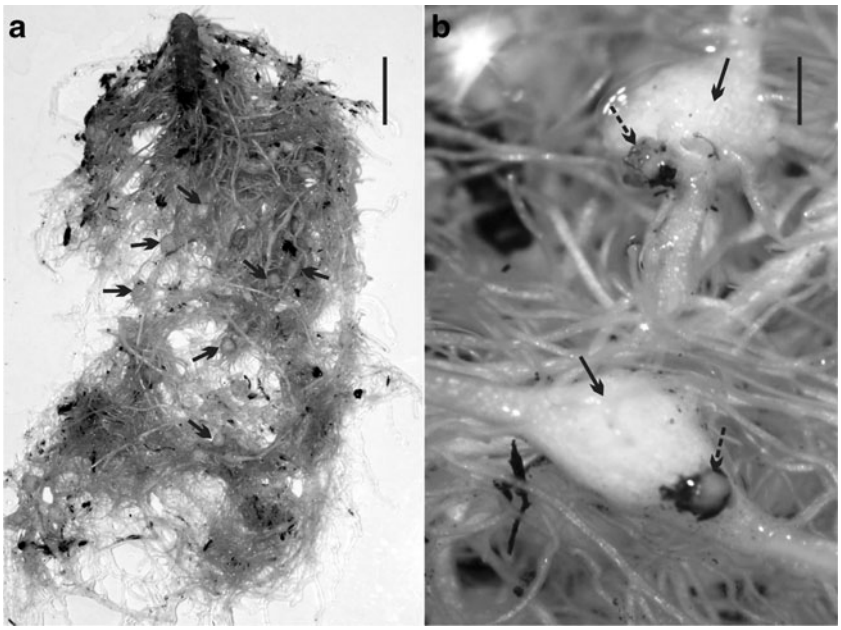

Fig. 1 Root system of tomato infected by *N. aberrans*. **a** Roots with galls (*arrows*) induced by the nematode. **b** Detail of galls (*arrows*) with egg masses (*arrow with dashed line*). *Scale bars*: **a** 2 cm, **b** 2 mm

higher dose (200 IJ/cm^2) of *S. riobrave* and *S. feltiae* did not reduce the number of galls produced by *Meloidogyne partityla* in pecan (*Carya illinoinensis*) (Shapiro-Ilan et al. 2006). In that work, the number of egg masses was also not affected in plants treated with EPNs, which is consistent with findings obtained using a much lower dose in the present work.

Jagdale and Grewal (2008) reported a reduction of a population of the foliar nematode *Aphelenchoides fragariae* in plants of *Hosta* sp. In that work, the inoculation of the rhizosphere with insect cadavers infected with *S. carpocapsae* reduced the multiplication of the nematode on leaves, despite a lack of direct contact with the EPNs. Such effect was attributed to induced systemic resistance (ISR) in the plant by insect-parasitic nematodes. Later, Jagdale et al. (2009) observed that the application of IJs of *S. carpocapsae* and its bacterial symbiont (*X. nematophila*) to the rhizosphere of the same plant stimulated the activity of P-peroxidase, G-peroxidase, and catalase in leaves; these enzyme activities would be responsible for ISR. As mentioned above, no differences in number of galls were observed between treatments; this finding indicates that IJs of EPNs did not affect *N. aberrans* penetration to the roots. However, *S. rarum* and *H. bacteriophora* were efficient in reducing the PPN final population, and, therefore, the RF (with a decrease of 53–57 %). Consequently, these variables should be evaluated in similar studies to have a better understanding of the suppressive effect of EPNs. The reduction of the reproductive potential of *N. aberrans* might be attributed to the possible ISR as suggested by Jagdale et al. (2009).

The pathogenicity of the nematode–bacterium complex varies with the EPN species and its symbiotic strain (Simoes and Rosa 1996). Hence, similar studies should be conducted using other isolates to select the most efficient ones in the management of *N. aberrans.* Furthermore, populations of this species from different geographical areas exhibit differences in their host range. According to their capacity or incapacity to infest certain plants (known as differential hosts), the existence of physiological races within the species is considered (Costilla et al. 1977; Inserra et al. 1985; Lax et al. 2011b). For that reason, it would be useful to evaluate other populations of this parasite. As this experiment involved the use of sterile soil and was developed under controlled conditions, it would also be of great interest to continue evaluating the potential of the use of EPNs as biological control organisms of *N. aberrans* in naturally infested soils, both under greenhouse and field conditions.

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References

Abd-Elgawad MMM, Mohamed MMM (2006) Efficacy of selected bio-control agents on *Meloidogyne incognita* on eggplant. Nematol medit 34:105–109

Affokpon A, Coyne DS, Lawouin L, Tossou C, Agbèdè RD, Coosemans J (2011) Effectiveness of native West African arbuscular mycorrhizal fungi in protecting vegetable crops against root-knot nematodes. Biol Fertil Soils 47:207–217

Ashoub AH, Amara MT (2010) Biocontrol activity of some bacterial genera against root-knot nematode, *Meloidogyne incognita*. J Am Sci 6:321–328

Burkett-Cadena M, Kokalis-Burelle N, Lawrence KS, van Santen E, Kloepper JW (2008) Suppressiveness of root-knot nematodes mediated by rhizobacteria. Biol Control 47:55–59

Costilla MA, de Ojeda SG, de Gomez TH (1977) Contribución al estudio del falso nematodo del nudo *Nacobbus aberrans*. Nematropica 7:7–8

EPPO (1984) Data sheets on quarantine organisms. No. 144, *Nacobbus aberrans*. EPPO Bull 14:61–66

Fallon DJ, Kaya HK, Gaugler R, Sipes BS (2002) Effects of entomopathogenic nematodes on *Meloidogyne javanica* on tomatoes and soybeans. J Nematol 34:239–245

Fallon DJ, Kaya HK, Gaugler R, Sipes BS (2004) Effect of *Steinernema feltiae-Xenorhabdus bovienii* insect pathogen complex on *Meloidogyne javanica*. Nematology 6:671–680

Georgis R, Hague NGM (1991) Nematodes as biological insecticides. Pestic Outlook 2:29–32

Grewal PS, Lewis E, Venkatachari S (1999) Allelopathy: a possible mechanism of suppression of plant parasitic nematodes by entomopathogenic nematodes. Nematology 1:725–743

Grewal PS, Martin WR, Miller RW, Lewis EE (1997) Suppression of plant-parasitic nematode populations in turfgrass by application of entomopathogenic nematodes. Biocontrol Sci Techn 7:393–399

Hol HWG, Cook R (2005) An overview of arbuscular mycorrhizal fungi–nematode interactions. Basic Appl Ecol 6:489–503

Hu K, Jianxiong L, Webster JM (1999) Nematicidal metabolites produced by *Photorhabdus luminescens* (Enterobacteriaceae), bacterial symbiont of entomopathogenic nematodes. Nematology 1:457–469

Hussey RS, Barker KR (1973) A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant Dis Rep 57:1025–1028

InfoStat (2002) InfoStat versión 1.1. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina

Inserra RN, Griffin GD, Anderson JL (1985) The false root-knot nematode, *Nacobbus aberrans*. USA Research Bulletin 510. Utah State University, Logan, 14p

Jagdale GB, Grewal PS (2008) Influence of the entomopathogenic nematode *Steinernema carpocapsae* infected host cadavers or their extracts on the foliar nematode *Aphelenchoides fragariae* on *Hosta* in the greenhouse and laboratory. Biol Control 44:13–23

Jagdale GB, Kamoun S, Grewal PS (2009) Entomopathogenic nematodes induce components of systemic resistance in plants: biochemical and molecular evidence. Biol Control 51:102–109

Jagdale GB, Somasekhar N, Grewal PS, Klein MG (2002) Suppression of plant parasitic nematodes by application of live and dead entomopathogenic nematodes on Boxwood (*Buxus* spp.). Biol Control 24:42–49

Jenkins WR (1964) A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Dis Rep 48:692

Kaya HK, Stock P (1997) Techniques in insect nematology. In: Lacey LA (ed) Manual of techniques in insect pathology. Academic Press, San Diego, pp 281–324

Lax P, Becerra A, Soteras F, Cabello M, Doucet ME (2011a) Effect of the arbuscular mycorrhizal fungus *Glomus intraradices* on the false root-knot nematode *Nacobbus aberrans* in tomato plants. Biol Fertil Soils 47:591–597

Lax P, Becerra A, Caccia MG, Marro N, Valverde C, Agaras B, Cabello M, Doucet ME (2011b) Evaluation of biological alternatives for the control of *Nacobbus aberrans* populations. Nematropica 41:341

Lewis EE, Grewal PS (2005) Interaction with plant parasitic nematodes. In: Grewal PS, Ehlers RU, Shapiro-Ilan DI (eds) Nematodes as biocontrol agents. CAB International, Georgia, pp 349–362

Lewis EE, Grewal PS, Sardanelli S (2001) Interactions between the *Steinernema feltiae–Xenorhabdus bovienii* insect pathogen complex and the root-knot nematode *Meloidogyne incognita*. Biol Control 21:55–62

Manzanilla-López RH, Costilla MA, Doucet ME, Franco J, Inserra RN, Lehman PS, Cid del Prado-Vera I, Souza RM, Evans K (2002) The genus *Nacobbus* Thorne & Allen, 1944 (Nematoda: Pratylenchidae): systematics, distribution, biology and management. Nematropica 32:149–227

Manzanilla-López RH, Quénéhervé P, Brito JA, Giblin-Davis R, Franco J, Román J, Inserra RN (2008) Contributions by Latin American nematologists to the study of nematode plant disorders and related impact on crop production. In: Webster JM, Eriksson JM, McNamara KB (eds) An Anecdotal History of Nematology. Pensoft, Sofia-Moscow, pp 191–218

Molina J, Dolinsky C, Souza RM, Lewis E (2007) Effect of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) on *Meloidogyne mayaguensis* Rammah and Hirschmann (Tylenchida: Meloidogynidae) infection in tomato plants. J Nematol 39:338–342

Pérez E, Lewis E (2002) Use of entomopathogenic nematodes to suppress *Meloidogyne incognita* on greenhouse tomatoes. J Nematol 34:171–174

Pérez E, Lewis E (2004) Suppression of *Meloidogyne incognita* and *Meloidogyne hapla* with entomopathogenic nematodes on greenhouse peanuts and tomatoes. Biol Control 30:336–341

Reid A, Manzanilla-López RH, Hunt DJ (2003) *Nacobbus aberrans* (Thorne, 1935) Thorne & Allen, 1944 (Nematoda: Pratylenchidae); a nascent species complex revealed by RFLP analysis and sequencing of the ITS-rDNA region. Nematology 5:441–451

Shapiro-Ilan DI, Gaugler R (2002) Production technology for entomopathogenic nematodes and their bacterial symbionts. J Ind Microbiol Biotechnol 28:137–146

Shapiro-Ilan DI, Nyczepir A, Lewis EE (2006) Entomopathogenic nematodes and bacteria applications for control of the pecan root-knot nematode, *Meloidogyne partityla,* in the greenhouse. J Nematol 38:449–454

Simoes N, Rosa JS (1996) Pathogenicity and host specificity of entomopathogenic nematodes. Biocontrol Sci Techn 6:403–412

Somasekhar N, Denardo EAB, Grewal PS (2000) Impact of inundative application of entomopathogenic nematodes on nontarget nematode communities in turfgrass ecosystem. J Nematol 32:461

Tabarant P, Villenave C, Risède JM, Roger-Estrade J, Dorel M (2011) Effects of organic amendments on plant-parasitic nematode populations, root damage, and banana plant growth. Biol Fertil Soils 47:341–347

White GF (1927) A method for obtaining infective nematode larvae from cultures. Science 66:302–303