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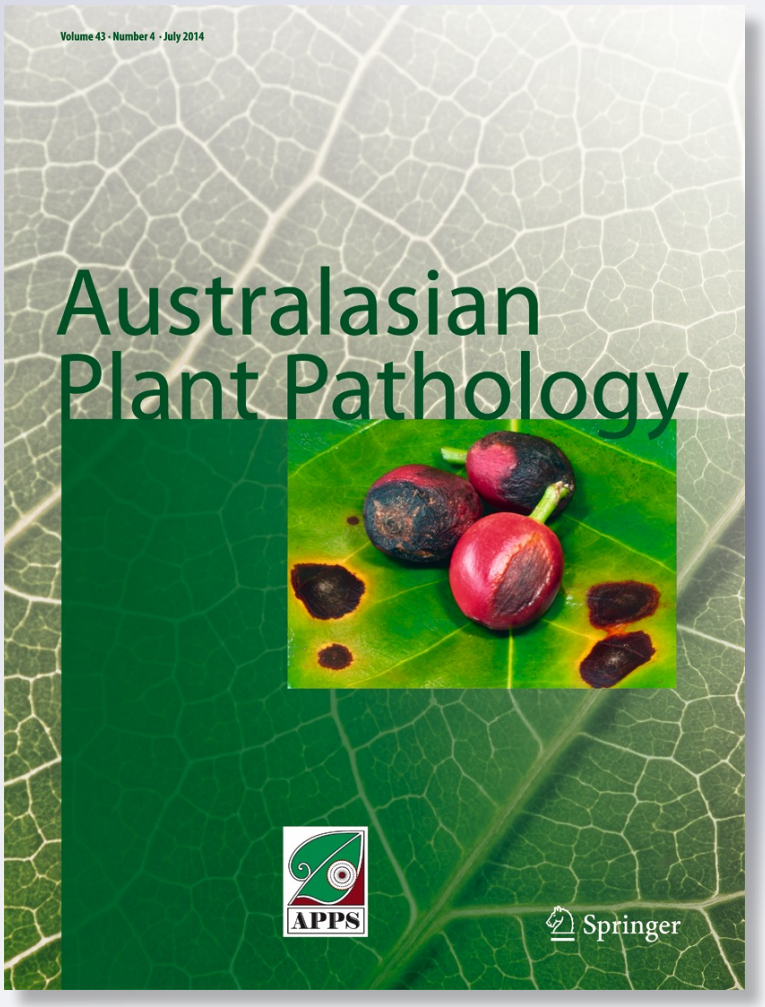

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Pochonia chlamydosporia controls *Meloidogyne incognita* on carrot

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Abstract The fungus *Pochonia chlamydosporia* var. *chlamydosporia* isolate Pc-10 (Pc-10) presents great potential for the management of root-knot nematodes on vegetable crops. However, there is no information about the use of Pc-10 on carrot. Thus, we evaluated in this study the effect of Pc-10 incorporated into the soil (PCI) or applied to the surface of the beds (PCS) on control of *Meloidogyne incognita* on carrot under field conditions, in comparison to an untreated control and the bionematicides *Paecilomyces lilacinus* + *Bacillus subtilis* (PLBS), mix of nematophagous fungi and *Bacillus* sp. (NFB) and *P. lilacinus* (PL). PCI increased the total and marketable production of carrot roots by 25.35 and 55.03 %, respectively. The production of unmarketable roots was reduced by about 50 % in plots treated with PCI, PCS and PLBS. All bionematicides reduced the number of unmarketable roots with galls, but only PCI reduced the reproduction factor of the nematode. The incorporation of Pc-10 in the soil of the beds controls *M. incognita* and improves carrot quality and yield.

Keywords Biological control · *Daucus carota* · Root-knot nematode · Nematophagous fungus

Introduction

Plant-parasitic nematodes, especially *Meloidogyne* spp., are one of the most important pathogens of carrot (*Daucus carota* L.) throughout the world, reducing both quantity and quality of marketable roots (Gugino et al. 2006). In Brazil, nematode management strategies on carrot include crop rotation, fallow, nematicides and biological control agents. However, human health safety and environmental concerns have resulted in the reduction of the use of chemical nematicides in many carrot farms, being replaced by biological products.

Among the various natural enemies of nematodes, the fungus *Pochonia chlamydosporia* var. *chlamydosporia* isolate Pc-10 (Pc-10) has great potential for the management of root-knot nematodes (Dallemole-Giaretta et al. 2012). This fungus parasites eggs and exposed females of root-knot nematode, and has the ability of producing a large amount of chlamydospores, resistant structures which allow this organism to persist in the soil during adverse conditions (Dallemole-Giaretta et al. 2012; Yang et al. 2012; Manzanilla-López et al. 2013).

Despite the potential of *P. chlamydosporia* for controlling root-knot nematodes on vegetable crops, there is no empirical evidence that Pc-10 can be used on the management of this nematode on carrot. Thus, we evaluated the effect of a bionematicide based on the isolate Pc-10 on the control of *M. incognita* on carrot under field conditions.

Materials and methods

A commercial carrot field in Rio Paranaíba, Minas Gerais, Brazil (19°18′ S; 46°09′ W; 1,160 m) was selected for the experiment. The area was naturally infested with *M. incognita*.

Pochonia chlamydosporia isolate Pc-10 (supplied as Rizotec, 3 kg/ha, Rizoflora Biotecnologia S.A., Viçosa - MG) was incorporated into the soil or applied to the surface of the seed beds. The effect of Pc-10 was compared to an untreated control and three bionematicides applied to the surface of the seed beds, namely *Paecilomyces lilacinus* +

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Bacillus subtilis (Rizos Plus, 2.0 kg/ha, Laboratório Farroupilha, Patos de Minas - MG), mix of nematophagous fungi and *Bacillus* sp. (Profix Max, 5 kg/ha, Agrivalle Biotecnologia Agrícola, Pouso Alegre - MG) and *P. lilacinus* (Nemout, 8.0 kg/ha, Improcrop do Brasil, Araucária - PR). The biological treatments will henceforth be referred to as PCI (for the incorporated treatment), PCS (for surface application of Pc-10), PLBS (*Paecilomyces lilacinus* + *Bacillus subtilis*), NFB (mix of nematophagous fungi and *Bacillus* sp.) and PL (*Paecilomyces lilacinus*).

Before sowing, soil samples were collected to evaluate the need for correction of soil pH and fertilizer application for crop maintenance. Based on the results of soil analysis (pH 6.05; P 17.24 mg/dm^3; Ca^{+2} 1.6 cmolc/dm^3; Mg^{+2} 0.92 cmolc/dm^3; 3.7 % organic matter; 41.5 % clay; 35 % sand; 23.5 % silt), lime was used to correct pH and provide calcium and magnesium (1,730 kg/ha).

The plots, 5.0 m long by 1.80 m wide, were marked before the preparation of the beds with the use of a GPS navigation receiver (Garmin, model Etrex H). The experiment consisted of 24 plots with 24 treatments distributed in four randomized blocks.

For PCI treatment, Pc-10 was diluted and applied to the surface of the soil with the aid of a backpack sprayer pressurized with CO_2 at 3.02 kgf/cm^2, equipped with a bar and two fan type nozzles 11002, spaced 0.5 m apart, with spray volume adjusted to 300 L/ha. Following this, the raised beds (1.60 m wide, 0.3 cm high and 0.2 m between beds) were mechanically prepared and a 02-24-12N-P-K formulation (2,200 kg/ha) was applied for starter fertilisation. Then, Pc-10, previously applied to the surface of the soil, was incorporated into the soil during the preparation of the beds.

Carrot cv. Juliana was sown (26 seeds/m) on November 15th, 2011. Each bed had four twin rows (12 cm between twin row and 14 cm between plants within the row), under centre pivot irrigation. Soil samples were collected from each experimental plot for determination of the initial population (Pi) of *M. incognita* before application of the bionematicides. Composite soil samples, consisted of three cores taken to a depth of 200 mm with a soil auger. Second-stage juveniles were extracted from soil samples by centrifugal-flotation technique (Jenkins 1964). Immediately after sampling, the products were applied to the surface of the beds (PCS, PLBS, NFB and PL) similarly as described above for PCI.

The plants were thinned 15 days after emergence, leaving 13 plants/m for final density of 750,000 plants/ha. Top-dressing fertilization was performed by application of urea (80 kg/ha) and potassium chloride (150 kg/ha) at 45 days after sowing (DAS), and a 25-00-25N-P-K formulation (180 kg/ha) was applied at 65 DAS.

At 104 DAS, plots were harvested by digging up the plants from each of two central double lines, discarding 2.5 m at each end. Foliage was removed and discarded.

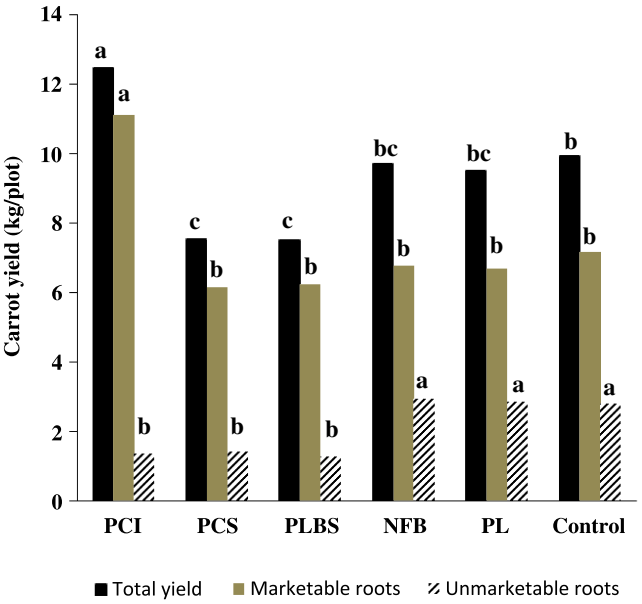

Fig. 1 Carrot (*Daucus carota* cv. Juliana) yield in plots infested with *Meloidogyne incognita* and treated with the *Pochonia chlamydosporia*-based bionematicide applied to the surface of the beds (PCS) or incorporated into the soil (PCI), in comparison with the control and the bionematicides *Paecilomyces lilacinus* + *Bacillus subtilis* (PLBS), mix of nematophagous fungi + *Bacillus* spp. (NFB) and *P. lilacinus* (PL). Means followed by the same letter within each class of roots do not differ by the Tukey test (P=0.05)

Taproot mass (kg/plot) were assessed, according to three categories: marketable, unmarketable without visible galls and unmarketable with visible galls (Walker 2004). Moreover, soil samples were collected from each plot after harvesting the roots to determine the final population of nematodes (Pf). The ratio between Pf and Pi was used to

Table 1 Population of *Meloidogyne incognita* in th soil and percentage of carrot (*Daucus carota* cv. Juliana) roots infected by the nematode in plots treated with *Pochonia chlamydosporia*-based bionematicide applied to the surface of the beds (PCS) or incorporated into the soil (PCI), *Paecilomyces lilacinus* + *Bacillus subtilis* (PLBS), mix of nematophagous fungi + *Bacillus* spp. (NFB) and *P. lilacinus* (PL)

Treatments	Initial population (Pi)	Final population (Pf)	Reproduction factor (R)	Production of unmarketable roots with galls (%)
PCI	18.2 ns	6.0 d	0.33 b	0 b
PCS	19.6	25.9 c	1.32 a	0 b
PLBS	22.6	17.6 cd	0.78 ab	0 b
NFB	27.0	41.6 b	1.54 a	3.4 b
PL	12.0	19.6 cd	1.63 a	2.1 b
Control	39.5	65.6 a	1.66 a	11.1 a

ns not significant by the F test (p=0.05). Means followed by the same letter within each column are not significantly different by the Tukey test (P=0.05). Reproduction factor = Final population/Initial population (Oostenbrink 1966)

calculate the reproduction factor (R) of the nematode (Oostenbrink 1966). The average minimum and maximum air temperatures during the experiment were respectively 25.2 and 34.0 °C.

All data were tested for normality of the error (Kolmogorov-Smirnov test), homogeneity of variances (Bartlett test) and subjected to analysis of variance (P=0.05). Reproduction factor and production of unmarketable roots data were transformed to square roots to achieve a normal distribution before ANOVA. Means were compared by the Tukey test (P=0.05).

Results and discussion

The incorporation of Pc-10 into the soil (PCI) increased the total and marketable production of carrot roots by 25.35 and 55.03 %, respectively (Fig. 1). *Pochonia chlamydosporia* may act as a plant growth promoter (Dallemole-Giaretta et al. 2008; Maciá-Vicente et al. 2009) and in carrot the benefit of the fungus was probably related to the improvement of the marketable product, that is, the taproot. However, when Pc-10 was applied on the surface of the beds already prepared (PCS), the total and marketable production of roots were similar to the control and the bionematicides PLBS, NFB and PL. It is likely that the incorporation into the soil protected Pc-10 from the deleterious effect of the solar radiation (Burges 1998) and also enhanced fungal survival. Additionally, the antagonist was placed in direct contact with or close to the seeds and nematode eggs. Thus, the probability of the fungus establishing itself in the soil and colonising the rhizosphere of carrot and the eggs of the pathogen was greater, in comparison to the application on the surface of the soil (PCS).

The production of unmarketable roots was reduced by about 50 % in plots treated with PCI, PCS and PLBS, and it was similar to the control when NFB and PL were applied on the surface of the beds (Fig. 1). All bionematicides reduced the number of unmarketable roots with galls when compared to the control, but only PCI reduced the reproduction factor of the nematode (Table 1). Root-knot nematodes can induce carrot defects, such as galling, forking, fasciculation, constriction and stubbing of roots (Walker 2004). These malformations are one of the main factors contributing to discarding of taproots in the field (Hay and Pethybridge 2005). The action of *P. chlamydosporia* in colonisation of nematode eggs in the soil prevented the hatching of juveniles (Kerry 2001), especially when the chlamydospores of the antagonist were incorporated into the soil. As a result, the amount of roots infected by *M. incognita*, the final population and the multiplication rate of the nematode were reduced.

In Brazil, previous studies have highlighted the efficiency of the isolate Pc-10 on the control of the root-knot nematode (Coutinho et al. 2009; Podestá et al. 2009; Dallemole-Giaretta et al. 2011, 2012). However, these investigations were carried out under controlled conditions and the plants were grown in sterilised soils. This study produced results which corroborate the findings of the researchers about the potential of *P. chlamydosporia* Pc-10 on the management of root-knot nematode, and it was also provided important information about the performance of the fungus on carrot under field conditions. The incorporation of Pc-10 in the soil of the beds controls *M. incognita* and improves carrot quality and yield. Nevertheless, the integration of the bionematicide with other control methods should be performed to maximize the effect of the fungus, especially under high pressure of nematode inoculum.

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