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Isolation of fungi associated with *Criconemoides* sp. and their potential use in the biological control of ectoparasitic and semiendoparasitic nematodes in sugar cane

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Abstract

Phytoparasitic nematodes are the important pests of sugar cane and controlled with the application of highly toxic chemicals. This study isolated fungi from the sugar cane phytoparasitic nematode *Criconemoides* sp. and tested the pathogenicity of one of these isolates on the nematode community of the sugar cane producing region of Veracruz, Mexico. One fungus was selected in order to monitor the *in vitro* infection process of *Criconemoides* sp. and the effect of this fungus on the density of nematodes associated with sugar cane in greenhouse was evaluated, using naturally infested soil and plants. Two treatments were established: biocontrol, applied with a spore suspension of fungus, and control treatment. A total of 42 fungal isolates were obtained, including *Purpureocillium lilacinum*, which was selected for use in the *in vitro* and greenhouse experiments. From 48 h after *in vitro* infection, blastospores and mycelia were observed within the body of the nematode. The most abundant phytoparasitic nematodes found in samples of the greenhouse experiment were *Criconemoides* sp. and *Helicotylenchus* sp. The initial phytoparasitic nematode populations in biocontrol and control treatments were 253±98 and 287±164 100 mL soil^{-1}, respectively. Ten days following application of the fungus, the population of phytoparasitic nematodes was significantly ($p < 0.01$) lower in the biocontrol (91±26) than in the control (230±5) treatment. The fungus used in the experiment efficiently reduced the population of ectoparasitic and semiendoparasitic nematodes. We recommend field-testing of this fungus in order to determine its potential effectiveness under field crop conditions.

Keywords: Nematofauna, phytoparasites, *Purpureocillium lilacinum*, *Saccharum officinarum*.
Abbreviations: OA_oatmeal agar culture medium; Pi_initial population density; Pf_final population density; Pf/Pi_multiplication rate.

Introduction

Sugar cane is a crop of great importance worldwide; it is grown mainly for the production of sugar, but also provides other products such as paper, fibers and ethanol (Moncada et al., 2013). The economic benefits of this crop have a great social impact, above all in developing countries. Mexico is the fifth largest producer in the world, accounting for almost 50 million tonnes of sugar cane (2.7% of the global total) annually (FAO, 2011). Sugar cane is cultivated in 15 Mexican states, of which the main producer is Veracruz (SIAP, 2011). As in other parts of the world, the monoculture of sugar cane is subject to intensive management featuring multiple applications of chemical pesticides and fertilizers (Pankhurst et al., 2003; Blair and Stirling, 2007; Desgarennes et al., 2011). This type of management leads to a reduction in the biodiversity of the agroecosystem, also reducing the populations of certain natural suppressors of the existing pathogens in the crop (Desgarennes et al., 2011; Severino et al., 2010).

Pests and diseases affecting sugar cane constitute one of the main problems for sugar production worldwide. The most significant pests include the phytoparasitic nematodes, which cause global economic losses estimated to be equivalent to 15% of the production of this crop (Koenning et al., 1999). These phytoparasites cause necrotic lesions and destruction of the secondary roots, causing galls, nodules and malformations that impede the uptake of water and nutrients necessary for development. Infested sugar cane plants present chlorotic foliage and thinner, shorter stalks, which in turn translates into reduced yields (Victoria et al., 1995). The most widely distributed phytoparasitic nematodes in sugar cane fields worldwide are *Criconemoides* spp., *Helicotylenchus* spp., *Meloidogyne* spp. and *Pratylenchus* spp. (Bond et al., 2000; Cadet et al., 2002; Cadet and Spaull, 2005). The phytoparasitic nematodes recorded in sugar cane in Mexico are *Pratylenchus* spp., *Criconema* sp., *Meloidogyne* sp. and *Tylenchus* sp. (Montes, 2000). In the central-coastal region of Veracruz state, studies have been conducted of the population density of phytoparasitic nematodes in various fields sown with sugar cane cultivar MEX-69-290. These studies have recorded *Criconemoides* sp., *Helicotylenchus* sp., *Xiphinema* sp. and *Tylenchus* sp. (85 individuals 100 mL soil^{-1}) as well as free-living nematodes such as *Acrobeles* sp., *Cruznema* sp., *Aphelenchus* sp., *Aporcelaimellus* sp. and *Thornenema* sp. (Desgarennes et al., 2011).

Phytoparasitic nematodes of sugar cane in Mexico are controlled by the application of chemical nematicides (e.g. carbofuran, aldicarb and oxamil). The use of these agrochemicals, although effective, is being restricted because of undesirable secondary effects, such as increased production costs, environmental contamination and risks to human health (Satar et al., 2005). At present, there is a tendency towards substituting these chemical pesticides, including the nematicides, with more ecologically sound methods such as biological control which, as well as presenting no environmental risks, is just as effective in

Table 1. Nematodes associated with the sugar cane rhizosphere in soil used in a greenhouse experiment.

Trophic group	Family	Subfamily	Genus
Phytophagous	Hoplolaimidae	Hoplolaiminae	*Helicotylenchus* sp.
	Criconematidae	Macroposthoniinae	*Criconemoides* sp.
	Ecphyadophoridae	Ecphyadophoroides	*Tenunemellus* sp.
	Tylenchidae	Tylenchinae	*Tylenchus* sp.
		--	morphotype 1
		--	morphotype 2
Bacteriophagous	Cephalobidae	Cephalobinae	*Acrobeles* sp.
			Acrobeloides sp.
	Rhabditidae	Rhabditinae	*Cruznema* sp.
	Panagrolaimidae	Panagrolaiminae	*Panagrolaimus* sp.
Mycophagous	Aphelenchidae	Aphelenchinae	*Aphelenchus* sp.
	Aphelenchoididae	--	morphotype 3
Omnivorous-predator	Aporcelaimidae	Aporcelaiminae	*Aporcelaimellus* sp.
			morphotype 4
	Qudsianematidae	Lordellonematinae	morphotype 5
		Qudsianematinae	morphotype 6
	Dorylaimidae	Thornenematinae	*Thornenema* sp.

reducing the numbers of individuals of the phytoparasitic nematode species (Cadet et al., 2007). Biological control of nematodes refers to the use of microbial agents, such as bacteria and nematophagous fungi (Kerry, 2000; Saxena, 2004). Some nematophagous fungi have high potential as biocontrol agents, however, the populations of these organisms that occur naturally in the soil have been diminished by the repeated application of inorganic fertilizers and chemical pesticides (Wachira and Okoth, 2009). At present, bionematicides are available that contain fungi such as *Arthrobotrys robusta*, *A. conoides*, *A. oligospora*, *Purpureocillium lilacinum* (=*Paecilomyces lilacinus*), *Paecilomyces fumosoroseus*, *Verticillium chlamydosporium* and *Myrothecium verrucaria* (Saxena, 2004); however, isolation of native fungi that are better adapted to the specific edaphoclimatic conditions of the zone of application could ensure higher efficiency in the biological control of nematodes (González et al., 1999). The aim of this study was therefore to isolate nematophagous fungi from individuals of *Criconemoides* sp. and utilize a greenhouse experiment to evaluate the pathogenicity of one of these fungi on nematodes associated with the sugar cane crop.

Results

Native fungi associated with Criconemoides sp. in sugar cane

Forty-two fungal isolates were obtained from the individuals of *Criconemoides* sp. (N=300).

Twenty of these isolates were obtained from areas under habitual management for sugar cane cultivation: *Aspergillus flavus* (1), *Aspergillus niger* (1), *Aspergillus* sp. (1), *Penicillium* sp. (7), *Trichoderma* sp. (1), *Fusarium* sp. (4), *Phoma* sp. (1), *Absidia cylindrospora* (1) and Coelomycetes (3), while the remaining 22 isolates were obtained from an area with no recent application of agrochemicals: *Aspergillus niger* (1), *Aspergillus* sp. (1), *Phoma* sp. (1), *Purpureocillium lilacinum* (3), *Penicillium* sp. (11), *Fusarium* sp. (3) and Coelomycetes (2). Due to the fact that it was the only isolate found that is a parasite of nematodes (Jatala et al., 1979), *P. lilacinum* was selected for observation of the infection process.

Infection process of Purpureocillium lilacinum

In all specimens inoculated with *P. lilacinum*, the conidia germinated and the mycelium developed. At 24 h after inoculation of *P. lilacinum*, development and penetration of the conidia germination tube (29 ± 13 µm in length) was observed on the cuticle of the nematode. Within the body of the nematode, the median bulb and esophageal glands were clearly visible. Movement was observed in all the specimens under examination (Fig. 1a). After 48 h, blastospores and mycelia were observed within the bodies of the nematodes, which no longer presented any movement. At 72 h, the mycelium had grown from the interior of the nematode. The stylet could be distinguished, but by then it was impossible to recognize any other organ in the nematodes due to corporal degradation (Fig. 1b). At 96 h, conidiophores and blastospores were observed protruding from the body of the nematode, which had turned dark brown in color. The stylet then began to degrade. After 120 h, the internal organs and stylet were completely degraded, and only the annulated cuticle allowed recognition of the body of the nematode. A mycelial mass growing through the cuticle was evident, along with blastospores within the body (Fig. 1c). Nematodes in the control treatment at this time showed no evidence of fungal and bacterial growth and it was possible to distinguish the annular cuticle, stylet, cephalic region and median bulb. On examination, the majority of these specimens were still alive and mobile (Fig. 1d).

Nematodes associated with the sugar cane rhizosphere

Seventeen nematode morphotypes of the orders Tylenchida, Dorylaimida, Rhabditida and Aphelenchida were identified, belonging to 12 families and to 11 genera. Four trophic groups of nematodes were found: phytophages, bacteriophages, mycophages and omnivorous-predators (Table 1). Following the classification of Yeates et al. (1993), three subgroups were recorded within the group of phytophagous nematodes: semiendoparasites (*Helicotylenchus*), ectoparasites (*Criconemoides* and *Tenunemellus*) and algae, lichen or moss feeders (*Tylenchus*). The free-living nematodes found included the genera *Acrobeles*, *Acrobeloides*, *Aporcelaimellus*, *Aphelenchus*, *Cruznema*, *Panagrolaimus* and *Thornenema*.

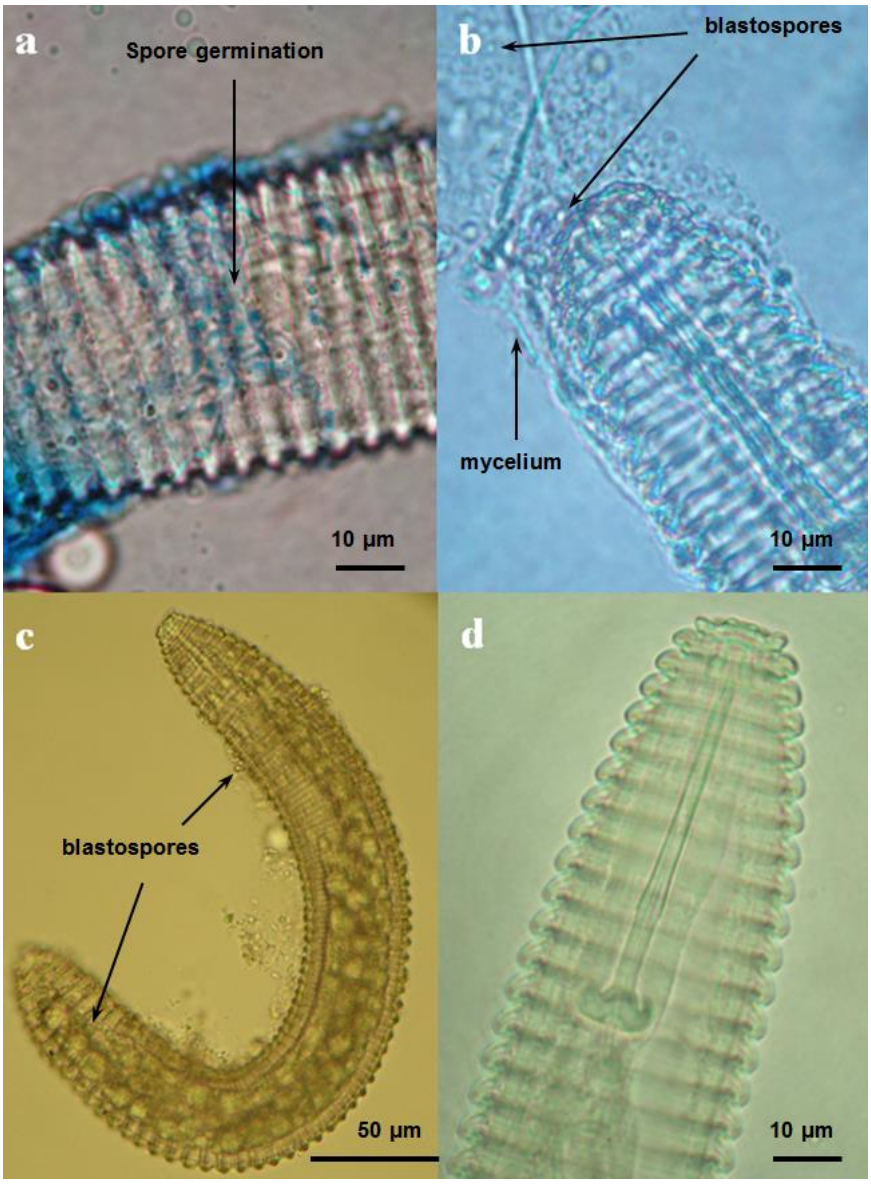

Fig 1. Infection process of *Purpureocillium lilacinum* on *Criconemoides* sp. **a** spores germinating on the nematode cuticle at 24 h after inoculation (1000x), **b** mycelium and blastospores emerging from the body 72 h after inoculation (1000x), **c** nematode completely degraded by the fungus at 120 h after inoculation (400x), **d** control specimen at 120 h after inoculation (1000x).

Population density of phytoparasitic nematodes in greenhouse experiment

The most abundant nematodes in both biocontrol and control treatments were the phytoparasites. Of these, *Criconemoides* sp. and *Helicotylenchus* sp. presented the highest number of individuals per experimental unit. The population density of *Criconemoides* sp. fell by 81% in the biocontrol treatment by the end of the experiment; however, the difference in density between initial population (Pi) and final population (Pf) was not significant (p = 0.07); in the control treatment, the population of *Criconemoides* sp. increased by 1.3% (p = 1). The population density of *Helicotylenchus* sp. fell by 50% in the biocontrol treatment, while in control it fell by 37%, although again no significant differences were found between Pi and Pf in each case. In the case of *Tylenchus* sp., the population density was significantly (93%) lower in the Pf in the biocontrol treatment (p < 0.05), while no significant differences were found between populations in the control treatment (p = 0.22). In *Tenunemellus* sp., the population density had reduced significantly (p < 0.05) in both treatments by the end of the experiment. In the case of the two morphotypes of the family Tylenchidae, no significant difference was observed in population density between the Pi and Pf in each treatment (Table 2).

No significant differences (p = 0.09) were found between treatments on comparison of the mean Pi of phytoparasitic nematodes: 253 and 287 nematodes 100 mL soil^{-1} were found in the biocontrol and control treatments, respectively. However, significant differences (p < 0.01) were found between treatments in terms of mean Pf of phytoparasitic nematodes: Mean Pf values were 91 and 230 nematodes 100 mL soil^{-1} in the biocontrol and control treatments, respectively. This represents a population reduction of 60% in the biocontrol treatment, relative to that of the control. The multiplication rate (Pf/Pi) was also reduced with application of *P. lilacinum*, relative to that of the control, although the difference was not significant. The percentage of reduction

Table 2. Number of phytoparasitic nematodes 100 mL soil-1 in the sugar cane rhizosphere by species (mean ± standard deviation) found in the treatments.

Nematodes	Treatments							
	Biocontrol				Control			
	Pi	Pf	Pf/Pi	*P*	Pi	Pf	Pf/Pi	*p*
Criconemoides sp.	63 ± 60a*	12 ± 6a	0.4	0.07	148 ± 98a	150 ± 67a	1.6	1
Helicotylenchus sp.	125 ± 47a	63 ± 21a	0.5	0.07	89 ± 43a	56 ± 17a	0.7	0.13
Tylenchus sp.	28 ± 19a	2 ±2b	0	0.04	17 ±19a	3 ± 1a	0.1	0.22
Tenunemellus sp.	11 ± 5a	0 ± 0b	0.1	0.04	6 ± 5a	0 ± 1b	0	0.04
Tylenchidae morphotype 1	4 ± 4a	3 ± 1a	0.6	0.58	3 ± 2a	4 ± 3a	1	0.36
Tylenchidae morphotype 2	22 ± 13a	11 ± 6a	0.7	0.20	23 ± 20a	17 ± 10a	1.3	0.50

*Different letters between initial and final populations indicate significant differences according to the Wilcoxon T test ($p \leq 0.05$) for dependent samples. Pi = initial population; Pf = final population. Pf/Pi multiplication rate of nematodes (mean multiplication rate from the five experimental units).

presented in the biocontrol treatment was 64%, while this value was only 20% in the control (Table 3).

Population density of free-living nematodes in greenhouse experiment

In general, the initial population density of free-living nematodes was low, with mean Pi values of 49.4 and 60.8 nematodes 100 mL soil^{-1} in the biocontrol and control treatments, respectively. The most abundant free-living nematodes in both treatments were the bacteriophages, of which *Acrobeloides* sp., *Cruznema* sp. and *Panagrolaimus* sp. presented the greatest number of individuals per experimental unit. However, the population density of these nematodes declined in both treatments. The most abundant of the mycophagous nematodes was the morphotype of the family Aphelenchoididae, which presented Pi values of 13 and 12 nematodes 100 mL soil^{-1} in the biocontrol and control treatments, respectively. In both treatments, population density had declined in the Pf values. Few examples of the omnivorous-predator nematodes were found, but the most abundant was *Thornenema* sp. with Pi values of six and nine individuals 100 mL soil^{-1} in the biocontrol and control treatments, respectively (Table 4). On comparison of the data between treatments, no differences were found in either the initial or final populations. At the end of the experiment, the mean multiplication rate in the biocontrol treatment was 0.12, which represents a reduction of 89.7% from the beginning of the experiment. In the control treatment, the multiplication rate was 0.07, representing a 94.7% reduction relative to the initial population (Table 5).

Discussion

Purpureocillium lilacinum is considered one of the most functional biocontrol agents for reducing populations of phytoparasitic nematodes (Saxena, 2004). It is also recognized as a nematophagous fungus that can survive in the soil despite the practices of intensive agricultural management (Núñez-Camargo et al., 2012). In the present study, however, *P. lilacinum* was only isolated in the area where no agrochemicals had been applied for at least a year (a sugar cane plantation used solely for the production of fodder). It has been shown in certain studies that numerous isolates of nematophagous fungi are obtained in arable soils where agrochemicals are not applied, compared to those that are treated with chemical fertilizers (Jaffee et al., 1998; Wachira et al., 2011). For this reason, it is considered that pest management and fertilization of the soil using techniques such as biological control and the use of biofertilizers constitutes a method by which to achieve the restoration of edaphic diversity (Garcia-Alvarez et al., 2005).

In this study, the *P. lilacinum* conidiospores that adhered to the cuticle of *Criconemoides* sp. germinated after 24h, in a process that is characteristic of endoparasitic fungi (Lopez-Llorca, et al., 2008). This supports the findings of other studies carried out on the eggs of *Meloidogyne javanica*, where *P. lilacinum* infection was recorded one day after inoculation (Holland et al., 1999). Similarly, the rapid growth of *P. lilacinum* on *Criconemoides* sp. allowed the degradation of all the organs within the body of the individual nematode to take place within five days. These results agree with those described by Khan et al. (2006), who recorded total *P. lilacinum* infection in females of *Meloidogyne javanica* four days after exposure to the fungus, under laboratory conditions.

The phytoparasitic nematodes found in the soil in this experiment were similar to those of the principal sugar cane producing countries worldwide. However, even when the roots were examined for endoparasitic nematodes, there was no presence detected of *Pratylenchus* and *Meloidogyne*, genera that are widely distributed in sugar cane (Cadet and Spaull, 2005; Berry et al., 2009; Desgarennes et al., 2011). *Tenunemellus* sp. is reported here for the first time as associated with sugar cane. Regarding the free-living nematodes, Desgarennes et al. (2011) and Mondino et al. (2010) reported *Acrobeles*, *Acrobeloides*, *Cruznema*, *Aphelenchus*, *Aporcelaimellus*, *Eucephalobus*, *Prismatolaimus*, *Rhabditis* and *Thornenema*, among others, as forming part of the nematofauna associated with the sugar cane rhizosphere. In addition to the previously described genera, *Panagrolaimus* was found to be associated with the rhizosphere of sugar cane in this study.

With the application of *P. lilacinum*, the final population density of phytoparasitic nematodes in the biocontrol treatment approached a level (<50 nematodes 100 mL soil^{-1}) that would have no effect on crop yield (Cadet and Spaull, 2005). Under controlled conditions, Mucksood and Khan, (2010) showed that *P. lilacinum* is more effective as a control of phytoparasitic nematodes in soil when the application of the fungus precedes the inoculation of *Meloidogyne javanica*, reducing the initial population (2000 J2 juveniles) by up to 61.2%, unlike inoculation of this fungus into soil previously infested with *M. javanica*, where the reduction is 47.13%. In this study, a reduction of 64% was observed in the population density of phytoparasitic nematodes (253 nematodes 100 mL soil^{-1}), even when *P. lilacinum* was applied once to naturally infested soils. This result supports that described by González et al. (2009) where application of commercial *P. lilacinum* strains was observed to reduce populations of *Helicotylenchus*,

Table 3. Population density of phytoparasitic nematodes 100 mL soil^{-1} (mean ± standard deviation) of sugar cane by treatment.

Treatment	Pi	Pf	Pf/Pi	Reduction (%)
Biocontrol	253±98 a*	91±26 a	0.44±0.2 a	64
Control	287±164 a	230±53 b	1.15±0.8 a	20
U	12	0.0	5	--
P	0.9	< 0.01	0.1	--

*Different letters in each column indicate significant differences between treatments for the Mann-Whitney U test ($p \leq 0.01$). Pi= initial population density; Pf= final population density; Pf/Pi multiplication rate of nematodes (mean multiplication rate from the five experimental units).

among other phytoparasitic nematodes, by between 61 and 38% in cultivated banana. The results of this study suggest the potential utility of this fungus in sugar cane producing areas that present high densities of ectoparasitic and semiendoparasitic nematodes. Several other studies indicate that the fungus has a high potential for application in agricultural soils, where it can infect a range of phytoparasitic nematode genera (Khan et al., 2006; Mendoza et al., 2007; Pandey et al., 2011).

The reduction of free-living nematodes in both the biocontrol and control treatments indicates that *P. lilacinum* was not the cause of nematode mortality in this group. In this regard, Carrión and Desgarennes, (2012) recorded, *in vitro*, a low mortality of free-living nematodes associated with the potato crop when inoculated with a native *P. lilacinum* isolate and concluded that native strains of this nematophagous fungi could be used in the control of pest nematodes without posing a risk to the free-living nematofauna. In addition, free-living nematodes are highly susceptible to changes in the agroecosystem, such as soil tillage and crop establishment (Govaerts et al., 2006). We consider that the confined conditions to which the free-living nematodes were subjected in the greenhouse experiment influenced their mortality by altering their habitat stability, for example, in terms of food availability and increased temperatures of the soil in pots (Neher, 2010).

Materials and Methods

Study site selection and sampling strategy

The soil used in this study came from three 1 ha areas, each cultivated with sugar cane (cultivar MEX-69-290), in the municipality of Paso de Ovejas, in Veracruz, Mexico. This municipality is located in the semi-arid part of the central region of the state (19° 17' N, 96° 22' W), 40 m above mean sea level and features a vertisol soil type. Two of the areas were subjected to habitual crop management with cultivation activities and multiple applications of agrochemicals, while the third area was used exclusively for the production of fodder. This area presented infrequent cultivation activity and had no application of agrochemicals for at least one year prior to the experiment. Five samples (500 mL) were taken from each area, at a depth of 15 cm, following a zig-zag pattern throughout the plot.

Isolation and identification of fungi

For the isolation of fungi, soil samples (N= 15) were taken from the three areas described previously. Nematodes were extracted from the soil samples using the sieving-centrifugation technique (s'Jacob and van Bezooijen, 1984) and specimens of *Criconemoides* sp. were selected (N= 300). Previous studies report that this is the most abundant phytoparasite in the area (Desgarennes et al., 2011). Each specimen was separately placed in pre-packaged blisters of oatmeal agar culture medium (OA). The mycelium that grew on the surface of the nematodes was resown on OA plates to obtain a pure culture and a collection of fungi associated with *Criconemoides* sp. was thus formed. Fungi were identified using the keys of Booth (1971), Samson (1974), Domsch et al. (1980), Sutton (1980), Samson and van Reenen-Hoekstra (1988) and Boerema et al. (2004).

Purpureocillium lilacinum infection process

Purpureocillium lilacinum was selected from the obtained strains in order to observe the infection process. The fungus was reproduced on OA for 10 days and a spore suspension of concentration 1 x 10^5 mL^{-1} prepared. Concurrently, ten live individuals of *Criconemoides* sp., extracted from recently taken field soil samples, were placed in Petri dishes (five replicates) and 1 mL of the fungal spore suspension added to each Petri dish. For the control, the same number of individuals was distributed in five petri dishes and 1 mL of sterile distilled water was added to each dish. Each Petri dish was inspected at 24, 48, 72, 96 and 120 h after inoculation in order to monitor the infection process of *P. lilacinum* on *Criconemoides* sp.

Test of pathogenicity of Purpureocillium lilacinum on nematodes associated with sugar cane

One of the sugar cane plots under habitual management was chosen to provide the soil and plants used for the test in the greenhouse (temperature 30±5 ºC; relative humidity 65%) because these presented the highest number of phytoparasitic nematodes. Two treatments were established: biocontrol and control, with five experimental units each. Experimental units consisted of 1.5 L pots with soil naturally infested with nematodes and apparently healthy cultivar MEX-69-290 sugar cane plants. In the biocontrol treatment, 100 mL of a *P. lilacinum* spore suspension ($2x10^6$ spores mL^{-1}) was added to each pot. In the control treatment, 100 mL of sterile distilled water was added to each pot. In both treatments, extraction of nematodes was carried out on two occasions: prior to and ten days after application of the fungus. According to the results of the infection process, the fungus had completely degraded the nematodes by the end of this ten-day period.

Extraction and identification of nematodes

The ectoparasitic and semiendoparasitic nematodes of the greenhouse experiment were extracted using the sieving-centrifugation technique (s'Jacob and van Bezooijen, 1984). Specimens were fixed and cleared (Seinhorst, 1962), then quantified and grouped according to the morphological differences observed in the microscope. For identification of the nematodes, taxonomic keys were used to determine the orders Dorylaimida (Jairajpuri and Ahmad, 1992), Rhabditida

Table 4. Number of free-living nematodes 100 mL soil^{-1} in the sugar cane rhizosphere by species (mean ± standard deviation) found in the treatments.

Nematodes	Treatments							
	Biocontrol				Control			
	Pi	Pf	Pf/Pi	*p*	Pi	Pf	Pf/Pi	*p*
Bacteriophagous								
Acrobeles sp.	4 ± 2a*	0 ± 0b	0	0.04	3 ± 1a	0 ± 0b	0	0.04
Acrobeloides sp.	3 ± 4a	0 ± 0a	0	0.10	10 ± 9a	0 ± 1b	0	0.04
Cruznema sp.	10 ± 10a	0 ± 0b	0	0.04	12 ± 8a	0 ± 0b	0	0.04
Panagrolaimus sp.	8 ± 4a	1 ± 1a	0.3	0.06	8 ± 6a	1 ± 1b	0.1	0.04
Mycophagous								
Aphelenchus sp.	3 ± 3a	1 ± 2a	1.1	0.46	5 ± 3a	1 ± 0b	0.2	0.04
Aphelenchoididae (morphotype 3)	13 ± 4a	0 ±1b	0	0.04	12 ± 9a	0 ± 0b	0	0.04
Omnivorous-predators								
Aporcelaimellus sp.	2 ± 1a	0 ± 1b	0.2	0.04	2 ± 2a	0 ± 0a	0.2	0.10
Aporcelaimidae (morphotype 4)	0 ± 0	0 ± 0	0	--	0 ± 1	0 ± 0	0	--
Qudsianematidae (morphotype 5)	0 ± 0	0 ± 0	0	--	1 ± 1a	0 ± 0a	0	0.10
Qudsianematidae (morphotype 6)	0 ± 0	0 ± 1	0.4	--	0 ± 0	0 ± 0	0	--
Thornenema	6 ± 3a	1 ± 1a	0.5	0.07	9 ± 5a	0 ± 0b	0	0.04

*Different letters between initial and final populations indicate significant differences according to the Wilcoxon T test ($p \leq 0.05$) for dependent samples. Pi= initial population density; Pf= final population density; Pf/Pi multiplication rate of nematodes (mean multiplication rate from the five experimental units).

Table 5. Population density of free-living nematodes 100 mL soil^{-1} (mean ± standard deviation) of sugar cane by treatment.

Treatment	Pi	Pf	Pf/Pi	Reduction (%)
Biocontrol	49.4±20.3 a*	5±1.2 a	0.12±0.1 a	89.7
Control	60.8±37.8 a	3.2±1 a	0.07±0.05 a	94.7
U	9	12.5	9	--
p	0.46	1.0	0.46	--

*Different letters in each column indicate significant differences between treatments for the Mann-Whitney U test ($p \leq 0.01$). Pi= initial population density; Pf= final population density; Pf/Pi multiplication rate of nematodes (mean multiplication rate from the five experimental units).

(Andrássy, 1984) and Tylenchida (Siddiqi, 2000). Specimens were identified to family and to genus level where possible. Similarly, the roots of the plants were examined in order to verify the presence or absence of endoparasitic nematodes (*Meloidogyne* spp. and *Pratylenchus* spp.). Finally, the nematodes were classified into trophic groups, following Yeates et al. (1993).

Statistical analysis

Due to the fact that the obtained data did not fulfill the assumptions of normality (Shapiro-Wilks test) and homogeneity of variance (Levene test), non-parametric Wilcoxon T tests ($p \leq 0.05$) were used for comparison between initial and final populations in the same treatment, while a Mann-Whitney U test ($p \leq 0.01$) was used for comparisons between treatments. All analyses were conducted using the program STATISTICA 8.0 for Windows.

Conclusions

The nematophagous fungi could not be isolated in the rhizosphere of the sugar cane crop that had undergone repeated use of agrochemicals. Rational use of agrochemicals allows the proliferation of native fungi that can act as nematode controllers. *Purpureocillium lilacinum* has potential as a controller of ectoparasitic and semiendoparasitic nematodes of the sugar cane crop. No evidence was found to suggest that *P. lilacinum* affects free-living nematodes.

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