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RESEARCH ARTICLE

89 % reduction of a potato cyst nematode population using biological control and rotation

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Abstract A major issue of potato cultivation in temperate zones is the potato cyst nematode *Globodera rostochiensis*. Population density of *G. rostochiensis* is high in Mexican potato fields. Control currently consists of the inefficient application of high doses of chemical nematicides. We evaluated the population density of *G. rostochiensis* in potato production plots in central Veracruz, Mexico. Plots were treated with the biocontrol agent *Paecilomyces* sp. and rotation with two different leguminous crop plants, *Pisum sativum* and *Vicia faba*. A random block experimental design was used with four different treatments over two crop cycles: (1) biological control with crop rotation, (2) crop rotation only, (3) biological control applied to soil left in fallow, and (4) soil left in fallow only. We measured the number and content of cysts, and the number of J2 juveniles of *G. rostochiensis* in the soil. We then estimated the infestation level in soil and the multiplication rate (Pf/Pi). The number of free-living nematodes was also quantified. Results show that the highest mitigation of *G. rostochiensis* was observed for the biological control rotation, with 89.2 % reduction, and for the biological control fallow treatments with 84.4 % reduction. In rotation plots, infestation level decreased by 30.7 %. In the biological control rotation and biological control fallow treatments, the Pf/Pi was 0.1 and 0.15, respectively. The highest Pf/Pi of 0.93 was found in the fallow plots. The biological control agent did not significantly affect the free-living nematode populations. In this study, the nematophagous fungus *Paecilomyces* sp. was used for the first time to efficiently reduce the population of *G. rostochiensis* in two crop cycles.

Keywords Potato cyst nematode · Golden nematode · Integrated management · Nematophagous fungi

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1 Introduction

The potato cyst nematode *Globodera rostochiensis* (Woll. 1923) Skarbilovich, 1959 is considered to be a pest of great economic significance to the cultivation of *Solanum tuberosum* worldwide (van Riel and Mulder 1998). It is estimated to account for up to 83 % of losses in susceptible potato crops in temperate zones (Cunha et al. 2004; Franco 1994). Once established in the fields, it is very difficult to eliminate due to its high multiplication rate (Desgarennes et al. 2006; Turner 1996).

The main symptoms of infection are yellowing and hydric stress (EPPO 2009). When juveniles in the infective stage (J2) enter the root, they move through the cortex, dissolving the cell walls. They subsequently become sedentary (Fig. 1a, b), forming feeding sites known as syncytia (Sobczak and Golinowski 2011).

In Mexico, the potato cyst nematode is found in nine states and is responsible for estimated annual potato production losses of up to 70 % (Brodie 1998; Tovar et al. 2006). In order to avoid the introduction, establishment, and dispersal of *G. rostochiensis* to new cultivation areas, several phytosanitary measures have been implemented. These prevent farmers in infested sites from selling their product as seed; however, the authorities have no control to stop movement of the infected tubers, and this has allowed the dispersal of

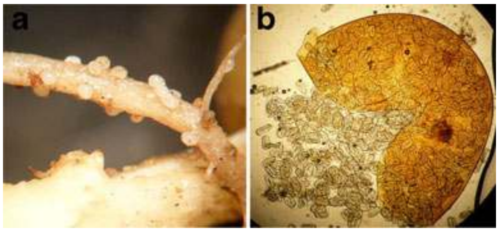


Fig. 1 *Globodera rostochiensis* **a** females in potato roots, **b** eggs within a cyst

this nematode to new areas (Núñez-Sánchez et al. 2003; SAGARPA 2002). Combat of the potato cyst nematode involves the application of chemical nematicides of the carbamate group (e.g., carbofuran, aldicarb, oxamyl); however, this nematicide application has not resulted in a reduced population of *G. rostochiensis* (Desgarennes et al. 2006). Moreover, due to the high mobility of these chemicals in the soil, there are many problems associated with their use; these include their negative effect on beneficial organisms (Haydock et al. 2006) and the fact that they can contaminate groundwater, and because of their residual effect (120 days), they can remain in the tuber until after harvesting, representing a risk not only to the farmers who apply these chemicals in the fields but also to the end consumers of the product (Mendes et al. 2005).

In the region of Cofre de Perote in Veracruz, Mexico, chemical control has produced poor results, and as a consequence of the potato monoculture, populations of this nematode have increased in some fields to more than 6,000 cysts kg soil^{-1} since initial detection in 1983 (Desgarennes et al. 2006). These values surpass the tolerance limit of 40 cysts kg soil^{-1} at which the nematode is considered to have no effect on crop yield (EPPO 1996). For this reason, the implementation of integrated management that includes the control of this pest with agroecological methods such as biological control and crop rotation has been suggested (Lichtfouse et al. 2009).

Integrated management can favor the control of pests through the combination of diverse control methods, since this enhances the efficacy of each method on its own (Gurr et al. 2004). This study evaluated, over two crop cycles, the effect of the combination of two control strategies (biological control and crop rotation) to reduce a population density of *G. rostochiensis*. The fungus *Paecilomyces* sp. was used as a biological control agent for the first time in the experimental plots, and species identification was corroborated using genetic sequences. This fungus was isolated in specimens of *G. rostochiensis* in the study area, and its pathogenicity has been demonstrated in vitro. Furthermore, this fungus is the subject of a patent of use, which is currently in process (PCT-MX 2012-000032). *Pisum sativum* and *Vicia faba* were used as (non-host) rotation crops.

2 Materials and methods

2.1 Study area

The study was conducted in the community of Los Pescados, in the municipality of Perote, in Veracruz, Mexico (19° 33′41″N, 97°08′53″W, 2,980 m above sea level), over two spring–summer crop cycles (April–October 2010 and 2011).

2.2 Experimental design

Twenty experimental plots of 50 m^2 were established in random blocks in a site with a high soil infestation of *G. rostochiensis* and subjected to four treatments with five replicates each: application of the biological control agent with peas (*P. sativum*) sown as a rotation crop in the spring–summer cycle of 2010 and beans (*V. faba*) in the 2011 cycle; the second treatment consisted of rotation only with no application of biological control; the third treatment consisted of application of the biological control agent and letting the soil lie fallow; the fourth treatment consisted of letting the soil lie fallow only with no application of the biological control. No tillage or weeding was carried out in either of the two fallow treatments. Potato plants remaining from the previous crop cycle were removed from all the plots. The rotation and fallow plots were used as controls of the cultivated and fallow plots, respectively. For those plots sown with peas, the variety "Canadiense" was used at a density of 180 plants per plot, and for beans, the variety "Major" was used at a density of 108 plants per plot. The nematophagous fungus *Paecilomyces* sp. was used as the biological control agent and was applied 10 days prior to sowing and at the time of sowing, in each crop cycle, with one further application made between the two crop cycles.

2.3 Inoculum preparation

The fungus was allowed to reproduce in a liquid medium until reaching a concentration of 2×10^7 spores ml^{-1}. The resulting suspension of spores was made up to 4 L with tap water and applied directly to the soil with a manual spray pump. Application dose was 4×10^{10} spores per plot.

2.4 Population density of *G. rostochiensis* and free-living nematodes

In each plot, composite soil samples were taken, comprising five subsamples (each of 500 ml). Eight such composite samples were taken throughout the experiment (N=160), samples 1 to 4 taken during the first crop cycle (pea) with samples 5 to 8 taken in the second cycle (bean). In each cycle, the first sample was taken 10 days prior to sowing; the second was taken at the time of sowing and the rest at 60 and 120 days after sowing. All samples were taken following a zig–zag pattern throughout each plot.

The cysts were obtained using the Fenwick can (1940) technique while nematodes were extracted from the soil using the sieve–centrifuge technique (s'Jacob and van Bezooijen 1984) (100 ml of soil in each technique). To determine the cyst content, the juveniles (J2) and eggs within the cysts were quantified. The emerged J2 of *G. rostochiensis* and free-living nematodes in the soil were fixed and transferred to glycerin, following the Seinhorst (1962) method.

G. rostochiensis was the only phytoparasitic nematode considered since in previous studies, it was shown to be the dominant species of potato crops in the study area (Desgarennes et al. 2009). In each plot, the number of cysts, their contents (eggs and J2 per cyst), and emerged J2 juveniles extracted from the soil were quantified in order to estimate the infestation level of *G. rostochiensis*: (number of juveniles and eggs 100 ml soil^{-1})=average cyst content× number of cysts+J2 100 ml soil^{-1}. Initial population (Pi) and final population (Pf) values of both *G. rostochiensis* and free-living nematodes in all treatments were used to establish the multiplication rate (Pf/Pi)=final nematode population/initial nematode population.

2.5 Statistical analysis

Because the data obtained did not conform to assumptions of normality (Shapiro–Wilk's test) or to equality of variance (Levene's test), they were analyzed with the non-parametric Kruskal–Wallis (H, $P\leq0.01$) test and a multiple comparison test to compare between all the treatments, as well as the Wilcoxon T test (Tw, $P\leq0.05$) to compare the initial and final populations in each treatment. All analyses were carried out using the statistical STATISTICA 8.0 for Windows.

3 Results and discussion

3.1 Population density of *G. rostochiensis*

An average of 203±75 cysts were found per 100 ml soil in the experiment plots at the beginning of the experiment. This is in broad agreement with the results obtained by previous studies in the same community of Los Pescados, where reports range from 1,656 to 6,200 cysts kg soil^{-1} (Desgarennes et al. 2006; Núñez-Sánchez et al. 2003). At the end of the experiment, depending on treatment, the number of cysts had either fallen or remained constant (127±51), and no significant differences were found between the four treatments. Nonetheless, the average number of cysts fell over the course of the experiment in the biological control fallow (Tw=0.0, N=10, P=<0.05) and fallow (Tw=10, N=10, P=<0.05) treatments.

The average cyst content at the beginning of the experiment was 12±6 eggs and J2 cyst^{-1}, with lower quantities of eggs and J2 per cyst in the biological control fallow (7.1±2.8) than in rotation (16.2±3.5). The variation found in the content of the cysts in the crop fields at the beginning of the experiment is partly due to the fact that, of the eggs contained within cysts that had been in the soil for a longer period of time, as little as 2 % may be viable while the cysts that had formed in the previous crop cycle could present 50 % live eggs and juveniles (Desgarennes et al. 2006). For this reason, using the number of cysts to estimate population density of *G. rostochiensis* is inexact. Towards the end of the experiment, both the eggs and J2 contents of the cysts were reduced in the biological control rotation (1.7±1.5) and biological control fallow (2.2±1.6) treatments compared to fallow (23.2±12.6) (H=14.45, N=20, P=<0.01). Moreover, the biological control rotation and biological control fallow plots presented reduced quantities of eggs and J2 per cyst over the course of the experiment (Tw=0.0, N=10, P=<0.05). In both of these biological control treatments, the content of the cysts was reduced by 86 and 68 %, respectively. This differs from the rotation plots, where the content of the cysts was reduced by 31 % following two cultivation cycles. In contrast, the content of the cysts in the fallow treatment increased by 54 %. Our results from the rotation treatment plots concur with other studies that have shown populations decreasing by up to 40 % after the second year of crop rotation in the absence of the host potato (Devine et al. 1999).

The average number of emerged J2 juveniles in the soil samples in all treatments was low at the beginning of the experiment (5±4 individuals per 100 ml soil). At the end of the experiment, the lowest number of *G. rostochiensis* J2 juveniles was found in plots that had been treated with the nematophagous fungus (biological control rotation=1±1; biological control fallow=0.2±0.4). Of these, the number in biological control fallow was significantly lower than that of the rotation (6±4) and fallow (5±1) treatments (H=14.7, N=20, P=<0.01). In rotation treatment, the number of J2 remained low throughout the experiment, which could indicate that the pea and bean crops utilized in the experiment did not stimulate the emergence of *G. rostochiensis*. In experiments carried out in the study area (unpublished data) in potato crop without any control, we found higher than 500 J2 100 ml soil^{-1}. This is in broad agreement with the results obtained by Brodie (1996) who reports 350 individuals 100 ml soil^{-1} in the presence of the host potato. Furthermore, in the treatments with biological control, we found on revision of the cyst interiors that the eggs and J2 were damaged, bound together by fragments of mycelia. In contrast, the eggs and J2 of the rotation and fallow treatments were found to be healthy.

Mean infestation level recorded in the treatments at the beginning of the experiment was 2,147±957 potentially infective individuals per 100 ml soil. The effect of bionematicide application was seen in the biological control rotation and biological control fallow treatments from the fifth sample onwards (beginning of the second year of rotation) until the end of the experiment where infestation level was very low (Table 1). In biological control rotation, the infestation level was reduced by 89.2 % (Tw=0.0, *N*=10, *P*=<0.05). In contrast, in the treatment rotation, it diminished by only 30.7 %, which was a non-significant difference with respect to the initial value (Fig. 2a). Related studies report that rotation with a non-host crop (*Avena sativa*) reduces the number of eggs 100 ml soil^{-1} by 30–40 %; however, subsequent sowing of a susceptible variety of potato allows the population to recover (Brodie 1996). In some studies, it has been demonstrated that rotation with leguminous crops can achieve a population reduction of *G. rostochiensis* and *Globodera pallida* by between 25 and 30 % (Iriarte et al. 1999; Pacajes et al. 2002). From the first crop cycle, potato plants growing from tubers remaining from the previous crops in our experimental plots were removed; however, we still had to remove potato plants during the second cycle. This was due to the large quantity of tubers that are left in the soil following harvest. For this reason, with these potato plants growing in the fields sown with non-host rotation crops, a certain amount of food was still available for the potato cyst nematode, which resulted in patches of high population density for the following crop cycle. Reduction of infestation level in the biological control fallow treatment was of 84.4 % (Tw=0.0, *N*=10, *P*=<0.05) relative to the initial values, while it was only 6.8 %, (Tw=0.94, *N*=10, *P*=<0.34) in the fallow treatment (Fig. 2b). These results are in agreement with studies conducted in Bolivia, where leaving a soil fallow over one crop cycle produced a reduction of 11 % in the infestation of *Globodera* spp. (Pacajes et al. 2002). The use of soil fallow periods and crop rotation as control methods for *G. rostochiensis* has not been effective because this nematode can survive for up to 20 years in the absence of its host (Evans 1993), and its population can still be viable after 10 years of cultivation of non-host plants or of soil left in fallow (Esprella et al. 1994). Similarly, the capacity of *G. rostochiensis* to survive in the soil in the absence of a host crop should not be underestimated, because it can reproduce in many wild host species that may be present in the cultivated area (Sullivan et al. 2007).

3.2 *G. rostochiensis* multiplication rate

The lowest multiplication rates were found in the treatments biological control rotation (0.1±0.09) and biological control fallow (0.15±0.09) (*H*=14.58, *N*=20, *P*=<0.01) (Table 1). Although the Pf/Pi reduced by more than 80 % (Tw=0.0, *N*=10, *P*=<0.05) in both the biological control treatments, it is more efficient to use the bionematicide along with crop rotation using non-host crops for two consecutive years. In studies using the fungus *Pochonia chlamydosporia* as a biological control agent in potato cultivation, a Pf/Pi of 8.9 was obtained (Tobin et al. 2008). At the end of the experiment, a multiplication rate of 0.69±0.40 was observed in the treatment rotation, similar to that found by Iriarte et al. (1999) and Pacajes et al. (2002) who recorded a multiplication rate of 0.7 with rotation of beans for 1 year. In our experiment, the highest Pf/Pi was in treatment fallow (0.93±0.25), but no significant differences were observed relative to the initial values (Tw=4, *N*=10, *P*=< 0.34). Pacajes et al. (2002) found a multiplication rate of 0.89 after leaving soil in fallow for 1 year. For this reason, allowing soil to lie fallow without carrying out some additional form of nematode control is not an alternative in the short term for *G. rostochiensis* population reduction. From these results, we consider that control of *G. rostochiensis* should be conducted from the point of view of integrated management and requires the use of biological control with the rotation of non-host crops or with fallow periods at the same time, in order to lower the population density of the potato cyst nematode prior to the introduction of a new potato crop.

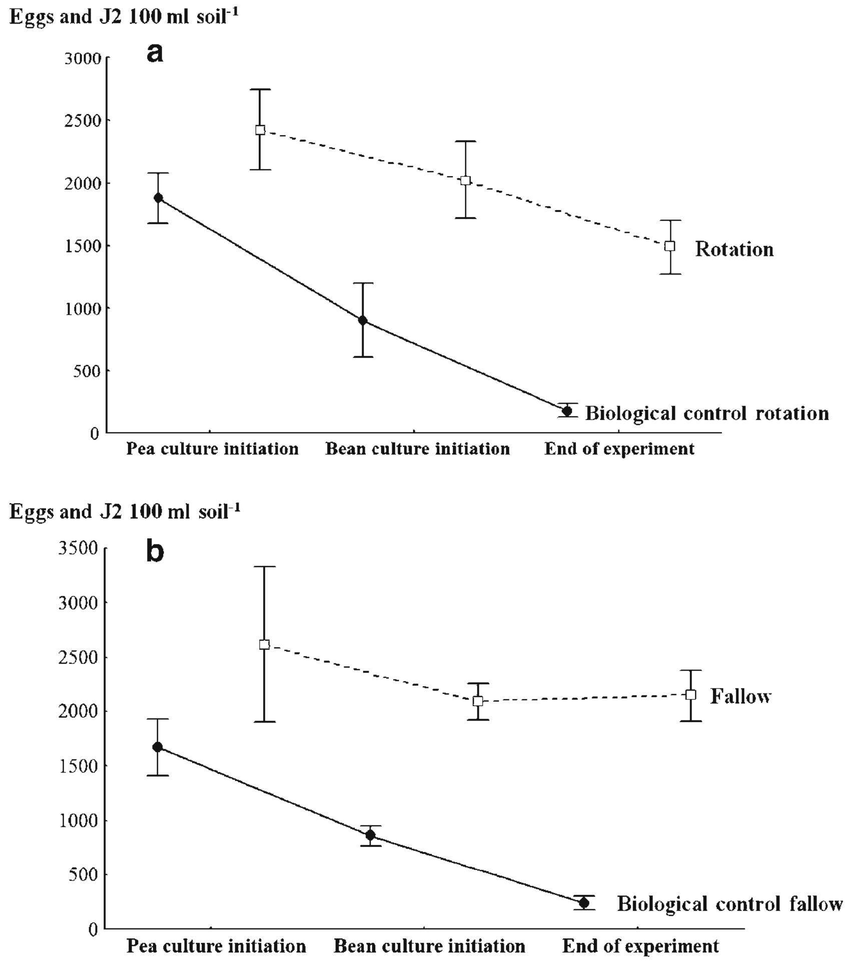
Table 1 Populations of *Globodera rostochiensis* by treatment (eggs and J2±standard deviation 100 ml soil^{-1})

Treatment	initial population density (Pi)	final population density (Pf)	Pf/Pi	% reduction
Biological control rotation	1,878±444a	181±130a	0.10±0.09a	89.2
Rotation	2,425±717a	1,486±483ab	0.69±0.4a	30.7
Biological control fallow	1,671±577a	240±134a	0.15±0.9a	84.4
Fallow	2,615±1602a	2,145±526b	0.93±0.2a	6.8
H	2.65	15.33	14.58	–
P	0.44	<0.01	<0.01	–

Different letters in each column denote significant differences between treatments indicated by a multiple comparisons test

H Kruskal–Wallis test (*P*≤0.01), *Pf/Pi* nematode multiplication rate

Fig. 2 Infestation level (±standard error) at the start of each cultivation cycle and at the end of the experiment. **a** Biological control rotation and rotation treatments, **b** biological control fallow and fallow treatments



3.3 Population density of free-living nematodes

At the beginning of the experiment, 83±31 free-living nematodes were recorded per 100 ml soil. At the end of the second crop cycle, this number had increased in all treatments, with the exception of biological control rotation where the number had reduced by 23 %. The fungus used as a biological control agent against *G. rostochiensis* had no significant effect on the populations of free-living nematodes (Table 2). In general, the average number of free-living nematodes was lower in the plots with crop rotation than in the fallow plots, where the numbers increased by more than 200 % in biological control fallow and fallow (Tw=0.0, N=10, P=<0.05). Thus, the lowest multiplication rate values were found in biological control rotation (0.84±0.5) and rotation (1.5±0.5). Conversely, the highest multiplication rates were found in biological control fallow (3.2±3) and fallow (3.7±2.1) (H=7.83, N=20, P=0.04). The difference in Pf/Pi between

Table 2 Population of free-living nematodes per treatment (number of individuals±standard deviation 100 ml soil^{-1})

Treatment	initial population density (Pi)	final population density (Pf)	Pf/Pi	% increase
Biological control rotation	105±23	82±34	0.84±0.51	−15
Rotation	73±27	117±70	1.51±0.56	51
Biological control fallow	84±31	212±184	3.29±3.49	229
Fallow	70±37	221±83	3.78±2.17	278
H	3.55	7.59	7.83	–
P	0.31	0.05	0.04	–

Different letters in each column denote significant differences between treatments indicated by a multiple comparisons test

H Kruskal–Wallis test (P≤0.01), *Pf/Pi* nematode multiplication rate the biological control rotation and rotation treatments was 44 %, while the difference between biological control fallow and fallow treatments was 13 % (Table 2).

This indicates that the tillage and sowing of the crop has an influence on the reduction of free-living nematode numbers, as has been shown in a study focused on tillage effects on soil organisms (Govaerts et al. 2006). In our experiment, no tillage or weeding in biological control fallow and fallow plots provided organic matter enough to increase free-living nematode populations. The susceptibility of these organisms to changes in their environment makes them potential bio-indicators of soil conditions. The free-living nematodes play an important role in the agroecosystem through their participation in the decomposition of organic material and mineralization of nutrients in the soil as well as through their function as regulators of populations of fungi, bacteria, and insects (Bongers and Bongers 1998). For this reason, it is important that control methods for phytoparasitic nematodes do not significantly affect populations of free-living nematodes.

4 Conclusions

Biological control, in combination with rotation of non-host crops, can be a management strategy for the reduction of high populations of *G. rostochiensis*. We recommend the initial sanitizing of the soils in highly infected areas prior to sowing new potato crops. This strategy opens the possibility of rehabilitating and reactivating areas that are suitable for potato cultivation but are under quarantine at present. At the same time, the lack of impact on free-living nematodes by the biological control agent can still permit the biological diversity necessary for nutrient cycling. We have shown that the practice of leaving soils to lie fallow, while helping increase the diversity of edaphic organisms, is not a viable alternative for reduction of *G. rostochiensis* population density. The combination of both the strategies evaluated together in this study, namely that of biological control in conjunction with non-host crop rotation, was designed to reduce the population of potato cyst nematodes to thresholds of no economic significance at the same time generating a food product for human and/or animal consumption. These practices can be relatively easily incorporated by producers into the system of potato production that exists at present.

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