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Natural occurrence of fungal egg parasites of root-knot nematodes, *Meloidogyne* spp. in organic and integrated vegetable production systems in Spain

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Abstract A survey was conducted to assess the biodiversity and frequency of infection of fungal egg parasites of *Meloidogyne* spp. and relate results to soil properties in organic and integrated vegetable production in Spain. Forty sites were sampled at the end of the cropping cycle, 30 under integrated and ten under organic production. Fungal egg parasites were isolated from all organically managed sites and from 73 % sites under integrated production. Species richness and Shannon–Wiener index did not differ between production systems but the percentage of fungal egg parasitism did, as well as soil properties. Percentages of egg parasitism higher than 40 % were found in five and three sites under organic and integrated production, respectively. In all these sites, *Pochonia chlamydosporia* was present alone or co-occurring with other fungal species. The relative frequency of *P. chlamydosporia* was positively related to the percentage of parasitism in both production systems.

Keywords Biological control · Fungal egg parasites · Integrated production · *Meloidogyne* spp. · Organic farming · Vegetable crops

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Introduction

Root-knot nematodes (RKN), *Meloidogyne* Göldi (Rhabditida: Meloidogynidae), are a major limiting factor for vegetable production worldwide. In Spain, these nematodes cause yield losses of up to 60 % in cucumber and tomato and 30 % in lettuce (Verdejo-Lucas et al. 1994; Ornat et al. 1997; Sorribas et al. 2005). In conventional agriculture, control of RKN has been mainly based on the use of soil fumigants and nematicides. However, the need to reduce dependence on some agro-chemicals due to their negative impact on human health and the environment has impelled researchers to explore non-chemical alternative methods for nematode control. The European Commission Directive 2009/128/EC on the sustainable use of pesticides established a framework for Community action to promote the use of integrated pest management and non-chemical alternatives to pesticides. One of these approaches is the conservation and

enhancement of beneficial organisms. Sustainable production systems (i.e. integrated and organic farming) are good candidates for developing biologically based control methods because microbial diversity and activity could be enhanced by promoting soil fertility and restricting (integrated production) or excluding (organic farming) the use of synthetic pesticides. In Spain, the agricultural land under organic and integrated production of vegetable crops in 2010 was 10,156.06 ha and 29,210 ha, respectively, and has steadily increased during the last decade (Generalitat de Catalunya 2012; Ministerio de Medio Ambiente y Medio Rural y Marino 2011).

Nematode antagonists, including fungi, bacteria, and invertebrates are commonly associated to plant-parasitic nematodes, fungal parasites being the most abundant group (Stirling 1991). All soils have an inhabitant microbial community able to restrict disease progression, and some possess specific microorganisms able to act against the pathogen, leading to specific suppressiveness (Cook and Baker 1983). The density or biomass and functionality of the soil's microbiota can be estimated by measuring the enzymatic activity of the soil. Thus, fluorescein diacetate hydrolysis (FDA) has been related to total microbial activity (Swisher and Carroll 1980), β-glucosaminidase to fungal biomass (Miller et al. 1998), protease to density of culturable bacteria (Asmar et al. 1992), and urease to bacterial and fungal biomass (Nannipieri et al. 1978). Furthermore, some specific enzymatic activities of soil can be of interest as indicators of the occurrence and functionality of putative biological control agents, as these enzymes affect key components of the structure of the nematode. Among them, protease and β-glucosaminidase activities could be indicators of microbial egg antagonists because the main components of the external egg shell layer are proteins and chitin, respectively. In addition, environmental factors can influence the presence of selected antagonists (Kim et al. 1998) as well as the level of suppressiveness (Kerry et al. 1980; Gené et al. 2005). Therefore, it is important to identify key factors in growing areas, with similar environmental and agronomic practices, in order to enhance biological control by managing the antagonistic potential of the soil, which is known as "the capacity of a soil ecosystem, through biotic factors, to prevent or reduce the spread of a pathogen, parasite, or other deleterious agent" (Sikora 1992).

The objectives of this study were (1) to determine and compare the biodiversity of naturally occurring fungal egg parasites of *Meloidogyne* in vegetable crops under integrated and organic production; (2) to assess the percentage of fungal egg parasitism and (3) to investigate the relationship between percentage of fungal egg parasitism, soil properties, and relative frequency of fungal egg parasites for each production system.

Materials and methods

Sampling and isolation of *Meloidogyne* spp. eggs

Forty vegetable growing sites, 30 under integrated and ten under organic production, with a history of RKN infestation, were sampled at the end of the cropping season from May to September 2010. The number of sampled sites under each production system corresponded to the actual proportion (3:1; integrated:organic production) in Spain. Sites located in the provinces of Barcelona, Tarragona (north-eastern) and Valencia (eastern Spain) were selected in collaboration with agricultural field advisors who provided information on site characteristics and history. None of the 30 sites under integrated production had been treated with chemical nematicides before planting the chosen crop although three sites had been treated the year before, 20 sites two years before, and the rest three years before. Fertilization in integrated production was based on pellets of composted manure combined with chemical fertilizers, whereas, in organic production, it was done by annual applications of composted manure and cover crops that are incorporated into the soil every four years. Weeds were managed by mulching, mechanically, and to a lesser extent by herbicides (integrated production). The annual air temperatures in the sampled area ranged from 9 to 26 °C.

At each site, 20 plants were removed from the ground at the end of the cropping season with a pitchfork to take roots and surrounding soil. Soil was sieved through a 4-mm aperture screen to remove stones and separate roots from soil, and carefully mixed to extract nematodes from two 250-cm^3 soil subsample using the sieving and centrifugation-flotation method (Jenkins 1964).

Roots were carefully washed free of soil, chopped, and root-knot nematode eggs extracted from two

10 g-subsamples by macerating them for 10 min in a blender containing a 5 % solution of commercial bleach (40 g l^{-1} of NaOCl) (Hussey and Barker 1973). Eggs were counted and expressed per g root.

Plant-parasitic nematodes were identified at the genus level. *Meloidogyne* species were identified according to the morphology of the perineal pattern of the females, and by SCAR-PCR markers (Zijlstra et al. 2000).

Isolation of egg parasites and identification

Fungal egg parasites of RKN were isolated according to the de Leij and Kerry (1991) procedure modified by Verdejo-Lucas et al. (2002). Briefly, per each site, 25–30 egg masses were handpicked from roots and placed in a watchglass containing sterile, distilled water. The outer part of the gelatinous matrix was removed from the egg masses with tweezers to eliminate potential surface colonizers. Egg masses were then placed in an Eppendorf microcentrifuge tube containing 1 ml of sterile distilled water. Eggs were dispersed from the egg masses using a pestle and 333 µl-aliquots of the eggs' suspension were spread onto each of three replicated Petri dishes (9-cm diam) containing a growth restricting medium (streptomycin, 50 mg l^{-1}; chloramphenicol, 50 mg l^{-1}; chlortetracycline, 50 mg l^{-1}; Rose Bengal, 50 mg l^{-1}; triton, 1 ml l^{-1}; and 1 % agar) (Lopez-Llorca and Duncan 1986). In all cases, more than 100 eggs per plate were dispensed. Plates were incubated at 25 ± 0.5 °C. The number of parasitized eggs was recorded after 24 h and 48 h under a dissecting microscope and percentage of parasitism was then calculated as the number of parasitized eggs per plate/number of eggs per plate. Eggs were considered parasitized if fungal hyphae grew from inside. The parasitized eggs were then individually transferred to corn meal agar (CMA) to establish pure cultures of the fungi. Cultures were stored in 1 % (w/v) water-agar slants, as well as lyophilized and stored at 4 °C. A single spore culture was established per each isolate and they were identified by cultural and morphological characteristics and/or molecular analyses by PCR amplification and sequencing of the internal transcribed spacers (ITSs) of the rDNA regions. DNA was extracted from 50 mg of mycelium collected from single spore cultures on potato dextrose agar (PDA). The extraction was carried out using the E.Z.N.A kit® Plant MiniPrep (Omega Bio-Tek) following the protocol described by the manufacturer. The PCR reaction was performed in 25 µl mix that contained 1 µl of the DNA extraction, 10.5 µl MiliQ water (Qiagen), 12.5 µl Taq PCR Master Mix (Qiagen) and 0.5 µl of each primer (5 pmol), ITS1F (CTTGGTCATTT AGAGGAAGTAA) (Gardes and Bruns 1993) and ITS4 (TCCTCCGCTTATTGATATGC) (White et al. 1990). The amplification consisted of an initial 5 min denaturation step at 94 °C, five amplification cycles at 94 °C for 30 s, 54 °C for 30 s, and 72 °C for 60 s, followed by 33 amplification cycles at 94 °C for 30 s, 48 °C for 30 s, 72 °C for 60 s, and a final extension step of 72 °C for 10 min (White et al. 1990). PCR products were cleaned using MinElute PCR Purification Kit (Qiagen) and sequenced by Secugen (Madrid, Spain). DNA sequences were analysed using the BLAST database (September 2011) and assigned to the reference isolate sequences with the highest bit score.

Assessment of species diversity of egg parasites

Fungal diversity at each site was estimated according to the species richness (S), Shannon–Wiener diversity index, and species evenness. The Shannon–Wiener diversity index (H') was calculated according to the formula $H' = -\sum (n_i/N) \times \log_2 (n_i/N)$, where N is the number of eggs parasitized and n_i is the number of eggs parasitized by the species i. The species evenness (relative abundance of each fungal egg parasite species in a given site) was calculated according to the expression $J' = H'/H'\,max$, where H' is the index of Shannon–Wiener and $H'\,max$ is the maximum value of H' ($H'\,max = \log_2 S$, where S is the species richness).

Soil properties

A 1 kg soil sample from each surveyed site was sent to AGQ Agroalimentaria y Medio Ambiente (Sevilla, Spain) to determine the physical and chemical characteristics that are described in Table 2. Four soil enzymes associated with microbial activity, β-glucosaminidase (Parham and Deng 2000), urease (Sastre-Conde and Lobos 2003), fluorescein diacetate hydrolysis and protease (Fernández et al. 2001) were analyzed following the above listed protocols.

Statistical analysis

Statistical analysis was carried out with the SAS system software V9. Variables were transformed when required to $\log_{10}(x + 0.1)$, or $\log_{10}(x)$. An analysis of variance using the general linear model (proc glm) was carried out to compare soil properties, richness of fungal species, biodiversity index, and percentage of parasitized eggs between integrated and organic production. Correlation analysis (proc corr) was performed separately per production system to determine single relationships between variables including soil properties, percentage of egg parasitism, relative frequency of fungal species, and RKN population densities in soil and in roots. In addition, the variable time elapsed since the last chemical soil treatment was also included in the correlation analysis for sites under integrated production. If no single strong relationships were found between soil properties and percentage of fungal egg parasitism, then a multiple regression analysis (proc reg) was performed with the stepwise model selection at a significance level of $P = 0.05$ to determine if any combination of variables could explain the variability.

Results

Survey of plant parasitic nematodes

Seven genera of plant-parasitic nematodes were common to both production system: *Meloidogyne* (40 sites), *Tylenchorhynchus* Cobb (20), *Aphelenchus* Bastian (10), *Telotylenchus* Siddiqi (8), *Aphelenchoides* Fischer (7), *Pratylenchus* Filipjev (6), and *Paratylenchus* Micoletzky (2). Three additional genera were only identified in organic production: *Helicotylenchus* Steiner (6 sites), *Ditylenchus* Filipjev (3) and *Heterodera* Schmidt (2). The species of *Meloidogyne*, in order of occurrence, were *M. incognita* (Kofoid and White) Chitwood (42.4 %), *M. javanica* (Treub) Chitwood (30.3 %), and *M. arenaria* (Neal) Chitwood (27.3 %). Population densities of *Meloidogyne* were higher than those of the other plant parasitic nematodes irrespective of the production system. In sites under organic production, RKN densities ranged from 124 to 4,000 J2 250 cm^{-3} of soil (1,197 ±1,230; mean ± SD), and from 1,330 to 22,455 eggs per gram of root (10,056 ± 8,811), and those of the other nematodes from 14 (*Helicotylenchus*) to 528 nematodes 250 cm^{-3} of soil (*Tylenchorhynchus*). In integrated production, RKN population densities ranged from 57 to 13,247 J2 250 $cm^{-3}$of soil (2,826 ± 7,651), and from 122 to 39,592 eggs per gram of root (6,154 ± 8,250), and the densities of the other nematodes from 11 (*Telotylenchus*) to 4,300 nematodes 250 $cm^{-3}$of soil (*Pratylenchus*).

Biodiversity of fungal egg parasites of *Meloidogyne* spp.

Fungal parasites of RKN egg were found in 100 % of the sites under organic, and in 73 % of the sites under integrated production. Fungal species common to both production systems were *Fusarium* sp., *F. oxysporum* Schlecht, *F. solani* (Mart.) Sacc., *Paecilomyces lilacinus* (Thom) Samson, *Plectosphaerella cucumerina* (Lindfors) Gams, *Pochonia chlamydosporia* (Goddard) Zare and Gams, and *Thielavia* sp. Fungi only found in organic production sites were *Cladosporium tenuissimum* Cooke, *Colletotrichum coccodes* (Wallr.) Hughes, and *F. equiseti* (Corda) Sacc. In contrast, *Chaetomium* sp. *Cladosporium sphaerospermum* Penz., *Cylindrocarpon olidum* (Wollenw.) Wollenw., *Dactylella oviparasitica* Stirling and Mankau, *F. verticillioides*, *Monacrosporium thaumasium* (Drechsler) de Hoog and Oorschot, *Myrothecium verrucaria* (Alb. & Schwein.) Ditmar, *Penicillium citrinum* Thom, *P. olsonii* Bainier and Sartory, and *Verticillium* sp. were only found in sites under integrated production. The most frequent fungal species isolated from RKN eggs in both production systems were *P. chlamydosporia*, *Fusarium* spp. and *P. cucumerina* (Table 1).

Species richness ($F = 3.31$; df = 1, 38; $P = 0.077$) and the biodiversity index of Shannon–Wiener ($F = 0.26$; df = 1, 31; $P = 0.3996$) did not differ between production systems. In organic production, species richness ranged from 1 to 3, the biodiversity index from 0 (sites with a single fungal species) to 1, and the evenness from 0.41 to 1. In integrated production, species richness ranged from 1 to 5, the biodiversity index from 0 to 1.74, and the evenness from 0.44 to 1. In the majority of the sites with more than one fungal species, *P. chlamydosporia* was the dominant species (evenness lower than 1) irrespective of the production system (Table 1).

Table 1 Vegetable crop, percentage of fungal egg parasitism of *Meloidogyne* spp., species richness, Shannon–Wiener diversity index (H') and evenness (J') in vegetable production sites under organic and integrated production

Production system	Site	Crop	Parasitism (%)[a]	Fungal species (richness)	H'	J'
Organic	M10.2	Tomato	24.4 ± 7.8	*Cladosporium tenuissimum*, *Fusarium oxysporum*, *Paecilomyces lilacinus* (3)	0.92	0.58
	M10.3	Tomato	56.9 ± 8.9	*Plectosphaerella cucumerina*, *P. chlamydosporia.* (2)	1	1
	M10.4	Tomato	4.0 ± 4.0	*Plectosphaerella cucumerina*, *Thielavia* sp. (2)	1	1
	M10.16	Tomato	65.4 ± 28.2	*Fusarium equiseti*, *P. chlamydosporia* (2)	1	1
	M10.19	Eggplant	4.5 ± 2.3	Unidentified (1)	0	–
	M10.23	Eggplant	14.4 ± 5.1	*Plectosphaerella cucumerina*, *P. chlamydosporia* (2)	0.76	0.76
	M10.41	Tomato	80.2 ± 3.5	*Colletotrichum coccodes*, *Fusarium solani*, *P. chlamydosporia* (3)	0.65	0.41
	M10.55	Eggplant	61.0 ± 7.8	*P. chlamydosporia* (1)	0	–
	M10.56	Pepper	9.4 ± 2.5	*Fusarium oxysporum*, *Paecilomyces lilacinus* (2)	1	1
	M10.58	Eggplant	41.7 ± 7.3	*Fusarium* sp., *P. chlamydosporia* (2)	0.92	0.92
Integrated	M10.13	Faba bean	3.3 ± 3.3	*Plectosphaerella cucumerina* (1)	0	–
	M10.21	Carrot	1.0 ± 0.5	*Thielavia* sp. (1)	0	–
	M10.24	Pepper	15.0 ± 0.0	*Penicillium olsonii*, *P. chlamydosporia* (2)	0.50	0.50
	M10.25	Cucumber	15.0 ± 1.7	*Plectosphaerella cucumerina*, *P. chlamydosporia* (2)	0.44	0.44
	M10.26	Tomato	0.9 ± 0.9	*Plectosphaerella cucumerina* (1)	0	–
	M10.27	Cucumber	1.4 ± 0.8	*Chaetomium* sp., *Cladosporium sphaerospermum* (2)	1	1
	M10.28	Courgette	0.7 ± 0.7	Unidentified (1)	0	–
	M10.29	Tomato	0.4 ± 0.4	Unidentified (1)	0	–
	M10.32	Cucumber	4.8 ± 2.9	Unidentified (1)	0	–
	M10.33	Cucumber	4.1 ± 2.4	*Plectosphaerella cucumerina*, *Monacrosporium thaumasium*, Unidentified (3)	1.58	1
	M10.35	Cucumber	4.0 ± 0.7	*Fusarium oxysporum* (1)	0	–
	M10.36	Tomato	13.5 ± 7.1	*Fusarium verticillioides*, *Fusarium oxysporum*, *Fusarium solani.* (3)	1.54	0.77
	M10.37	Tomato	1.5 ± 0.7	*P. chlamydosporia*, *Verticillium* sp. (2)	1	1
	M10.39	Tomato	2.6 ± 1.3	*Fusarium* sp., *Myrothecium verrucaria* (2)	1	1
	M10.43	Tomato	69.3 ± 4.2	*Cylindrocarpon olidum*, *Dactylella oviparasitica*, *Paecilomyces lilacinus*, *Penicillium citrinum*, *P. chlamydosporia* (5)	1.16	0.5
	M10.44	Tomato	2.3 ± 1.6	*Fusarium solani*, *Fusarium* sp., Unidentified (3)	1.15	0.72
	M10.45	Tomato	6.5 ± 1.0	*Chaetomium* sp., *Paecilomyces lilacinus*, *P. chlamydosporia* (3)	1.37	0.86
	M10.46	Eggplant	31.2 ± 7.4	*Fusarium* sp., *Paecilomyces lilacinus*, *P. chlamydosporia*, Unidentified (4)	1.74	0.87
	M10.51	Pepper	49.4 ± 8.0	*P. chlamydosporia*, Unidentified (2)	1	1
	M10.52	Tomato	2.1 ± 2.1	*Fusarium oxysporum* (1)	0	–
	M10.53	Cucumber	3.0 ± 3.0	Unidentified (1)	0	–
	M10.54	Pepper	49.7 ± 12.1	*P. chlamydosporia* (1)	0	–
Organic[b]			36.2 ± 8.9	2.0 ± 0.21	0.7 ± 0.13	0.8 ± 0.08

Table 1 continued

Production system	Site	Crop	Parasitism (%)[a]	Fungal species (richness)	H'	J'
Integrated[c]			9.4 ± 3.2	1.4 ± 0.24	0.6 ± 0.13	0.8 ± 0.06
Organic versus Integrated[d]			$F = 13.25$	$F = 3.31$	$F = 0.26$	$F = 0.08$
			df = 1, 38	df = 1, 38	df = 1, 31	df = 1, 18
			$P = 0.0008$	$P = 0.077$	$P = 0.399$	$P = 0.780$

Richness: number of fungal species in a given site; H': diversity index of Shannon–Wiener $(-\Sigma\ (n_i/N) \times \log_2\ (n_i/N)$, where N is the density of all the species and n_i is the density of species i; J': evenness index when number of species >1 ($H'/H'\ max$; where H' is the index of Shannon–Wiener and $H'\ max = \log_2(S)$ where S is the total number of species. [a] Data per each site are mean ± SE of three replicates. [b] Data for organic production are mean ± SE of ten sites for parasitism, richness, and H', and eight for J'. [c] Data for integrated production are mean ± SE of 30 sites for parasitism, and richness, 22 for H', and 12 for J'. [d] F-values, degrees of freedom, and P values to compare parasitism, richness, H', and J' between organic and integrated production

Percentage of fungal egg parasitism, soil properties and their relationships

The percentage of fungal egg parasitism was higher in organic (36.2 ± 8.9; mean ± SE) than in integrated production (9.2 ± 3.3) ($F = 13.25$; df = 1, 38; $P = 0.0008$) (Table 1). The relative frequency of *P. chlamydosporia* was positively related to the percentage of fungal egg parasitism both in organic ($r = 0.838$; $P = 0.0024$) and integrated production ($r = 0.575$; $P < 0.0001$).

Soils under organic production had higher content of clay ($F = 11.78$; df = 1, 38; $P = 0.0015$), silt ($F = 6.67$; df = 1, 38; $P = 0.014$), organic matter ($F = 9.16$; df = 1, 38; $P = 0.004$), exchangeable Mg ($F = 8.86$; df = 1, 38; $P = 0.005$), exchangeable K ($F = 19.48$; df = 1, 38; $P < 0.0001$), available K ($F = 14.09$; df = 1, 38; $P = 0.0006$), cationic exchange capacity ($F = 32.30$; df = 1, 38; $P < 0.0001$), lime ($F = 15.96$; df = 1, 38; $P < 0.0003$), exchangeable Ca ($F = 17.66$; df = 1, 38; $P < 0.0002$), pH ($F = 4.54$; df = 1, 38; $P = 0.040$) and FDA ($F = 9.63$; df = 1, 38; $P = 0.004$), urease ($F =$ 6.36; df = 1, 38; $P = 0.016$) and β-glucosaminidase ($F = 5.64$; df = 1, 38; $P = 0.023$) activities, and lower content of sand ($F = 11.06$; df = 1, 38; $P = 0.002$), Fe ($F = 19.25$; df = 1, 38; $P < 0.0001$), Mn ($F = 5.28$; df = 1, 38; $P = 0.027$), Cu ($F = 8.98$; df = 1, 38; $P = 0.005$), Zn ($F = 20.83$; df = 1, 38; $P < 0.0001$), as well as P/N ($F = 5.14$; df = 1, 38; $P = 0.029$), and (Ca + Mg)/K ($F = 7.20$; df = 1, 38; $P = 0.011$) ratios than those under integrated production (Table 2).

In the organic production sites, the percentage of fungal egg parasitism ranged from 4.0 to 80.2 %, and was correlated with the clay content of soil ($r = 0.694$; $P = 0.026$). Multiple regression analysis indicated that the percentage of parasitism was positively related to the clay content of soil and the P/N relationship (percentage parasitism $(\log_{10}(x + 0.1)) = -0.38 + 0.0596$ clay (%) + 3.897 P/N; $R^2 = 0.689$; $P = 0.0168$). In sites conducted under integrated production, the percentage of fungal egg parasitism ranged from 0.0 to 69.3 %, and did not correlate with any soil parameter nor with the time elapsed from the last nematicide treatment. In this system, multiple regression analysis did not reveal any relationship between fungal parasitism and soil properties.

Discussion

Twenty fungal species belonging to 15 genera, and nine unidentified fungi, were isolated from RKN eggs. Most of the isolated fungi have the capacity to parasitize or be antagonists of RKN or cyst nematode eggs in vitro, pot or field experiments like *Cladosporium sphaerospermum* (Meyer et al. 2004), *Dactylella oviparasitica*, *Fusarium equiseti* (Nitao et al. 2001), *F. oxysporum* (Olatinwo et al. 2006), *F. solani* (Zareen et al. 2001), *Monacrosporium thaumasium*, *Myrothecium verrucaria*, *Chaetomium* sp., *Thielavia* sp., (Sun et al. 2006), *Paecilomyces lilacinus* (Kiewnick and Sikora 2006; Kiewnick et al. 2011), *Plectosphaerella cucumerina* (Atkins et al. 2003), and *P. chlamydosporia* (Sorribas et al. 2003; Kerry and Hidalgo-Diaz 2004). *Colletotrichum coccodes* was reported associated to potato cyst nematode by van der Laan (cited in Jones et al. 1986) although it was not

Table 2 Physicochemical properties and enzymatic activity of soils conducted under integrated and organic vegetable production systems surveyed to detect fungal egg parasitism of *Meloidogyne* spp.

Variable	Integrated production	Organic production
B (ppm)	2.58 ± 0.25	2.63 ± 0.43
Exchangeable Ca (meq 100 g^{-1})	**9.71 ± 1.11**	**15.43 ± 0.69** *
Lime (% $CaCO_3$)	**2.97 ± 0.29**	**5.06 ± 0.36** *
Cation exchange capacity (meq 100 g^{-1})	**13.12 ± 1.11**	**25.95 ± 2.32** *
Cu (ppm)	**11.03 ± 3.41**	**2.61 ± 0.11** *
Electric conductivity (μmhos cm^{-1})	656.71 ± 150.8	397.77 ± 32.55
Available P (ppm)	157.94 ± 18.5	129.21 ± 3.24
Sand (%)	**69.52 ± 4.15**	**44.8 ± 3.24** *
Clay (%)	**11.65 ± 1.73**	**23.6 ± 2.34** *
Silt (%)	**18.84 ± 2.62**	**31.6 ± 3.81** *
Fe (ppm)	**101.23 ± 27.22**	**5.64 ± 0.64** *
Exchangeable Mg (meq 100 g^{-1})	**1.74 ± 0.14**	**2.67 ± 0.33** *
Available Mg (meq 100 g^{-1})	2.29 ± 0.25	2.92 ± 0.36
Mn (ppm)	**74.36 ± 8**	**41.51 ± 11.97** *
Organic matter (%)	**2.08 ± 0.16**	**3.12 ± 0.45** *
N (ppm)	1229.69 ± 113.78	1639.35 ± 210.76
pH (1:2.5 in water)	**8.08 ± 0.05**	**8.28 ± 0.04** *
Exchangeable K (meq 100 g^{-1})	**0.33 ± 0.05**	**0.78 ± 0.10** *
Available K (meq 100 g^{-1})	**0.52 ± 0.08**	**1.08 ± 0.14** *
C/N	10.59 ± 0.47	11.11 ± 0.51
Exchangeable Na (meq 100 g^{-1})	0.35 ± 0.02	0.41 ± 0.03
Available Na (meq 100 g^{-1})	1.07 ± 0.25	1.04 ± 0.27
Zn (ppm)	**35.04 ± 8.62**	**5.88 ± 1.91** *
Ca/Mg	5.9 ± 0.48	6.47 ± 0.71
Ca + Mg/K	**49.54 ± 4.93**	**25.32 ± 2.03** *
P/N	**0.14 ± 0.01**	**0.09 ± 0.02** *
Fluorescein diacetate hydrolysis (μg fluorescein h^{-1} × g soil)	**2.67 ± 0.25**	**4.4 ± 0.71** *
β-glucosaminidase (μmols *p*-nitrophenol h^{-1} × g soil)	**0.19 ± 0.03**	**0.31 ± 0.05** *
Urease (μmols N–NH_4 h^{-1} × g soil)	**0.91 ± 0.22**	**1.24 ± 0.15** *
Protease (μg tyrosine h^{-1} × g soil)	7.97 ± 0.32	6.85 ± 0.90

Each data are mean ± SE of 30 replicates (integrated production) or ten replicates (organic production). Values in bold and followed by * in the same row are different statistically according to the analysis of variance ($P < 0.05$)

consider a parasite. Three out the four remaining fungi, *Cladosporium tenuissimum*, *Penicillium citrinum*, and *P. olsonii*, have been reported as endophytes (Fisher and Petrini 1992; Vega et al. 2006; Maciá-Vicente et al. 2008), and *F. verticillioides* as a plant pathogen, but as far as we know, their capacity to parasitize nematode eggs has not been investigated.

Higher levels of parasitism were found in organic than integrated production. The former had more content of organic matter, finer textured particles, FDA, β-glucosaminidase, and urease activities of soil, which suggest that these factors may play a key role in increasing the antagonistic potential of the soils. It is widely accepted that organic matter improves soil structure, fertility, and enhances biological control because it acts as a substrate to microbial inhabitants of soil, including nematode antagonists (Akhtar and Malik 2000; Oka 2010). This idea is also supported by this study, because soils under organic production showed higher microbial activity (FDA), probably due to fungi (urease activity) which in turn had chitinolitic activity (β-glucosaminidase). Furthermore, clay and

silt content of soil influences pore size (30–90 μm), and affects the habitability of nematodes in the soil (Hassink et al. 1993). Eggs' hatching of *Meloidogyne* in soils with large pore size occurs earlier and in greater numbers than in soils with smaller pores (Evans and Perry 2009). Thus, in fine textured rather than sandy soils, unhatched eggs in the egg masses could be exposed to fungal egg parasites for a longer time, which would increase the likelihood of being parasitized. Also, fine-textured soils have a higher water holding capacity than coarse soils, which could maintain soil's humidity at sufficiently high levels to allow the activity of the fungal-egg parasites. For instance, parasitism of *Heterodera avenae* Wollenweber by *Nematophora gynophila* Kerry and Crump decreased at drier conditions (Kerry et al. 1980).

Fungal species co-occurred (Shannon–Wiener index >0) more frequently in sites under organic (80 %) than under integrated production (54.5 %). However, the biodiversity index was not related to percentage of parasitism irrespective of the production system. This suggests that the success of biological control might be more influenced by the functionality than by the diversity of fungal species. Thus, *P. chlamydosporia* was the only fungal species present alone or co-occurring with other fungal species that positively related to the percentage of fungal egg parasitism in both organic and integrated production, which suggest that *P. chlamydosporia* may be the main fungal species involved in the antagonistic potential of the surveyed soils. In California, Borneman and Becker (2007) reported that *D. oviparasitica* was the main biotic factor responsible for the suppressiveness against *H. schachtii* in sugar beet, despite the fact that *F. oxysporum* and other egg parasitic fungi co-occurred in the same field. Soil suppressiveness might be achieved in undisturbed systems, such as perennial crops, or when the nematode resistance form remains in the soil for long periods, as is the case of cyst nematodes. Such conditions may favour long-term associations of the nematode with microbial parasites that require direct contact with their hosts. On the contrary, soil suppressiveness might be less likely to occur in cropping systems with high soil disturbances, as in intensive vegetable production, or where nematode eggs are surrounded by a gelatinous matrix (egg mass) or remain unexposed to microbial parasites within the root. Nonetheless, suppressive soils have been reported in agricultural fields in California, USA (Pyrowolakis et al. 2002).

The sites sampled in this survey were selected for their previous history on RKN infestation. However, a diversity of plant parasitic nematodes genera were found, being higher under organic (ten genera) than in integrated production (7), and with similar levels of infestation for a given crop. Studies to compare nematode impact on organic production in comparison with integrated or conventional production have not been done in the country although yield losses caused by *Meloidogyne* can reach up to 60 % depending on the crop (Verdejo-Lucas et al. 1994; Ornat et al. 1997; Sorribas et al. 2005). In Germany, plant parasitic nematodes are widely spread in organic vegetable production and can cause severe damage (Hallmann et al. 2007). Despite yield losses due to plant parasitic nematodes are related to the population densities at planting and the plant tolerance (Seinhorst 1965), the cropping system could also influence the population dynamics of the nematodes, due to changes in the antagonistic potential of soils, and can thus affect the relationship between yield losses and nematode population densities. Long-term studies should be done to determine the effect of native fungal egg parasites on the population dynamics of RKN and crop yield of the most common rotation sequences, and to improve agronomical practices that enhance the level of suppression of plant parasitic nematodes.

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