NEW RECORDS OF *POCHONIA CHLAMYDOSPORIA* FROM MEXICO: ISOLATION, ROOT COLONIZATION AND PARASITISM OF *NACOBBUS ABERRANS* EGGS

F. Franco-Navarro[1*], K. Vilchis-Martínez[2], and J. Miranda-Damián[2]

[1]Phytopathology Program, Colegio de Postgraduados-Campus Montecillo, Mexico State, Mexico; [2]Agricultural Engineering, FES-Cuautitlán, U. N. A. M. P. O., Mexico State, Mexico. *Corresponding author: ffranco@colpos.mx

ABSTRACT

Franco-Navarro, F., K. Vilchis-Martínez, and J. Miranda-Damián. 2008. New records of *Pochonia chlamydosporia* from Mexico: Isolation, Root colonization and Parasitism of *Nacobbus aberrans* eggs. Nematropica 39:133-142.

One hundred and six soil samples were taken from the buffer zone of the Biosphere Reserve of "Los Tuxtlas", Veracruz, Mexico, and examined for the presence of native isolates of the fungus *Pochonia chlamydosporia*. Samples were collected from locations with different land uses (i.e. natural forest, secondary forest, pasture fields and maize fields) and were processed using a selective medium to isolate the fungus. Two varieties of the fungus (alone or in combination), were found in a total of 30 soil samples: *P. c.* var. *chlamydosporia* was present in 25 samples and *P. chlamydosporia* var. *catenulata* in 10 of the samples. Six isolates were present in pasture fields, 4 in maize fields, 13 in secondary forest and 12 in natural forest. All isolates were tested on maize for their ability to colonize roots, and to parasitize eggs of the nematode *Nacobbus aberrans*. There were highly significant differences in the proportion of eggs parasitized by the different isolates (Tukey, $\alpha < 0.01$). Eight isolates parasitized >80% of the eggs, 16 parasitized between 70-80%, and 11 parasitized <70%. Root colonization ranged from 75-100%: 12 isolates colonized all root segments, 14 colonized 90-99% of them, six colonized 80-89% and three colonized only 75%. This is the first record of *P. c.* var. *catenulata* being present in Mexican soils and the first record of *P. chlamydosporia* in natural habitats—non-disturbed soils—from Mexico.

Key words: false root-knot nematode, Los Tuxtlas, microbiological control, nematophagous fungi, plant-parasitic nematodes.

RESUMEN

Franco-Navarro, F., K. Vilchis-Martínez, and J. Miranda-Damián. 2008. Nuevos registros de *Pochonia chlamydosporia*: Aislamiento, Colonización de raíces y Parasitismo de huevos de *Nacobbus aberrans*. Nematropica 39:133-142.

Se procesaron 106 muestras de suelo tomadas de la zona de amortiguamiento de la Reserva de la Biosfera "Los Tuxtlas", Veracruz México, con el fin de detectar la presencia de aislamientos nativos del hongo *P. chlamydosporia*. Las muestras se colectaron en sitios con diferente uso de suelo (bosque natural, bosque secundario, pastizal y maizal), y se procesaron utilizando métodos estándares y un medio semi-selectivo para aislar al hongo. En un total de 30 muestras de suelo se identificó a *P. chlamydosporia*, presentándose sus dos variedades individualmente o en combinación: *P. c.* var. *chlamydosporia* estuvo presente en 25 muestras y *P. c.* var. *catenulata* en 10 de las muestras. En pastizal se encontraron 6 aislamientos, 4 en maizal, 13 en bosque secundario y 12 en bosque natural. De todos los aislamientos, se probó su capacidad para colonizar raíces de maíz y parasitar huevos del nematodo *Nacobbus aberrans*. Hubo diferencias altamente significativas en la proporción de huevos parasitados por los diferentes aislamientos (Tukey, $\alpha < 0.01$). Ocho aislamientos parasitaron >80% de los huevos, 16 parasitaron entre 70-80%, y 11 parasitaron <70%. La colonización de raíces varió entre 75-100%: 12 aislamientos colonizaron todos los segmentos de raíces, 14 colonizaron 90-99% de ellos, seis colonizaron 80-89% y tres colonizaron solamente 75%. Este es el primer registro de la presencia de *P. c.* var. *catenulata* en suelos mexicanos y el primer registro de *P. chlamydosporia* en ambientes naturales—con suelos no perturbados—de México.

Palabras clave: control microbiológico, hongos nematófagos, Los Tuxtlas, nematodo falso nodulador, nematodos fitoparásitos.

Pochonia chlamydosporia (Goddard) Zare and W. Gams (= *Verticillium chlamydosporium*) is a nematophagous fungus widespread and a natural parasite of cyst and root-knot nematodes (Morgan-Jones *et al.*, 1981; Kerry *et al.*, 1984; de Leij *et al.*, 1992, 1993; Hidalgo-Diaz *et al.*, 2000). Its potential as a biological control agent for use in integrated pest management strategies for *Meloidogyne incognita* in vegetable crops has repeatedly been studied (Atkins *et al.*, 2003a, b; Kerry and Hidalgo-Diaz, 2004). *Pochonia chlamydosporia* has been isolated from the rhizospheres of clover and ryegrass, from snail eggs and from oospores of some fungi and does not seem to depend on nematodes for its nutrition (Kerry *et al.*, 1984). This fungus is a facultative parasite of nematode eggs, and can proliferate in the rhizoplane and rhizosphere of a wide range of crop plants; although the fungus does not cause lesions or affect root growth, some endophytic colonization has been reported (Kerry *et al.*, 1984; de Leij and Kerry, 1991; Bourne *et al.*, 1994). Such colonization prolongs the survival of the fungus in soil and enables it to multiply close to developing female nematodes (de Leij and Kerry, 1991). Different strains can vary in their pathogenicity, growth and chlamydospores production in laboratory media (Kerry, 1981; Kerry *et al.*, 1984).

There have been few studies on the biological control of nematodes in Mexico using nematophagous fungi. The first survey was made by Lappe and Ulloa (1982), who isolated *Arthrobotrys oligospora* Fresen. and *A. conoides* Drechsler from Mexican soils. Mendoza-de Gives *et al.* (1994) tested the ability of *Arthrobotrys* spp. to infect two species of nematodes, one of them being the false root-knot nematode, *Nacobbus aberrans*. Later, Mexican isolates of *Pochonia chlamydosporia,* were screened against *N. aberrans* to identify the most effective for use them in developing integrated pest management strategies (Flores, 2003).

Nacobbus aberrans is a significant pest in Mexico, as it damages important food crops such as tomato (*Lycopersicon esculentum* Mill.), chili (*Capsicum annum* L.) and bean (*Phaseolus vulgaris* L.) (Manzanilla-López *et al.*, 2002). In fields, where control measures have not been implemented, tomato yield losses up to 83% have been reported (Cristóbal-Alejo *et al.*, 2001). Because of its wide host range, including many weeds, and its high reproductive capacity under field conditions, *Nacobbus aberrans* has become an important and difficult pest to control (Cristóbal-Alejo *et al.*, 2001).

This study was carried out to extend the search for additional native isolates of *P. chlamydosporia* in order to select those with potential as biological control agents against *N. aberrans*. The project is part of ongoing research of soil nematode communities and indigenous populations of microorganisms potentially antagonistic to plant-parasitic nematodes. The Biosphere Reserve of "Los Tuxtlas", Veracruz, Mexico was chosen because it is amongst the better studied natural reserves in Latin America regarding plant and animal ecology studies (Dirzo and García, 1992).

Soil samples were collected from the buffer zone of the Biosphere Reserve of "Los Tuxtlas" (18°10'-18°45'N, 94°42'-95°27'W), located in Veracruz, Mexico. Three localities of the buffer zone of the reserve were chosen as replicate sites, and representative areas of four land use inten-

sities were selected from each one: natural forest, secondary forest, pasture and maize fields. Eight sampling points were georeferenced in each land use area from each locality, following a grid pattern with a distance between points of at least 200 m in order to ensure that there was no dependence among them. Sampling points were positioned where grid lines crossed and eight subsamples (each of 100 g) were taken from each sampling point at a depth of 10 to 30 cm, following two concentric circles around each point. Four subsamples were taken from the inner circle (3 m diameter), and another four from the outer circle (6 m diameter). All subsamples from each sampling point were mixed in plastic bags and 32 soil samples were taken per locality making 96 samples in total. Additional soil samples (50g) were taken from each sampling point to determine soil moisture, pH and organic matter, which were analyzed at the Ecology Institute from Xalapa, Ver.

Each soil sample was mixed thoroughly and 100 g soil subsamples were taken for the isolation of *P. chlamydosporia* using standard methods and a semi-selective medium (de Leij and Kerry, 1991). To isolate the fungus, three 1 g aliquots were individually added to 9 ml of 0.05% sterile Water-Agar (WA) (dilution 10^{-1}) and shaken on a vortex shaker during a few seconds. From each sample, a second dilution (10^{-2}) was prepared and finally a 0.2 ml aliquot was plated onto a 9 cm Petri dish containing a semi-selective medium (de Leij and Kerry, 1991). The plates were incubated at 25°C for 3 weeks before colonies of *P. chlamydosporia* were detected (Hidalgo-Diaz *et al.*, 2000). Colonies of the fungus were picked from each sample and cultured separately on Potato-Agar (PA) plates (Hidalgo-Diaz *et al.*, 2000) to confirm identification. The fungal isolates were identified morphologically and both varieties of *P. chlamydosporia* distinguished following descriptions of the fungus (Kerry, 1997; Zare *et al.*, 2001). Identifications were made in open Petri dishes using a compound microscope (400X) to seek the production of conidia and dictyochlamydospores. Thirty samples contained one or both varieties of *P. chlamydosporia*, and one subculture of each variety was preserved from each sample, with storage at -80°C using 25% sterile glycerol as the storage medium.

The isolates were screened to determine their potential as biological control agents according to standardized methods (Hidalgo-Diaz, *et al.*, 2000). The characteristics assessed in this study were the capacity to parasitize *N. aberrans* eggs and the ability to colonize the rhizosphere of plants, as the first tests to select isolates to be used as potential biological control agents.

To assess the ability of each isolate to parasitize eggs of *N. aberrans*, plates of PA colonized by the fungus were washed off using 2-5 ml of sterile distilled water and the surface of the agar plate scraped with a sterile glass rod to make a suspension of the fungal material. A 0.2 ml aliquot of this fungal suspension was then spread on a 9 cm Petri dish containing WA with antibiotics and incubated at 25°C for two days (Hidalgo-Diaz *et al.*, 2000). Roots from 70 days-old tomato plants (*Lycopersicon esculentum* Mill. cv. Rio Grande), infected under glasshouse conditions with a pure population of *N. aberrans* (from Tecamachalco, Puebla, Mexico), were washed and three hundred egg masses were picked off by hand using fine syringes. The eggs masses were crushed mechanically using the syringes, to release eggs from the gelatinous matrix (Hidalgo-Diaz *et al.*, 2000). About 300 eggs were added to each plate and incubated at 25°C for four days, after which time the percentage of eggs parasitized was estimated by counting at random

100 eggs using a compound microscope (400X). The test consisted of five replicates of each isolate plus eggs of the nematode and five plates with eggs but without the fungus (negative control).

To assess the ability of the isolates to colonize the rhizosphere, maize seeds were surface-sterilized in 3.5% NaOCl for 1 min on a mechanical shaker, washed in sterile distilled water and transferred to Petri dishes with sterile, damp paper and left to germinate at 27°C for one week. Test tubes (20 × 3cm) were filled two-thirds with vermiculite, moistened, closed with cotton wool and autoclaved (Hidalgo-Diaz *et al.*, 2000). When cooled, three 1 cm plugs of the fungus on PA were inserted just below the surface of the vermiculite and two surface-sterilized germinated maize seeds placed on top of the fungus. Tubes were incubated at 27°C in the dark for one week, after which time the roots were shaken free of vermiculite, cut into 1 cm sections and placed on PA plates + antibiotics (Hidalgo-Diaz *et al.*, 2000). After four days incubation at 27°C, root segments colonized by the fungus were counted. There were three replicates for each isolate.

Each experiment was repeated and data were analyzed by ANOVA using the Tukey test for comparisons of the means. Statistical procedures were performed using Statistical Analysis System (SAS) software. Differences at ($P \leq 0.05$) were considered significant. *Pochonia chlamydosporia* was recovered from 30 locations; 20 samples contained only the variety *chlamydosporia*, five samples contained only the variety *catenulata* and five samples contained both. Twelve of the thirty five isolates came from natural forest, 13 from secondary forest, 6 from pasture and 4 from maize fields (Table 1). All isolates from maize fields, and all but one from pastures, were identified as *P. c.* var. *chlamydosporia*. Four of twelve isolates from natural forest and five of thirteen isolates from secondary forest were *P. c.* var. *catenulata*. Both varieties of *P. chlamydosporia* were found in two samples from the natural forest and three from the secondary forest (Table 1). The various isolates of *P. chlamydosporia* are shown in Table 1 in relation to altitude, soil moisture, pH and organic matter content of the soil.

There were differences in the proportion of eggs parasitized by the various fungal isolates (Tukey, $\alpha = 0.01$). Eight isolates parasitized more than 80% of the eggs, sixteen between 70-80% and eleven less than 70% (Figs. 1 and 2). Colonization of maize roots ranged from 75-100%. Twelve isolates colonized all root segments including two from the natural forest, six from the secondary forest, three from pasture and one from maize fields. Root colonization levels between 90-99% were observed in 14 isolates: seven from natural forest, four from secondary forest, two from pasture fields and one from maize fields. Six isolates colonized between 80-89% of the root segments including one from the natural forest, three from the secondary forest and two from the maize fields (Figs. 1 and 2).

According to Gams and Zare (2001), and Zare *et al.* (2001), about six nematophagous species and four varieties in the genus *Pochonia* have been recorded as parasites of cyst and root-knot nematodes. *Pochonia chlamydosporia* is the best studied species in respect to its potential as a biological control agent of plant-parasitic nematodes. The two varieties of *P. chlamydosporia* found in "Los Tuxtlas", differed in conidial aggregation as described by Zare *et al.* (2001), although the biological activities of both varieties were similar. Kerry *et al.* (1993) consider that *P. chlamydosporia* is not only the most common *Pochonia* species, but also the most complex with variations in morphology and physiology between both varieties and even among isolates of the same variety.

Table 1. Isolates of *P. chlamydosporia* extracted from soils within the Biosphere Reserve of "Los Tuxtlas", Mexico.

Land use	Locality	Sampling point	*Pochonia chlamydosporia* varieties	Geographical position		Altitude (m.s.l.)	SH (%)	pH	OM (%)
				N	W				
NF	L1	LM22	*P. c. catenulata*	18° 25' 73"	94° 57' 10"	255	53.33	5.17	12.48
		LM07	*P. c. catenulata & chlamydosporia*	18° 25' 84"	94° 57' 08"	264	55.47	5.29	8.26
		LM09	*P. c. chlamydosporia*	18" 26' 13"	94" 57' 25"	246	45.94	5.83	na
		LM29	*P. c. chlamydosporia*	18" 26' 04"	94" 57' 35"	324	45.55	5.47	na
	L2	SF10	*P. c. catenulata & chlamydosporia*	na	na	1000	47.45	5.15	na
		SF07	*P. c. chlamydosporia*	na	na	1035	60.31	4.86	6.69
		SF09	*P. c. chlamydosporia*	na	na	980	51.24	5.18	8.71
		SF05	*P. c. chlamydosporia*	na	na	1035	45.26	5.86	13.07
		SF01	*P. c. chlamydosporia*	18° 18' 57"	94° 53' 45"	1060	49.93	4.27	7.53
	L3	VC25	*P. c. catenulata*	18° 20' 48"	94° 45' 55"	220	45.38	4.58	8.21
SF	L1	LM05	*P. c. catenulata & chlamydosporia*	18° 25' 60"	94° 57' 99"	316	32.10	4.98	5.45
		LM17	*P. c. catenulata & chlamydosporia*	18° 26' 24"	94° 57' 93"	230	44.45	5.29	10.67
		LM16*	*P. c. chlamydosporia*	18° 26' 10"	94° 57' 83"	238	37.59	5.13	na
		LM18	*P. c. chlamydosporia*	18° 25' 25"	94° 57' 06"	278	49.14	5.01	na
SF	L1	LM15	*P. c. chlamydosporia*	18° 26' 13"	94° 57' 78"	248	49.18	5.24	7.67
	L2	SF15	*P. c. catenulata*	18° 17' 45"	94° 53' 36"	870	67.08	4.71	9.78
		SF16	*P. c. chlamydosporia*	18° 17' 39"	94° 53' 38"	830	59.70	4.65	na
		SF11*	*P. c. catenulata & chlamydosporia*	18° 16' 83"	94° 53' 30"	800	66.98	5.17	7.86

NF = Natural forest; SF = Secondary forest; PF = Pasture field; MF = Maize field.
L1-L3 = Localities 1-3; SH = soil humidity; OM = organic matter.
na = not available
*sampling point where *Meloidogyne* was present.

Table 1. (Continued) Isolates of *P. chlamydosporia* extracted from soils within the Biosphere Reserve of "Los Tuxtlas", Mexico.

Land use	Locality	Sampling point	*Pochonia chlamydosporia* varieties	Geographical position		Altitude (m.s.l.)	SH (%)	pH	OM (%)
				N	W				
SF	L2	SF19*	*P. c. chlamydosporia*	na	na	1069	58.79	6.16	12.14
		SF10	*P. c. catenulata*	na	na	na	na	na	na
PF	L1	LM25	*P. c. chlamydosporia*	18° 26' 06"	94° 57' 45"	320	51.60	5.29	7.65
		LM06	*P. c. chlamydosporia*	18° 25' 73"	94° 57' 04"	302	47.91	5.26	8.10
		LM10	*P. c. chlamydosporia*	18° 26' 23"	94° 57' 55"	209	49.77	5.16	8.02
		LM11	*P. c. chlamydosporia*	18° 26' 21"	94° 57' 50"	206	47.38	5.23	na
		LM13	*P. c. catenulata*	18° 26' 23"	94° 57' 69"	191	44.17	5.28	6.38
	L2	SF15*	*P. c. chlamydosporia*	na	na	na	na	na	na
MF	L1	LM36	*P. c. chlamydosporia*	18° 25' 96"	94° 58' 00"	263	36.83	5.58	na
		LM34	*P. c. chlamydosporia*	18° 26' 05"	94° 57' 99"	262	34.25	5.17	na
		LM37	*P. c. chlamydosporia*	18° 25' 92"	94° 58' 02"	314	46.58	5.40	4.97
	L2	SF5M	*P. c. chlamydosporia*	na	na	990	67.26	5.60	5.88

NF = Natural forest; SF = Secondary forest; PF = Pasture field; MF = Maize field.
L1-L3 = Localities 1-3; SH = soil humidity; OM = organic matter.
na = not available
*sampling point where *Meloidogyne* was present.

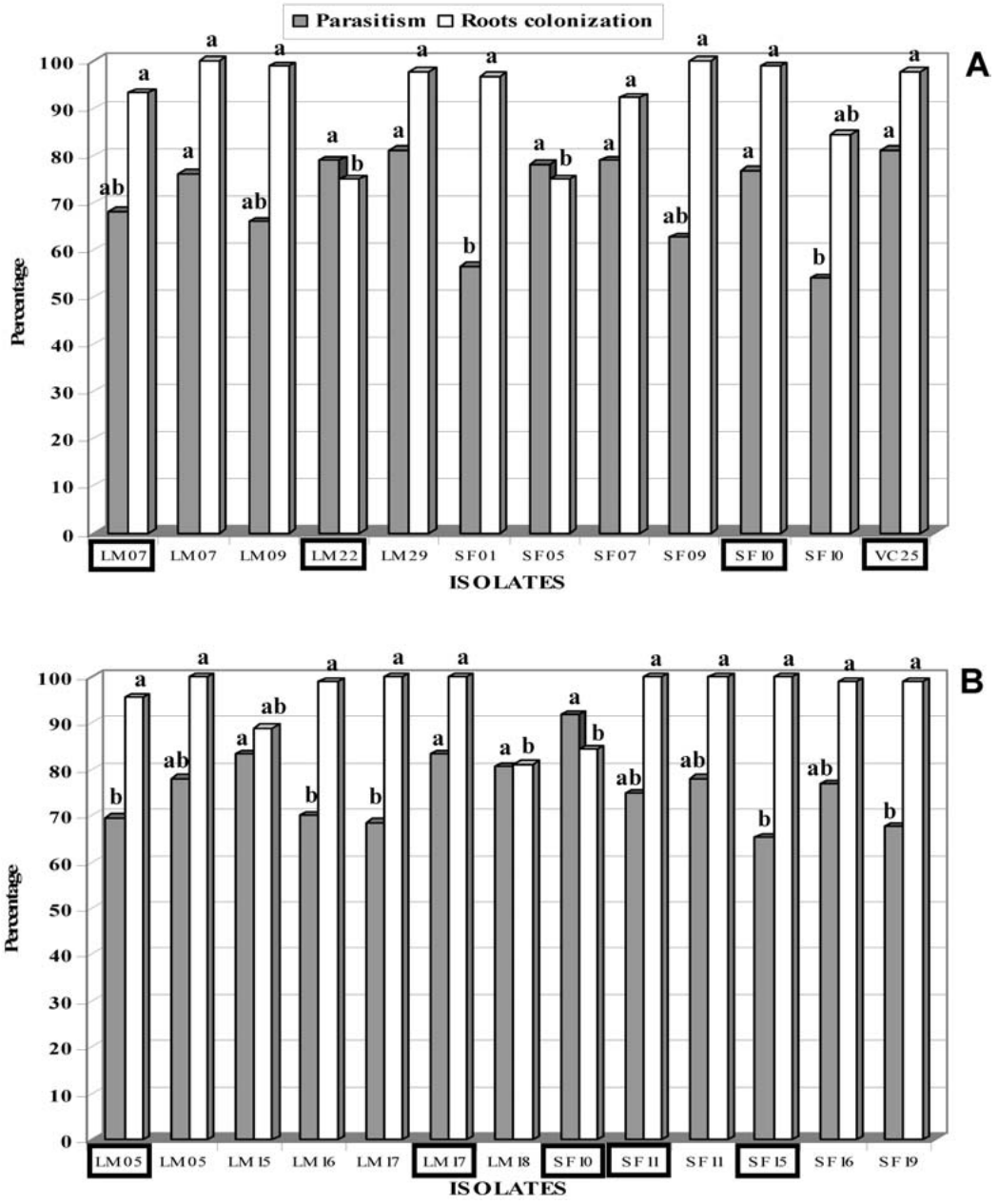

Fig. 1. Parasitism of *N. aberrans* eggs and root colonization by isolates of *P. chlamydosporia* from undisturbed sites: natural forest (A) and secondary forest (B). Keys framed show *P. c.* var. *catenulata* isolates. Isolates with the same key correspond to the same sampling point. Numbers with similar letters are not different significantly (Tukey $\alpha < 0.01$).

Our survey isolated both *P. c.* var. *chlamydosporia* and *P. c.* var. *catenulata* and provided the first evidence of *P. c.* var. *catenulata* in Mexican soils. The variety

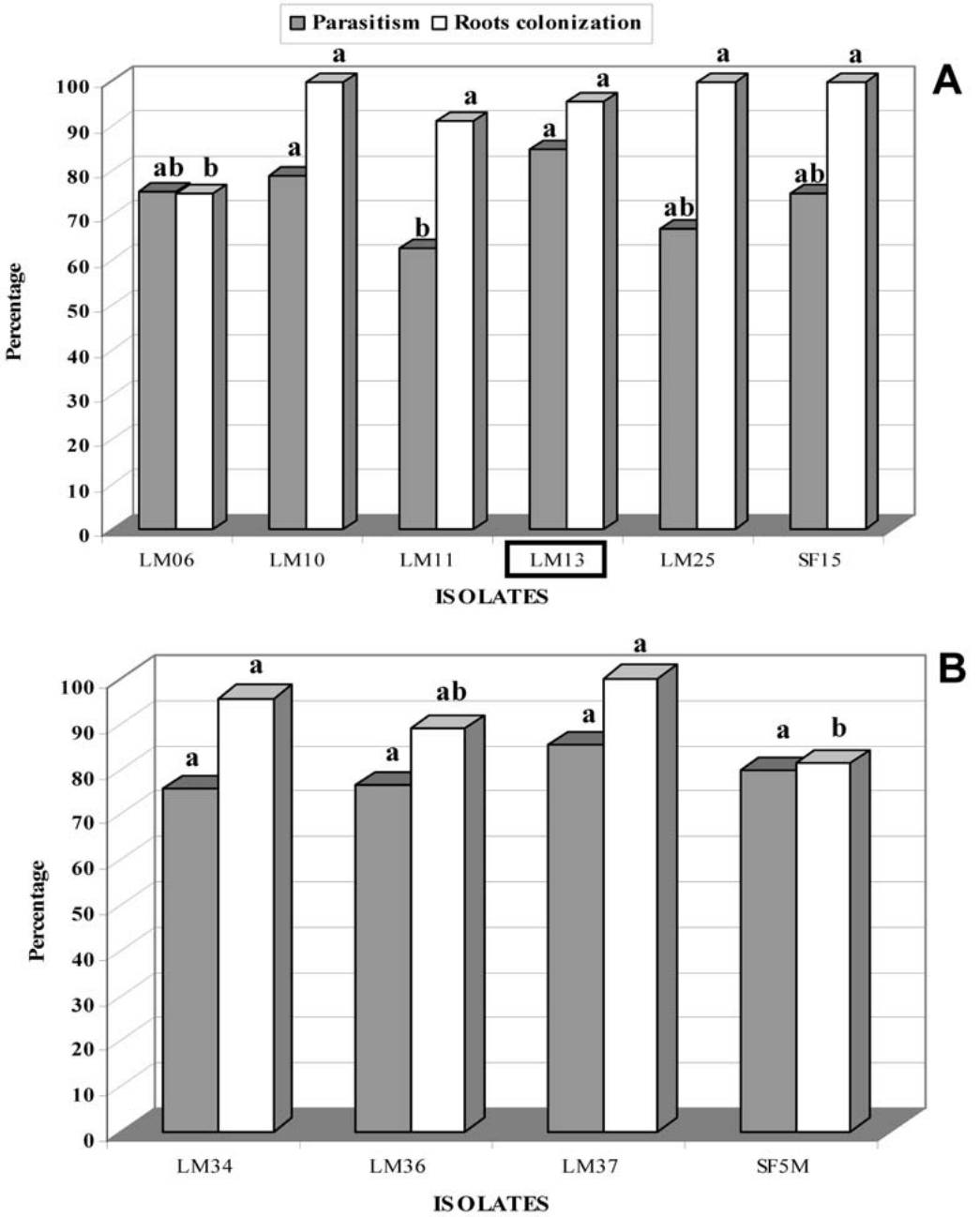

Fig. 2. Parasitism of *N. aberrans* eggs and root colonization by isolates of *P. chlamydosporia* from disturbed sites: pasture (A) and maize fields (B). Keys framed show *P. c.* var. *catenulata* isolates. Numbers with similar letters are not different significantly (Tukey $\alpha < 0.01$).

catenulata was recovered more frequently from undisturbed sites, than from sites used for pasture or maize cultivation; however, other site characteristics such as altitude, soil pH and organic matter appeared not to influence the frequency of either fungus.

Isolates of *P. chlamydosporia* from different geographical areas can vary in their pathogenicity to nematodes eggs, their ability to spread on roots, and in their chlamydospore production in laboratory media (Bourne *et al.*, 1994; de Leij and Kerry, 1991; Gaspard *et al.*, 1990; Hidalgo-Diaz *et al.*, 2000; Kerry, 1981; Kerry *et al.*, 1984; Kerry *et al.*, 1986). Bioassays with our isolates showed egg parasitism ranging from 54-92%, similar to results obtained by Hidalgo-Diaz *et al.* (2000). Furthermore, our data showed that thirty two isolates colonized more than 80% of roots and two of the most efficient egg parasites colonized the roots completely including isolate LM17 of *P. c.* var. *catenulata* from secondary forest and isolate LM37 of *P. c.* var. *chlamydosporia* from maize fields. Because of their ability to colonize roots, another six isolates could also be considered as potential biological control agents of *N. aberrans* including SF10, LM15, LM18, VC25, LM29 and LM13. De Leij and Kerry (1991) demonstrated that it is necessary to carefully select isolates in the development of biological control agents and Bourne *et al.* (1994) found that isolates that colonize greater than 80% of the root segments in a standard bioassay were not effective colonizers of the rhizosphere when tested in non-sterile field soils. According to this criterion, almost 50% of our isolates could be discarded as potential biocontrol agents.

These Mexican isolates will be studied further in order to corroborate these findings and characterize them deeper by molecular methods, and secondly to select the best ones for application in integrated management strategies against *Nacobbus aberrans*.

ACKNOWLEDGEMENTS

The research was funded by the European Union, project "Microbial Pest Control for Sustainable Peri-urban/Urban Agriculture in Latin America (Cuba and Mexico)—MiCoSPA—" (ICA 4-CT-2002-10044) and Global Environment Facility (GEF), project "Conservation and Sustainable Management of Below-Ground Biodiversity (CSM-BGBD)". Our appreciation for manuscript review is given to Dr. Brian Kerry, Nematodes Interaction Unit, Rothamsted Research, UK and Dr. Aurelio Ciancio, Istituto per la Protezione delle Piante, CNR, Bari, Italy. We also are indebted to Drs. Isabelle Barois, Dr. José Antonio García-Pérez and Biol Martín Santos for their support during field work.

LITERATURE CITED

Atkins, S. D., L. Hidalgo-Diaz, H. Kalisz, T. H. Mauchline, P. R. Hirsch, and B. R. Kerry. 2003a. Approaches for monitoring the release of *Pochonia chlamydosporia* var. *catenulata*, a biocontrol agent of root-knot nematodes. Mycological Research 107:206-212.

Atkins, S. D., L. Hidalgo-Diaz, H. Kalisz, T. H. Mauchline, P. R. Hirsch, and B. R. Kerry. 2003b. Development for a new management strategy for the control of root-knot nematodes (*Meloidogyne* spp.) in organic vegetable production. Pest Management Science 59:183-189.

Bourne J.M., B. R. Kerry, and F. A. A. M. de Leij. 1994. Methods for the study of *Verticillium chlamydosporium* in the rhizosphere. Supplement of Journal of Nematology 26:587-591.

Cristóbal-Alejo, J., I. Cid del Prado-Vera, P. Sánchez-García, N. Marbán-Mendoza, R. H. Manzanilla-López, and G. Mora-Aguilera. 2001. Nutritional disorders in tomato (*Lycopersicon esculentum* Mill.) due to infestation by *Nacobbus aberrans*. Nematropica 31:221-228.

de Leij, F. A. A. M., and B. R. Kerry. 1991. The nematophagus fungus *Verticillium chlamydosporium* Goddard, as a potencial biological control agent for *Meloidogyne arenaria* (Neal) Chitwood. Revue de Nématologie 14:157-164.

de Leij, F. A. A. M, B. R. Kerry, and J. A. Dennehy. 1992. The effect of fungal application rate and nematode density on the effectiveness of *Verticil-*

lium chlamydosporium as a biological control agent for *Meloidogyne incognita*. Nematologica 38:112-122.

de Leij F. A. A. M., B. R. Kerry, and J. A. Dennehy. 1993. *Verticillium chlamydosporium* as a biological control agent for *Meloidogyne incognita* and *M. hapla* in pot and micro-plot test. Nematologica 39:115-126.

Dirzo, R., and M. García. 1992. Rates of deforestation in Los Tuxtlas, a Neotropical area in southeast México. Conservation Biology 6:84-90.

Flores, C. R. 2003 Búsqueda de aislamientos de algunos hongos nematófagos para el control de *Nacobbus aberrans* (Thorne, 1935) Thorne y Allen, 1944. Tesis de Maestría. Colegio de Postgraduados, Montecillo, Estado de México. 99 p.

Gams, W., and R. Zare. 2001. A revision of *Verticillium* sect. Prostrata. III. Generic classification. Nova Hedwigia 72:329-337.

Gaspard, J. T., B. A. Jaffee, and H. Ferris. 1990. Association of *Verticillium chlamydosporium* and *Paecilomyces lilacinus* with root-knot nematode infested soil. Journal of Nematology 22:207-213.

Hidalgo-Díaz, L., J. M. Bourne, B. R. Kerry, and M. G. Rodriguez. 2000. Nematophagus *Vertillium* spp. in soil infested with *Meloidogyne* spp. in Cuba: isolation and screening. International Journal of Pest Management 46:277-284.

Kerry, B. R. 1981. Fungal parasites: a weapon against cyst nematodes. Plant Disease 65: 390-393.

Kerry, B. R. 1997. A workshop manual for research on *Verticillium chlamydosporium* as a biological control agent for root-knot nematodes. Approach to Crop Research. Rothamsted Long Ashton Broom's Barn UK. 90 pp.

Kerry, B. R., and L. Hidalgo-Díaz. 2004. Application of *Pochonia chlamydosporia* in the integrated control of root-knot nematodes on organically grown vegetable crops in Cuba. Multitrophic Interactions in Soil and Integrated Control. ROBC-WPRS Bulletin 27:123-126.

Kerry, B. R., F. Irving, and J. C. Hornsey. 1986. Variation between strains of the nematophagous fungus *Verticillium chlamydosporium* Goddard. I Factors affecting growth *in vitro*. Nematologica 32:461-473.

Kerry, B. R., I. A. Kirkwood, F. A. A. M. de Leij, J. Barba, M. B. Leijdens, and P. C. Brookes. 1993. Growth and survival of *Verticillium chlamydosporium* Goddard: a parasite of nematodes in soil. Biocontrol Science and Technology 3:355-365.

Kerry, B. R., A Simon, and A. D. Rovira. 1984. Observations on the introduction of *Verticillium chlamydosporium* and other parasitic fungi into soil for control of the cereal cyst-nematode *H. avenae*. Annals of Applied Biology 105:509-516.

Lappe, P., and M. Ulloa. 1982. Hongos destructores de nematodos en algunos suelos de México. Boletín de la Sociedad Mexicana de Micología 17:99-113.

Manzanilla-López, R. H., M. A. Costilla, M. Doucet, J. Franco, R. N. Inserra, P. S. Lehman, I. Cid del Prado-Vera, R. M. Souza, and K. Evans. 2002. The genus *Nacobbus* Thorne & Allen 1944 (Nematoda: Pratylenchidae): Systematics, Distribution, Biology and Management. Nematropica 32(2):149–227.

Mendoza-de Gives, P., E. Zavaleta-Mejía, D. Herrera-Rodríguez, and H. Quiroz-Romero. 1994. *In vitro* trapping capability of *Arthrobotrys* spp. on infective larvae of *Haemonchus contortus* and *Nacobbus aberrans*. Journal of Helminthology 68:223-229.

Morgan-Jones, G., G. Godoy, and R. Rodríguez-Kábana. 1981. *Verticillium chlamydosporium* fungal parasite of *Meloidogyne arenaria* females. Nematropica 11:115-119.

Zare R., W. Gams, and H. C. Evans. 2001. A revision of *Verticillium* section *Prostata*. V. The genus *Pochonia*, with notes on *Rotiferophthora*. Nova Hedwigia 73:51-86.

Received: 15/V/2008

Recibido:

Accepted for publication: 2/II/2009

Aceptado para publicación