DISTRIBUTION OF STAGES AND *IN VITRO* LARVAL HATCHING IN *GLOBODERA ROSTOCHIENSIS* CYSTS

D. Desgarennes[1]*, G. Carrión[1], A. E. Núñez-Sánchez[2] and M. C. Núñez-Camargo[1]

[1]Instituto de Ecología, A. C., Departamento de Biodiversidad y Sistemática, km 2.5 Antigua Carretera a Coatepec No. 351, Congregación El Haya, Xalapa, Veracruz, México. C.P. 91070, [2]Universidad Veracruzana, Facultad de Ciencias Agrícolas, Zona Xalapa, Circuito Gonzalo Aguirre Beltrán s/n, Zona Universitaria, Xalapa, Veracruz, México. C.P. 91090. *Corresponding author: damaris@ecologia.edu.mx

ABSTRACT

Desgarennes, D., G. Carrión, A. E. Núñez-Sánchez, and M. C. Núñez-Camargo. 2006. Distribution of stages and *in vitro* larval hatching in *Globodera rostochiensis* cysts. Nematropica 36:251-260.

The classification of cysts as young (YC), mature (MC), and old (OC) on the basis of cyst wall color was shown to be a practical tool for the diagnosis of population status. Also important are differences in the stages that occur within cysts (eggs, J1, and J2), as they too are linked to differences in color. These data, from an ecological point of view, help us understand population structure. The density recorded for Los Pescados, Perote in the state of Veracruz, Mexico in one kilogram of soil was 2974 YC, 3004 MC, and 221 OC. On average, young cysts have 55-63% viable eggs, 37% of which contain a vitellus, without a developing embryo; MC have 38-54% viability, 39% of which are J1, with J2 also observed; in OC, 98% are empty eggs and have 2% viability with J1 and J2 juveniles. The greatest degree of hatching was observed in MC (19%), with juveniles hatching only four hours after being exposed to root exudates.

Key words: density, hatching, population structure, viability.

RESUMEN

Desgarennes, D., G. Carrión, A. E. Núñez-Sánchez, y M. C. Núñez-Camargo. 2006. Distribución de estadios y eclosión *in vitro* en quistes de *Globodera rostochiensis*. Nematropica 36:251-260.

La clasificación de los quistes en jóvenes (QJ), maduros (QM) y viejos (QV) con base en la coloración de la pared del quiste resulta una herramienta práctica en el diagnóstico del estado de la población. También son importantes las diferencias en los estadios contenidos en los quistes (huevos, J1 y J2) por estar ligadas a diferencias en la coloración. Esta información, desde el punto de vista ecológico, nos ayuda a entender la estructura de la población. La densidad registrada en la localidad de Los Pescados, Perote, Veracruz, México, en un kilogramo de suelo, fue de 2974 QJ, 3004 QM y 221 QV. Los QJ tienen en promedio un 55-63% de huevos viables, de estos el 37% contiene vitelo y se inicia la formación de juveniles J1; los QM tienen un 38-54% de viabilidad, del cual el 39% se encuentra en J1 y se registran juveniles J2; en QV el 98% son huevos vacíos y tienen un 2% de viabilidad, presentan juveniles J1 y J2. La mayor eclosión se registró en QM (19%) y los juveniles pueden eclosionar a las cuatro horas de exposición a los exudados radicales.

Palabras clave: densidad, eclosión, estructura de la población, viabilidad.

INTRODUCTION

The second juvenile stage (J2) of the golden potato nematode, *Globodera rostochiensis* (Mulvey and Stone, 1976) (Nematoda: Heteroderidae), is considered to be the infective stage (Turner and Evans, 1998). The root damage that is caused by the infection of this nematode reduces crop yield (Evans and Rowe, 1998). Inside plant roots, juveniles pass through two more juvenile stages (J3 and J4), during

which the sex organs are defined and become mature (Perry, 2002), becoming fifth-stage adult males and females. The latter emerge through the root surface as their bodies swell to a spherical shape, but they remain attached to the root and are fertilized by the males, which are vermiform, motile, and leave the roots in search of females. Eggs begin to form inside the females, which die and become cysts that then separate from the root and remain in the soil. Thus, the female body serves as a protective cover for the eggs, which can vary in number from 200 to 500 per female (Evans *et al.*, 1993; Franco, 1994; Perry, 1998). Within each egg, the first stage juvenile (J1) develops and then molts to stage J2 (Stone, 1979). Juveniles hatch when they are stimulated by potato root exudates, which include a series of inorganic ions such as Ca^{2+}, Mg^{2+}, Na^{+}, and K^{+}, along with organic substances such as fumaric and citric acids (Clarke and Shepherd, 1966; Clarke and Hennesy, 1983, 1984). The exudates trigger a series of events believed to change the permeability of the eggshell to trehalose, allowing trehalose to diffuse out and thus reducing its concentration in the vitelline fluid and osmotic stress on the juvenile. This allows the juvenile to take in sufficient water for normal metabolism to begin (Clarke and Perry, 1977). The activated juveniles carry out exploratory movements within the egg, mainly in the area around the head. The juvenile exerts pressure on the egg wall with its head and uses its stylet to pierce the eggshell repeatedly until a slit has opened, allowing the juvenile to hatch from the egg into the cyst interior (Clarke and Perry, 1977). The hatched juveniles continue to absorb water until they are completely hydrated. At this point, they emerge through the natural openings in the cyst (neck and vulva) or stay inside until emerging conditions are optimal (Sharma and Sharma, 1998).

The number of *G. rostochiensis* cysts in the soil has been used to evaluate population density of the nematode, because the cysts are easily extracted and handled. However, little has been written about differences in cyst wall color as a consequence of maturation and transition of generations in the nematode population. Núñez-Sánchez *et al.* (2003) addressed the practical need to know more about nematode population dynamics by classifying cysts into three categories: young, mature, and old. Today, methods exist for crushing cysts to count eggs, but they do not provide important information on the different stages contained in the cysts or on egg viability, data that would broaden knowledge of the population dynamics of this nematode.

A study was conducted to learn more about the population dynamics within cysts. For each cyst category, we recorded the number of individuals and the stages they contained (eggs, J1, and J2), as well as their viability (empty eggs, those with vitellus and developing embryo, J1, and J2). We hypothesize that there is a correlation between a change in cyst wall color and the stages that it contains. Furthermore, young and mature cysts have a larger number of juveniles capable of hatching than old cysts. Evidence is herein presented that with age, these organisms have decreased viability.

MATERIALS AND METHODS

Sampling

Soil samples containing *G. rostochiensis* were collected from potato fields in Los Pescados, Municipality of Perote in Veracruz, Mexico. Samples were collected during two crop periods: summer (29 September 2004, representing long days) and winter (28 January 2005, representing

short days). Summer samples were collected after tubers were harvested, while winter samples were collected before sowing. Ten 100 g soil samples were taken during each period; this was done following a zig-zag or W pattern. The potato cultivars Rosita and San José, both of which have a 120-day cropping cycle, were grown at the collection site. For the quantification of eggs and J1/J2 juveniles, only the summer samples were used; while for hatching analysis, both sets of samples (summer and winter) were considered. Samples were kept in the dark at a temperature of 18-20°C and 50% relative humidity to allow the cysts to continue maturing during storage.

Cyst Extraction and Quantification

To extract cysts from the ten soil samples, the Fenwick can method of extraction by flotation (Fenwick, 1940) was used. We took 100 g soil samples and recovered the material floating from the can in sieves of 20, 60, and 120 meshes/cm^2 (with 850, 250, and 125 µm openings, respectively). The material obtained from the second and third sieves was poured into a glass beaker that had a strip of moist filter paper around its interior wall. A drop of anionic surfactant (sodium dodecyl sulfate) was added to the center of the beaker to reduce surface tension, causing the cysts to move outward to the filter paper. Finally, the filter paper strips with cysts were placed in a Petri dish and, using a stereoscopic microscope, cysts were counted and classified into the following categories: young cysts (YC), of light brown to golden color, shiny and fresh, with eggs easily observed through the cyst wall; mature cysts (MC), with brown walls that are duller and egg clusters that are less easily observed; and old cysts (OC), that have an opaque, dark brown cyst wall and an unobservable egg mass within (Núñez-Sánchez *et al.*, 2003). Data are presented as average ± SD.

Quantification of Eggs and J1/J2 Juveniles

From the counted and classified cysts, ten were randomly selected on each extraction date from each category (YC, MC, and OC). Each cyst was opened and its contents placed on a slide with distilled water for examination under a compound microscope. Eggs were counted, as were first stage juveniles (J1—those found within the egg and in the process of stylet formation) and second stage juveniles (J2—those found outside the egg but within cysts, with a fully developed stylet). The latter were larger with a more turgid look and a well-defined cuticle. Ten samples were processed over a period of 11 weeks; while the first took two weeks, the processing of subsequent samples took three days/week. Quantification of individual data did not follow a normal distribution pattern; therefore, means were analyzed using the non-parametric Kruskal-Wallis test.

Tuber Rooting

Root exudates were collected through *in vitro* rooting of potato tubers from cultivars Rosita and San José. The tuber size chosen (6-8 cm in diameter) was convenient for handling and observation under a stereoscopic microscope. The tubers were placed on filter papers in Petri dishes within plastic incubation chambers, which were maintained at 21 ± 2°C. They were observed every other day, at which time the filter paper was changed and the formation of roots checked. Later, the rooted tubers were placed in beakers with sterile distilled water and the roots were maintained in contact with the water for 48 hours. The water with the root exudates was used to stimulate hatching of juveniles in the various treatments.

Hatching and Quantification of Globodera rostochiensis Juveniles

Cysts were extracted from the soil samples, separated by category (YC, MC, and OC), and placed on small plastic trays (Multidishes Nunclon Delta by NUNC™) for cell culture with six 35 mm diameter wells, one cyst per well. There were two root exudate treatments and a control, with three repetitions per treatment in the three categories (N = 27). In one of the exudate treatments, 1 ml of unmodified root exudate solution (EX) was added to cysts while in the other, we added 1 ml of root exudate solution supplemented with Ca^{2+} and Mg^{2+} (EXCaMg). In the control, cysts were placed in contact with 1 ml of sterile distilled water (SDW). Observation and evaluation of hatching and the presence of other stages within cysts was made at ten different times (4, 8, 12, 24, 36, 48, 60, 72, 84, and 96 hours) for all age categories (N = 270). The above treatments were applied to the cysts obtained from the soil samples collected in both summer and winter (N = 540) in order to observe differences in hatching and emergence for cysts obtained from soil exposed to both long and short days (LD and SD), as recorded by Franco and Evans (1979). The quantification of hatched juveniles was carried out by carefully opening cysts with ultra-fine needles in order to prevent egg damage. All individuals contained in the cysts were counted by category; to record their viability, they were divided into "viable" (J1 or J2 juveniles and eggs with vitellus—those that contained only vitelline fluid but without embryonic development) and "non-viable" (empty eggs—those lacking vitelline fluid but that had a complete eggshell).

All data were evaluated using analysis of variance (ANOVA) 2 × 3 × 3 factorial design for two day lengths (LD and SD), three cyst types (YC, MC, and OC), and three treatments (EX, EXCaMg, and SDW). Afterward, a Tukey test was carried out to compare the means of each variable studied. To obtain viability data, the Tukey test for comparison of means was applied only to those individuals considered viable (StatSoft, Inc., Tulsa, OK).

RESULTS

Cyst Density

The number of cysts extracted from the ten samples in Los Pescados varied from 441 to 1038 cysts/100 g of soil. Overall cyst density in the soil averaged 620 cysts/100 g of soil. Of the total cysts separated by age category, we recorded 297.4 ± 105.69 young cysts/100 g of soil (48%), 300.4 ± 85.33 mature cysts/100 g (48.4%), and 22.1 ± 6.47 old cysts/100 g (3.6%). Because the samples were processed over a long period of time, both internal and external changes were observed in the golden nematode cysts. These changes included modifications in cyst wall color and the formation of J1 and J2 juveniles within cysts. In samples 1 to 4 (1st to 7th week of observation after soil collection), the average number of YC (347) was greater than that of MC (254). Evaluation of the fifth sample (8th week of sample processing) yielded more MC (301) than YC (264). Thus, as expected, there was a significant negative correlation ($r = -0.84$; $n = 10$; $P < 0.05$) between numbers of YC and MC.

Individuals Found in Cysts

The average number of individuals found in cysts was different for each of the three categories. Some MC had up to 690 individuals inside, while some YC only had 150. On average, MC contained more individuals (353); the Kruskal-Wallis test ($P < 0.05$) showed a significant difference between MC compared to YC (271) and OC (253).

Stages within Cysts

For all three cyst categories, the average percentage of eggs with no discernible juveniles from samples 1 to 4 (7th week of observation after soil collection) was higher (YC = 77%, MC = 82%, and OC = 97%) than those with J1 (YC = 23%, MC = 18%, and OC = 3%) and J2 (0% in all three). However, for samples 5 to 10 (8th-11th week of sample processing), the percentage of eggs without discernible juveniles decreased in young and mature cysts (YC = 41%, MC = 71%, OC = 99%), but the percentage with J1 increased (YC = 59%, MC = 28%, OC = 1%), and J2 were detected only in MC (2%). Here too, a significant negative correlation was observed ($r = -0.75$; $n = 10$; $P < 0.05$): in MC the proportion of undifferentiated eggs decreased as the proportion of J1 increased.

Hatching of Globodera rostochiensis

The percentage of hatched J2 in young cysts for the two periods evaluated (long days, LD, and short days, SD) was greater for the root exudates plus Ca^{2+} and Mg^{2+} (EXCaMg), with an average of 17% of hatched juveniles for both periods. In the root exudate only (EX) treatment, 16% of juveniles hatched from both short and long day cysts. In the control treatment (SDW), less than 5% of juveniles hatched from both periods (Table 1). For mature cysts, the percentage of hatched J2 was also higher with the EXCaMg treatment: 19% was recorded for SD and 12% for LD. With the EX treatment, 42 juveniles/cyst (17%) hatched from SD and 36 juveniles/cyst (11%) from LD cysts; in the control (SDW), less than 5% hatched from both periods. The percentage of hatched juveniles in old cysts for both periods (long and short days) was 1% (Table 1). The largest number of hatched J2 was recorded in mature cysts for both periods (Fig. 1). The hatching ranges observed for the three categories were YC = 20-40 juveniles/cyst, MC = 25-50 juveniles/cyst and OC = 1-10 juveniles/cyst. The number of emerged

Table 1. Average number of eggs and hatched J1 and J2 per cyst category from short and long day cysts.

	YC				MC				OC			
Treatments	0	E	J1	J2	0	E	J1	J2	0	E	J1	J2
						Short days						
EX	55	15	66	29 bc	99	8	97	42 b	204	0	4	1 d
EXCaMg	66	13	56	31 bc	103	5	86	46 a	226	0	3	1 d
SDW	58	19	99	5 d	127	9	87	5 a	202	0	3	0 d
						Long days						
EX	68	15	58	27 c	188	8	85	36 bc	190	0	4	1 d
EXCaMg	66	16	58	33 bc	178	7	82	36 bc	203	0	5	2 d
SDW	87	14	49	5 d	195	10	90	6 d	190	0	3	0 d

YC = young cysts, MC = mature cysts, and OC = old cysts. 0 = empty eggs, E = eggs with vitellus, J1 = first juvenile stage, J2 = second juvenile stage; a, b, c, and d = ranges per Tukey. Numbers marked with different letters are different ($P < 0.05$). No letters in a column indicate no differences at $P < 0.05$.

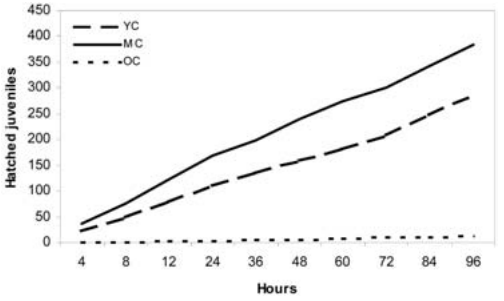

Fig. 1. Average number of hatched juveniles in YC, MC, and OC during a 96-hr period.

J2 was zero, as the observation period only lasted five days (96 hrs). In all cysts, hatch recording began four hours after exposure to root exudates and Ca^{2+} and Mg^{2} ions. There were no significant differences in the number of empty eggs (non-viable individuals), or eggs with vitellus and J1 (viable individuals), but there were significant differences in hatched J2. Analysis of J2 individuals showed that significant differences depended on certain factors: long days, observation of hatching made after four hours, mature cysts, and the treatment involving root exudates supplemented with Ca^{2+} and Mg^{2+} ions. The comparison of means indicated that more ($P < 0.05$) J2 hatched from MC exposed to the EXCaMg treatment than in any other treatment (Table 1).

Viability of Individuals Contained in Cysts

The number of viable individuals within cysts is not high as, on average, the number of eggs constitutes 76% of total individuals in a cyst, while J1 and J2 represent only 23% and 1%, respectively. This becomes more marked as cysts age. The percentage of viable individuals within YC collected in short day conditions was 63%, while in long day conditions it was 55% (Table 2). For MC, it was 54% on short days and 38% on long days. The percentage of viable individuals in OC was low for both types of day (2 and 3%, respectively).

The largest numbers of eggs with vitellus were found in young cysts, the largest numbers of J1s and J2s in mature cysts, and the largest number of empty eggs (i.e., non-viable individuals) in old cysts (Table 2). Based on these data, information was added to the classification established by Núñez-Sánchez *et al.* (2003). Young cysts contained an average of 270 individuals, of

Table 2. Viability of individuals in three categories of cyst.

Daylength at time of collection	Cyst age category	Number of individuals	
		Viable	Non-viable
Short Day	YC	103 ab	60
	MC	127 a	110
	OC	4 c	210
Long Day	YC	89 b	74
	MC	115 a	187
	OC	5 c	194

For each daylength type, means in columns followed by the same letter do not differ ($P < 0.05$) according to Tukey's test.

which 37% were eggs with vitellus; J1 were present (34%) and there were few empty eggs (29%). They had a hatching range of 20-40 juveniles/cyst. Mature cysts contained an average of 350 individuals, most of which were J1 (39%); J2 were also present (8%), there were a few eggs with vitellus (7%), more empty eggs (46%), and a range of 25-50 hatched juveniles/cyst. On average, old cysts contained 250 eggs, most of which were empty (98%); there were no eggs with vitellus, and hatching ranged from 1-10 juveniles/cyst.

DISCUSSION

The classification of cysts in various categories (YC, MC, and OC) based on the correlation of cyst wall color to cyst contents can be used to assess population dynamics and aid in management. Cyst color is a practical indicator of cyst category because as color changes, individuals within the cyst also change, from eggs to J1 and J2.

The dense population of *Globodera rostochiensis* in the Cofre de Perote region in Veracruz, Mexico is the result of constant potato cropping coupled with massive applications of nematicides, principally aldicarb and carbofuran (carbamates). Over the years, this may have caused a reduction in the nematode's natural enemies, favored soil microflora able to break down nematicides quickly, and selected for resistance to these chemical compounds (Tiyagi *et al.*, 2004; Choo *et al.*, 1998). Furthermore, carbamates, particularly carbofuran, are highly toxic to humans (Rendón *et al.*, 2004; Satar *et al.*, 2005). Schomaker and Been (1999) showed that when *G. rostochiensis* cysts are subjected to high doses of chemical compounds (1, 3-dicloropropene), they can recover mobility and normal hatching patterns 45-200 days after application. The high cyst density obtained at the study site (6200 cysts/kg of soil), where 22 cysts/kg of soil were recorded in 1991 and 1656 cysts/kg of soil in 2000, was well over the economic threshold (approximately 40 cysts/kg of soil) established by the European and Mediterranean Plant Protection Organization (Álvarez-Ríos, 1993; EPPO, 1997; Núñez-Sánchez *et al.*, 2003).

Although dense populations of *Globodera rostochiensis* are the result of these factors, as well as environmental conditions that are more favorable than those to which the species originally adapted (in the high Andes mountains of South America), it is also important to consider that increases in population density relate to two survival strategies developed by the nematode: 1) having numerous offspring and 2) forming cysts. The first provides enough individuals to re-populate if the host is present. With regard to the individual contents of a cyst, Evans *et al.* (1993) and Franco (1994) point out that it can vary from 200-500 individuals/cyst. In our study, cysts contained between 150-690 individuals. The second survival strategy of *G. rostochiensis*, cyst formation, is also observed in other nematodes (*Afenestrata*, *Cactodera*, *Dolichodera*, *Heterodera*, and *Punctodera*), both in natural habitats and where they have been introduced for the purpose of protecting offspring from environmental stress and natural enemies (Koenning and Sipes, 1998; McSorley, 2003). *Globodera rostochiensis* has adapted to respond to host stimuli as juveniles take only a brief period of time to move from the cyst to potato roots, where they feed and pass through juvenile stages J3 and J4 to reach the adult stage, when they mate. In Mexico, inadequate agricultural management and nematode survival strategies together with favorable environment conditions have contributed to high golden cyst nematode density. From an ecological point of view, cyst analysis can therefore be useful for

understanding *G. rostochiensis* population dynamics.

Turner (1996) states that, in the absence of a host, cysts can remain in soil for 20 to 30 years. However, we believe that after a lengthy period of time in soil, cysts are not completely viable, and that hatching and emergence diminish as cysts continue to mature, age, and degrade. In our results, neither the percentage of viable individuals nor those hatched per cyst is high, yet the large number of cysts present in soil permits enough juveniles to emerge to cause damage to potato crops. The "viability" recorded for YC (55-63%) occurs because this category has the highest number of eggs with vitellus. The number of individuals with hatching potential is 34%, as they are in the J1 stage. Viability decreases in MC (38-54%) because the number of eggs with vitellus is lower, although individuals with hatching potential account for 47% (J1 = 39% and J2 = 8%). In OC, viability is extremely low (1-2%), because only individuals that were late in hatching remain. This coincides with one of the cyst categories established by Brodie and Brucato (1993), which had 2 to 9 viable eggs/cyst. Robinson *et al.* (1985) found that juveniles contained in OC lose much of their lipid reserves, and when hatching stimulation occurs the lipid reserves are insufficient to reach the host's roots. We corroborated that viability is higher in YC and MC than in OC, which coincides with the hypothesis of Núñez-Sánchez *et al.* (2003). Perhaps due to the amount of time and conditions in which samples were stored in our laboratory, in this study day length at time of cyst collection was not observed to affect cyst viability, contrasting with the findings of Franco and Evans (1979).

The viability of YC and MC was not reflected in hatching percentage, which was under 20%. This occurs because although a certain proportion of individuals are considered viable, they may not have completed all developmental phases; however, it is interesting to note that if a J2 is mature, the juvenile will hatch after four hours of exposure to root exudates. The low hatching percentage of *G. rostochiensis* is attributable both to the high cyst density in the soil—as roots can support a large number of juveniles—and to their diminished viability due to age. Changes in viability occur because organisms degrade under the influence of natural enemies and various environmental factors, such as temperature, light, and moisture.

Factors that decrease viability and diminish cyst population density are rotation with non-host crops, efficient use of nematicides, and biological control. Crozzoli (1990) reported a 32% reduction in the final density of the golden nematode with low doses of aldicarb. Heavy nematicide application has negative consequences on nematode density because nematicides have only temporary effects on the emergence of juveniles from cysts (Whitehead and Turner, 1998). Other approaches include biological control with nematophagous fungi (*Acremonium incrustatum*, *Paecilomyces amoneoroseus*, and *P. carneus*), as described by Núñez-Camargo *et al.* (2003) and Núñez-Camargo (2005).

We believe that the high young cyst density obtained in this study is due to massive emergence of juveniles during the second half of the crop season, when the farmer suspends nematicide application and juveniles have the opportunity to complete their life cycle. On the other hand, the high young cyst density corroborates the hypothesis of Núñez-Sánchez *et al.* (2003) that YC result from golden nematode reproduction in the most recent crop. Rotation and chemical control must continue to be used until advances can be made in areas such as biological control.

ACKNOWLEDGMENTS

We are grateful to M. Sc. Alejandro Esquivel, Escuela de Ciencias Agrarias, Universidad Nacional, Heredia, Costa Rica and Manuel Canto-Saenz, U.N.A. La Molina Centro Internacional de la Papa, Lima, Peru for critically reviewing the manuscript. The research reported herein was funded by Instituto de Ecología, A. C. (902-10/138) and Facultad de Ciencias Agrícolas, Universidad Veracruzana (6953).

LITERATURE CITED

Álvarez-Ríos, M. S. 1993. Problemática del nematodo dorado (*Globodera rostochiensis* Woll. 1923, Mulvey & Stone 1976) (Nematoda: Heteroderidae) en el cultivo de papa (*Solanum tuberosum* L.) (Solanaceae) en la región de Perote, Veracruz. Tesis de Licenciatura. Universidad Veracruzana, Veracruz, Mexico. 114 p.

Brodie, B. B., and M. L. Brucato. 1993. Effects of inoculum density and egg age on establishment of *Globodera rostochiensis* populations. Journal of Nematology 25:286-290.

Choo, H. Y., H. H. Kim, and H. K. Kaya. 1998. Effects of selected chemical pesticides on *Agamermis unka* (Nematoda: Mermithidae), a parasite of the brown plant hooper, *Nilaparvata lugens*. Biocontrol Science and Technology 8:413-427.

Clarke, A. J., and J. Hennessy. 1983. The role of calcium in the hatching of *Globodera rostochiensis*. Revue de Nématologie 6:247-255.

Clarke, A. J., and J. Hennessy. 1984. Movement of *Globodera rostochiensis* (Wollenweber) juveniles stimulated by potato-root exudates. Nematologica 30:206-212.

Clarke, A. J., and R. N. Perry. 1977. Hatching of cyst nematodes. Nematologica 23:350-368.

Clarke, A. J., and A. M. Shepherd. 1966. Inorganic ions and the hatching of *Heterodera* spp. Annals of Applied Biology 58:497-508.

Crozzoli, R. 1990. Utilización de aldicarb y carbofuran para el control del nematodo dorado de la papa (*Globodera rostochiensis*). Fitopatología Venezolana 3:9-10.

European and Mediterranean Plant Protection Organization (EPPO). 1997. Pp. 601-606 *in* Data sheets on quarantine organisms. *Globodera rostochiensis* and *Globodera pallida*. Quarantine Pest for Europe CAB.

Evans, K., and J. A. Rowe. 1998. Distribution and economic importance. Pp. 1-30 *in* S. B. Sharma (ed.). The cyst nematodes. Chapman & Hall, London, UK.

Evans, K., D. L. Trudgill, and J. M. Webster. 1993. Plant Parasitic Nematodes in Temperate Agriculture. CAB International, Wallingford, UK. 648 p.

Fenwick, D. W. 1940. Methods for the recovery and counting of cysts of *Heterodera schachtii* from soil. Journal of Helminthology 18:155-172.

Franco, J. 1994. Problemas de Nematodos en la Producción de Papa en Climas Templados en la Región Andina. Nematropica 24:179-195.

Franco, J., and K. Evans. 1979. Effect of day length on the multiplication of potato cyst nematode (*Globodera* spp.) populations. Nematologica 25:237-244.

Koenning, S. R., and B. S. Sipes. 1998. Biology. Pp. 156-190 *in* S. B. Sharma (ed.). The cyst nematodes. Chapman & Hall, London, UK.

McSorley, R. 2003. Adaptations of nematodes to environmental extremes. Florida Entomologist 86:138-142.

Núñez-Camargo, M. C. 2005. Aislamiento y evaluación de la patogenicidad *in vitro* de hongos nematófagos asociados a larvas (J2) de *Globodera rostochiensis* (Woll). Tesis de Maestría. Universidad de Colima, Tecomán, Colima, Mexico. 45 pp.

Núñez-Camargo, M. C., G. Carrión, and A. E. Núñez-Sánchez. 2003. Fungi associated with *Globodera rostochiensis* cysts in Mexico. International Journal of Nematology 13:151-161.

Núñez-Sánchez, A. E., G. Carrión, M. C. Núñez-Camargo, and O. Rebolledo-Domínguez. 2003. Densidad de población de quistes de *Globodera rostochiensis* Woll. en el Cofre de Perote, Veracruz, México. Revista Mexicana de Fitopatología 21:207-213.

Perry, R. N. 1998. The physiology and sensory perception of potato cyst nematodes, *Globodera* species. Pp. 27-49 *in* R. J. Marks and B. B. Brodie (eds.). Potato cyst nematodes: Biology, distribution and control. CAB International, Wallingford, UK.

Perry, R. N. 2002. Hatching. Pp. 147-169 *in* D. Lee (ed.). The biology of nematodes. CAB International, London, UK.

Rendón-von Osten, J., R. Tinoco-Ojanguren, A. M. V. M. Soares, and L. Guilhermino. 2004. Effect of pesticide exposure on acetylcholinesterase activity in subsistence farmers from Campeche, Mexico. Archives of Environmental Health 59:418-425.

Robinson, M. P., H. J. Atkinson, and R. N. Perry. 1985. The effects of delayed emergence on infectivity of juveniles of the potato cyst nematode. Nematologica 31:171-178.

Satar, S., S. Satar, A. Sebe, and H. Yesilagac. 2005. Carbofuran poisoning among farm workers. The Mount Sinai Journal of Medicine 27:389-392.

Sharma, S. B., and R. Sharma. 1998. Hatch and emergence. Pp. 191-216 *in* S. B. Sharma (ed.). The cyst nematodes. Chapman & Hall, London, UK.

Schomaker, C. H., and T. H. Been. 1999. Compound models describing the relationship between dosage of (Z)- or (E)-isomers of 1-3 dichloropropene and hatching behaviour of *Globodera rostochiensis*. Nematology 1:19-29.

Stone, A. R. 1979. Co-evolution of nematodes and plants. Symbolae Botanicae 22:46-61.

Tiyagi, S. A., S. Ajaz, and M. F. Azam. 2004. Effect of some pesticides on plant growth, root nodulation and chlorophyll content of chickpea. Archives of Agronomy and Soil Science 50:529-533.

Turner, S. J. 1996. Population decline of potato cyst nematodes (*Globodera rostochiensis*, *G. pallida*) in field soils in Northern Ireland. Annals of Applied Biology 129:315-322.

Turner, S. J., and K. Evans. 1998. The origins, global distribution and biology of potato cyst nematodes (*Globodera rostochiensis* Woll. and *Globodera pallida* Stone). Pp. 2-26 *in* R. J. Marks and B. B. Brodie (eds.). Potato cyst nematodes: Biology, distribution and control. CAB International, Wallingford, UK.

Whitehead, A. G., and S. J. Turner. 1998. Management and regulatory control strategies for potato cyst nematodes (*Globodera rostochiensis* and *Globodera pallida*). Pp. 135-152 *in* R. J. Marks and B. B. Brodie (eds.). Potato cyst nematodes: Biology, distribution and control. CAB International, Wallingford, UK.

Received: *17/I/2006*

Recibido:

Accepted for publication: *7/III/2006*

Aceptado para publicación: