Journal of Nematology 47(4):290–295. 2015.

Life Cycle of the Golden Cyst Nematode, *Globodera rostochiensis*, in Quebec, Canada

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Abstract: In 2006, the golden cyst nematode, *Globodera rostochiensis*, was discovered in the province of Quebec, Canada. We report here the life cycle of *G. rostochiensis* under the climatic conditions of southwestern Quebec. Only one full generation was completed per year under these latitudes. On susceptible potato cv. Snowden, *G. rostochiensis* needed a minimum of 579 growing degree units (GDU) (base 5.9°C) to complete its life cycle and the first mature cysts were observed 42 to 63 days after planting (DAP). In soil, second-stage juveniles (J2) were first observed 14 to 21 DAP, whereas both white females on roots and males in soil appeared synchronously after 35 to 42 days. The duration of the life cycle was affected by temperature but not by soil type. A second wave of hatching systematically occurred later in the season and a second generation of males was observed during the 2011 growth season. No complete second cycle was observed before plant senescence. Climate change and later maturing cultivars/crops could allow the development of a full second generation in the future.

Key words: potato cyst nematodes, *Globodera*, life cycle, Canada.

Potato is a major industry in Canada with over 150,000 ha planted in 2012, representing a value of more than $1 billion (Statistics Canada, 2013). Potato cyst nematodes (PCN), *Globodera rostochiensis* (Woll.) and *G. pallida* (Stone) are the most important nematode threats to potato production worldwide (Turner and Evans, 1998). These nematodes have a remarkable ability to survive many decades in the absence of a suitable host and are capable of causing major yield losses (Baldwin and Mundo-Ocampo, 1991; Brodie et al., 1998). Thus, they are the subject of strict quarantine regulations in many countries, including Canada and the United States. Until recently, PCN presence in Canada was confined on the island of Vancouver and the province of Newfoundland and Labrador (Olsen and Mulvey, 1962; Orchard, 1965; Stone et al., 1977). In 2006, *G. rostochiensis* was detected near the town of Saint-Amable in the province of Québec (Sun et al., 2007; Mahran et al., 2010). This finding led to the enforcement of quarantine status on 4,750 ha of land by the Canadian Food Inspection Agency (CFIA, 2006).

The life cycle of PCN has been extensively described over the past century from many regions on the globe (Philis, 1980; Greco et al., 1988; Renčo, 2007; Bačić et al., 2011; Ebrahimi et al., 2014). Under temperate conditions, a single generation normally occurs during a growing season. Management of PCN generally implies the combination of several integrated approaches. Chemical treatments exist, but this option is costly and not environmentally desirable (Evans et al., 2002). Crop rotations with nonhost plants can be effective in preventing PCN multiplication, but cannot be used alone to obtain a rapid reduction below damaging threshold or detection levels (Brodie, 1996). Indeed, PCN had developed a strategy that limits hatch in the absence of its host, allowing eggs to persist in the soil for decades in the absence of potato (Evans and Stone, 1977). Several alternative approaches have also been used, but require a close monitoring of PCN behavior in a given region of the world to be successful. For example, both early harvesting (Webley and Jones, 1981; Ebrahimi et al., 2014) and trap cropping (Scholte and Vos, 2000) use susceptible hosts that are harvested or destroyed before completion of the nematode life cycle; this allows hatching without the formation of new cysts. Thus, a good knowledge of the life cycle is required to prevent failure of this method and multiplication of PCN. Among all, the most effective control method against PCN is through the use of resistant cultivars. It allows the reduction of populations under detection levels as long as PCN pathotype in a given field is susceptible to the resistance gene used (Whitehead and Turner, 1998). In this study, we present observations on the life cycle of *G. rostochiensis* populations under field conditions in Québec, Canada.

Material and Methods

From 2009 to 2012, two naturally infested fields with *G. rostochiensis* were cultivated with susceptible potato cv. Snowden on two quarantine experimental sites, one on a sandy soil (89% sand, 6% silt, and 6% clay) (Saint-Amable; 45°39′44.81″N 73°16′24.52″O) and a second on a muck soil (75% organic content) (Saint-Dominique; 45°35′7.31″N 72°54′4.08″O). At each site, 16 potato tubers on three rows with 30-cm spacing between plants and rows were sown in eight 1- × 2-m plots bordered with wooden planks. Tubers were planted on 15 May 2009, 12 May 2010, 18 May 2011, and 23 May 2012 in Saint-Amable and 14 May 2009, 13 May 2010, 19 May 2011, and 25 May 2012 in Saint-Dominique. Plants were fertilized according to the recommendations of the "Centre de référence en agriculture et agroalimentaire du Québec" (Reference Guide fertilization 1st edition,

Received for publication December 18, 2014.
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We thank Yvon Fournier and Catalin Cebuc for their technical assistance and also thank George Laplante for his expertise and guidance throughout the golden nematode project.
This work was supported by the DIAP program of Agriculture and Agri-Food Canada, the RPS program of the Canadian Food Inspection Agency (CFIA), and Centre de recherche Les Buissons.
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This paper was edited by Inga Zasada.

2003). Before planting and each week following planting, soil samples were collected from 12 evenly distributed points within the plot to a depth of 20 cm using a hand trowel. After drying, 300 cm^3 of soil was measured for each composite sample and used for cyst extraction using the Fenwick can procedure (Fenwick, 1940). For each sample, cysts were recovered on a milk filter (KenAG BREAK PROOF D-547) and enumerated visually under stereoscopic microscope. Egg viability was assessed on recovered cysts (maximum 50 cysts) deposited in a 1.5-ml microtube containing 1 ml of tap water. Using a pellet pestle, the cysts were ruptured, and the entire content was deposited in a counting dish for observation under an inverted microscope. Egg viability was determined visually based on the internal morphology of both living and dead eggs and juveniles (EPPO, 2013).

Three weeks after planting and weekly thereafter, one potato plant per plot was removed using a shovel and a sample of soil clinging to roots was collected from the harvested plant. To determine the number of second-stage juveniles (J2) and males, a 100-ml subsample of soil per plot was extracted using the Baermann pan (Townshend, 1963). The potato root system was dipped in a pail filled with clean water to remove adhering soil and then stored in plastic bag containing a moist paper towel. The number of white females (immature) and brown cysts (mature) on the entire root system of 16 plants (8 plants from each site) was determined using a ×10-power lens.

In order to standardize the nematode counts between plots, locations, and years, all counts (J2, male, and white female) were expressed as a ratio of abundance by dividing each count by initial number of viable eggs in a given plot. Soil temperature at 15-cm depth and precipitation were monitored hourly on both locations using HOBO H8 Pro Series and HOBO Rain gauge RG3-M data loggers, respectively. Growing degree units accumulation after plantation were also calculated using the basal temperature of 5.9°C proposed by Mugniéry (1978) and are presented in Fig. 1.

Results

Soil temperature and precipitation: The average daily soil temperatures are shown in Fig. 2. Overall, Saint-Amable was 1.0°C ± 0.1°C warmer than Saint-Dominique. In Saint-Amable, the GDU at plant senescence, 134 DAP, ranged from 1,590 (2009) to 2,005 (2012) with an average of 1,837 GDU (Table 1). In Saint-Dominique, the GDU ranged from 1,539 (2009) to 1,845 (2012) with an average of 1,757 GDU. Across experimental locations, the 2012 growing season was warmer than the three other years with higher temperatures during the spring and early summer (Fig. 2). The 2011 growth season was characterized by higher than average midsummer temperatures. The 2009 growth season was the coolest with 1,590 and 1,539 GDU in Saint-Amable and Saint-Dominique, respectively, whereas the 2010 season had an average of 1,819 and 1,759 GDU in Saint-Amable and Saint-Dominique, respectively.

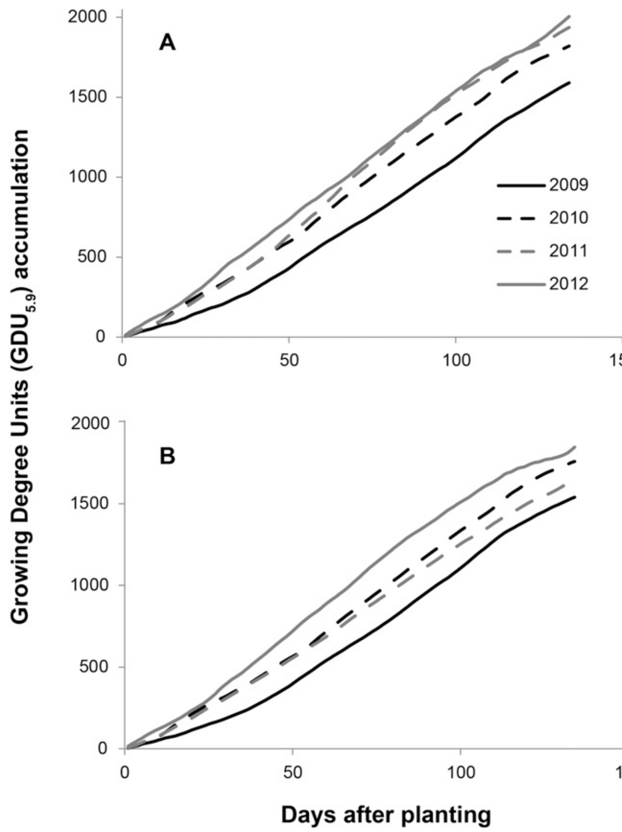

Fig. 1. Growing degree units (base 5.9°C) accumulated after planting of potato at A. Saint-Amable, Quebec, Canada. B. Saint-Dominique, Quebec, Canada.

Cumulative rainfall (in millimeters) at both locations for all the four years is also presented in Fig. 2. The 2011 growth season received the most precipitation with over 500 mm from planting to plant senescence mostly occurring late in the season. The 2012 growth season received the lowest amount of precipitation with approximately 350 mm at both sites.

Life cycle analysis: The initial population densities of *G. rostochiensis* ranged from 5 to 110 and 2 to 315 viable eggs/gram of soil at Saint-Amable and Saint-Dominique, respectively (Table 2). Potato shoots emerged a week after planting in each year.

The first second-stage juveniles were found in soil 14 to 28 DAP and required a minimum of 122 GDU to occur (Table 1). In the years when potatoes were planted early (2009 and 2010), the presence of J2 in soil was delayed (Fig. 3). A second peak of J2 was recorded annually on both sites, generally smaller than the first peak in a 1:2 proportion (Fig. 3). However, in 2011, the second peak of J2 in Saint-Dominique was found to be three times the size of the first one and also bimodal in shape (Fig. 3).

Males were detected in soil 35 to 42 DAP and required a minimum of 299 GDU (Table 1). Generally, a single peak of males was observed at both sites (Fig. 4). In

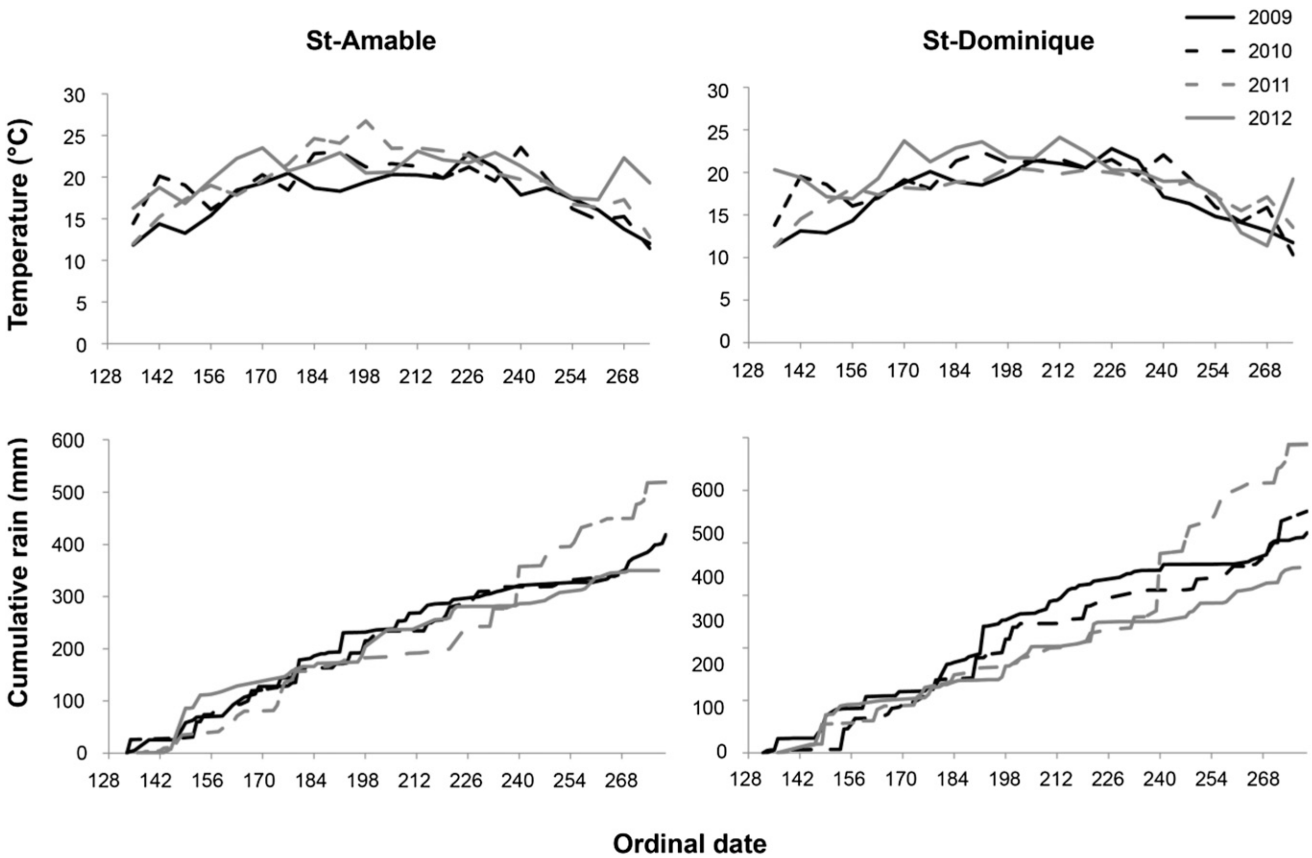

FIG. 2. Average soil temperature at 15-cm depth and cumulative rainfall from planting for both fields: Saint-Amable and Saint-Dominique, Quebec, Canada.

proportion, there were three to six times fewer males than J2 in soil. In 2011, the number of males in soil was particularly abundant in Saint-Dominique and a second peak was recorded but smaller in a 1:6 proportion (Fig. 4). A 55 days delay between the first observations of males and the second peak was measured that same year.

The first white females appeared 28 to 42 DAP and required a minimum of 299 GDU (Table 1). In 2009, the coolest season, white females were delayed by 1 and 2 weeks in Saint-Amable and Saint-Dominique, respectively, when compared to the three other years (Fig. 5). In 2012, the warmest season, the first white females were recorded 28 DAP and 357 GDU in Saint-Dominique (Table 1). Mature cysts were observed 42 to 63 DAP; a minimum of 579 GDU were required for complete development (Table 1).

DISCUSSION

Based on these observations, one full generation of *G. rostochiensis* per year was recorded at both locations in Quebec. These observations are in line with the general consensus that one generation occurs during

TABLE 1. Number of growing degree units using a base of 5.9°C ($GDU_{5.9}$) accumulated in soil (15 cm) after potato planting until the observation of different developmental stages of *Globodera rostochiensis* in St-Amable and Saint-Dominique, Quebec, Canada.

Developmental stage (soil type)	2009		2010		2011		2012	
	$GDU_{5.9}$	DAP	$GDU_{5.9}$	DAP	$GDU_{5.9}$	DAP	$GDU_{5.9}$	DAP
J2 (Saint-Amable)	190	28	237	21	213	21	267	21
J2 (Saint-Dominique)	122	21	224	21	122	14	161	14
Male (Saint-Amable)	329	42	394	35	391	35	501	35
Male (Saint-Dominique)	299	42	375	35	369	35	466	35
Female (Saint-Amable)	329	42	394	35	391	35	501	35
Female (Saint-Dominique)	299	42	375	35	369	35	357	28
Cyst (Saint-Amable)	616	63	585	49	618	49	718	49
Cyst (Saint-Dominique)	579	63	648	56	633	56	583	42

DAP = days after planting.

Table 2. Average number of initial viable eggs (range) in Saint-Amable and Saint-Dominique, Quebec, Canada.

	Sites	
Year	Saint-Amable	Saint-Dominique
2009	33 (16–50)	94 (45–180)
2010	53 (24–110)	154 (91–315)
2011	18 (5–30)	14 (2–39)
2012	34 (13–54)	102 (37–202)

a growing season under temperate climate (Morris, 1971; Evans and Stone, 1977; Greco et al., 1988; Bačić, 2011). The length of the life cycle differed between years and was influenced by heat accumulation. The minimum number of GDU required for completion of the life cycle was found to be 579. In comparison, Ebrahimi et al. (2014) reported a minimum of 401 GDU and 62 DAP for the same species but on a different cultivar in Belgium. This lower number of GDU could be explained by their use of sprouted seeds, which provided a head start to potato plants. Heat accumulation was faster under our conditions in Quebec and the life cycle was completed 42 DAP during the warmest year (2012). This finding is similar with the observations of Jones and Parrott (1969) who compared different years and planting dates and found that the development of *G. rostochiensis* is controlled more by the rate of heat accumulation than by temperatures except when extreme.

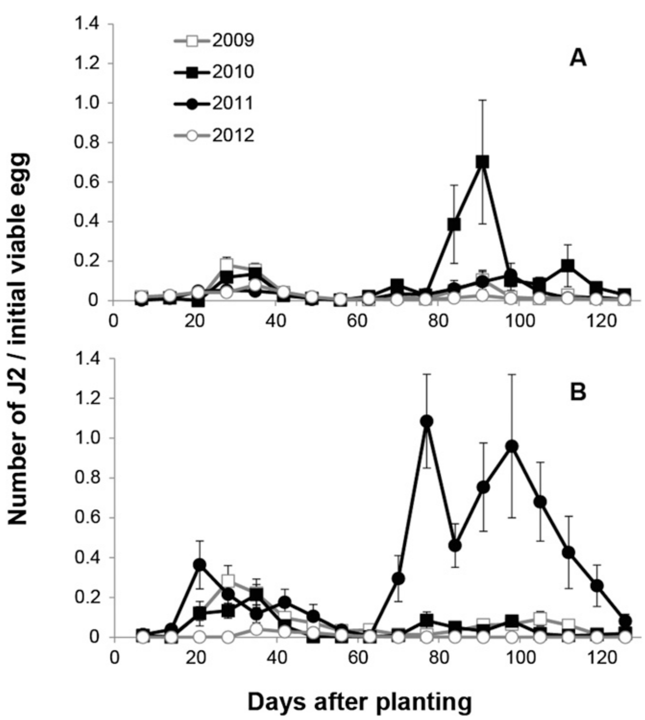

Fig. 3. Occurrence of *Globodera rostochiensis* second-stage juvenile (J2) at A. Saint-Amable, Quebec, Canada. B. Saint-Dominique, Quebec, Canada. Each point represents the average (n = 8) of the ratios of abundance of J2 divided by initial number of viable eggs per gram of soil.

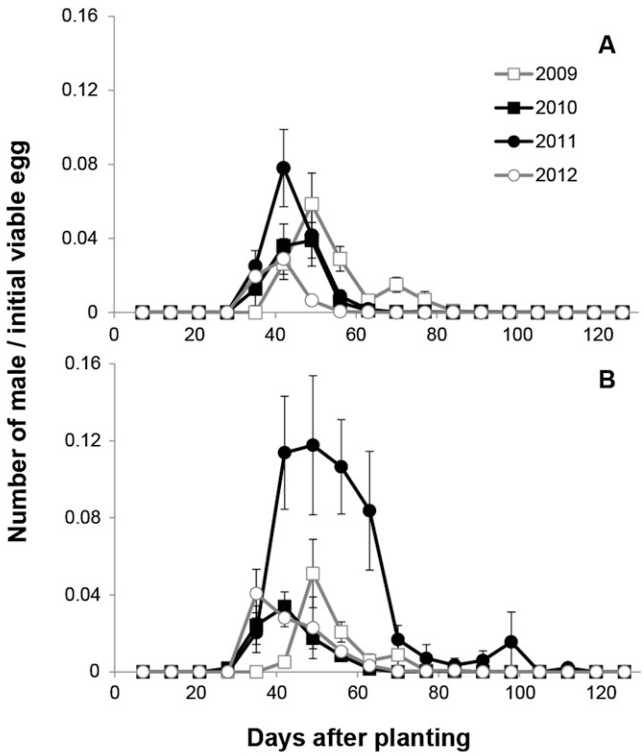

Fig. 4. Occurrence of *Globodera rostochiensis* males in soil at A. Saint-Amable, Quebec, Canada. B. Saint-Dominique, Quebec, Canada. Each point represents the average (n = 8) of the ratios of abundance of males divided by initial number of viable eggs per gram of soil.

We found that there was a minimum duration required for each developmental stage, regardless of the temperature. A minimum of 7 weeks was required before the observation of fully mature cysts. In the same manner, a minimum of 14 days was needed before any significant increase of J2 in soil after planting. In addition, a minimum of 14 days was required between the presence of J2 in soil and the appearance of the first white females and a minimum of 28 days was required between J2 in soil and the first mature cysts.

Soil temperature has been shown to have a significant impact on the hatching of PCN (Robinson et al., 1987; Kaczmarek et al., 2014). Optimum hatch of *G. rostochiensis* occurred between 15°C and 27°C but hatching was delayed at temperatures above 25°C (Kaczmarek et al., 2014). Based on the work of Kaczmarek et al. (2014), it is likely that increases in soil temperatures associated with climate change will increase hatching for *G. rostochiensis* in climates such as that found in Quebec.

In 2009, potatoes were planted early (15 May) because of a warm spring but temperature fell soon after and remained low for a few weeks. This delayed the first observation of J2 in soil to 28 DAP. In comparison, potatoes were planted at the same time in 2010 (12 May) and J2 were observed after only 21 days which is similar to years with later planting dates. This was also observed by Jones and Parrott (1969) who stated that the time required for egg hatch and invasion of the

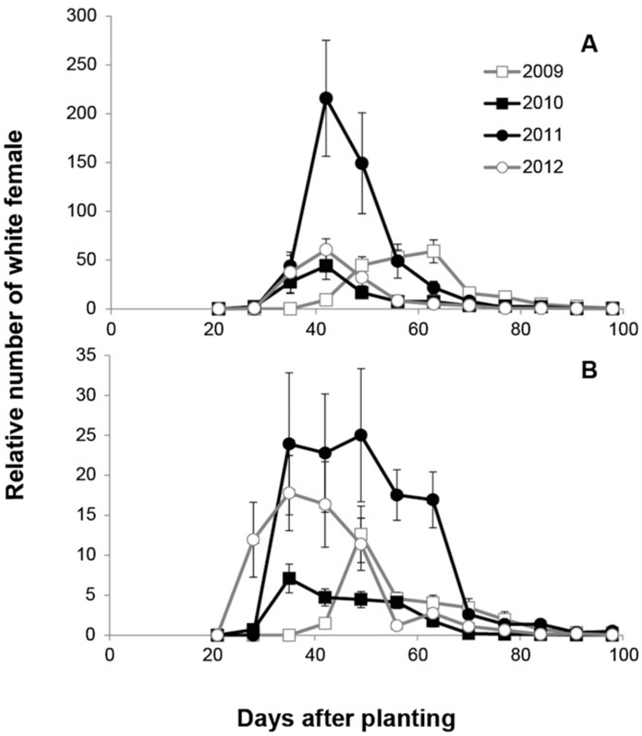

FIG. 5. Occurrence of *Globodera rostochiensis* white females on roots of potato at A. Saint-Amable, Quebec, Canada. B. Saint-Dominique, Quebec, Canada. Each point represents the average ($n = 8$) of the ratios of abundance of white female on the roots of one plant divided by initial number of viable eggs per gram of soil.

roots by J2 was longer when planting occurred early compared to late. At the opposite, the time lapse between the presence of J2 in soil and the apparition of the first females on roots was not influenced by temperature, probably because of more "temperate" conditions inside the roots. In this study, it appears that development of *G. rostochiensis* was not affected by soil type. Although the effect of soil type per se on the life cycle is not documented, no significant effect on the population decline of both *G. rostochiensis* and *G. pallida* in different soil types has been reported (Turner, 1996).

A second wave of J2 occurred late in the season at both locations during all years. This event has been previously described to occur elsewhere (Greco et al., 1988; Renco, 2007). In 2011, this event was particularly important in Saint-Dominique. It was interesting to notice that the plots selected that year harbored the lowest *G. rostochiensis* population densities and also provided a higher multiplication rate compared to other years, similarly to previous report (Jones and Parrott, 1969). When adding the J2 abundance ratio recorded that year, the total number of J2 recovered from the soil surpassed by one to three times the initial number of viable eggs recorded that spring. Thus, these observations would support the hypothesis that hatching of newly laid eggs could occur before undergoing dormancy. The results obtained by Jiménez-Pérez et al. (2009) also suggest that diapause is not obligatory for *G. rostochiensis* and that at least a small proportion of the eggs from the first generation can hatch directly. Blok et al. (2011) also observed hatching of newly formed eggs along with an incomplete second generation. In our experiments, the second appearance of J2 was not followed by a second wave of cysts due to the absence of small feeding roots on the senescing potato plants. However, a second occurrence of males in soil was recorded in 2011 at Saint-Dominique 21 days after the second wave of J2 was observed. This indicates that a second generation of *G. rostochiensis* could be possible if later maturing cultivars were used. It would be interesting to observe the development of *G. rostochiensis* on other host crop such as tomato which is able to grow later in the season depending on the frost-free period. In Italy, when growing the late-maturing potato cv. Majectic, a second generation of *G. rostochiensis* has been reported (Greco et al., 1988).

Our results provide insight into the importance on a good knowledge of the life of *G. rostochiensis* when cultural practice such as trap cropping is used for the management. The trap cropping technique relies on triggering PCN egg hatch with root exudates and by destroying the plants before any nematode reproduction occurs. When relying on a resistant potato cultivar, it was demonstrated that 2 or 3 years of trapping was needed to decrease populations below detectable levels in Quebec (unpubl. data). Unfortunately, the availability of resistant potato seeds remains very limited in Canada. Thus, the proper timing for destroying a susceptible potato cultivar when making use of a trap cropping will be much more critical to avoid increasing nematode populations. From our results, first white females were recorded between 28 and 42 DAP depending mainly on the number of GDU accumulated after planting. Ideally, the trapping crop should be destroyed at the appearance of the white females for maximum efficacy.

Literature Cited

Bačić, J., Barsi, L., and Štrbac, P. 2011. Life cycle of the potato golden cyst nematode (*Globodera rostochiensis*) grown under climatic conditions in Belgrade. Archives of Biological Sciences 63:1069–1075.

Baldwin, G. J., and Mundo-Ocampo, M. 1991. Heteroderinae, cyst- and non-cyst-forming nematodes. Pp. 275–362 *in* R. W. Nikle, ed. Manual of agricultural nematology. New York: Marcel Dekker.

Brodie, B. B. 1996. Effect of initial nematode density on managing *Globodera rostochiensis* with resistant cultivars and nonhosts. Journal of Nematology 28:510–519.

Brodie, B. B., Evans, K., and Franco, J. 1998. Nematode parasites of potato. Pp. 87–132 *in* K. Evans, D. L. Trudgill, and J. M. Webster, eds. Plant parasitic nematodes in temperate agriculture. Wallingford: CAB International.

Blok, V., Kaczmarek, A., and Palomares-Rius, J. E. 2011. Influence of temperature on the life-cycle of *Globodera* spp. Communications in Agricultural and Applied Biological Sciences 76:307–310.

Canadian Food Inspection Agency (CFIA). 2006. Golden nematode infested places order. (November 13, 2015). http://www.inspection.gc.ca/plants/plant-protection/nematodes-other/golden-nematode/order/eng/1337006958263/1337007108429.

Ebrahimi, N., Viaene, N., Demeulemeester, K., and Moens, M. 2014. Observations on the life cycle of potato cyst nematodes, *Globodera rostochiensis* and *G. pallida*, on early potato cultivars. Nematology 16:937–952.

EPPO. 2013. PM 7/40 (3) *Globodera rostochiensis* and *Globodera pallida*. Bulletin OEPP/EPPO Bulletin 43:119–138.

Evans, K., and Stone, A. R. 1977. A review of the distribution and biology of the potato cyst nematodes *Globodera rostochiensis* and *G. pallida*. International Journal of Pest Management 23:178–189.

Evans, K., Webster, R. M., Halford, P. D., Barker, A. D., and Russell, M. D. 2002. Site-specific management of nematodes—pitfalls and practicalities. Journal of Nematology 34:194–199.

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

Greco, N., Inserra, R. N., Brandonisio, A., Tirrò, A., and De Marinis, G. 1988. Life-cycle of *Globodera rostochiensis* on potato in Italy. Nematologia Mediterranea 16:69–73.

Huijsman, C. A. 1955. Breeding for resistance to the potato root eelworm. Euphytica 4:133–140.

Jiménez-Pérez, N., Crozzoli, R., and Greco, N. 2009. Life-cycle and emergence of second stage juveniles from cysts of *Globodera rostochiensis* in Venezuela. Nematologia Mediterranea 37:155–160.

Jones, J., and Parrott, D. M. 1969. Population fluctuations of *Heterodera rostochiensis* Woll. when susceptible potato varieties are grown continuously. Annals of Applied Biology 63:175–181.

Kaczmarek, A., MacKenzie, K., Kettle, H., and Blok, V. C. 2014. Influence of soil temperature on *Globodera rostochiensis* and *Globodera pallida*. Phytopathologia Mediterranea 53:396–405.

Mahran, A., Turner, S., Martin, T., Yu, Q., Miller, S., and Sun, F. 2010. The golden potato cyst nematode *Globodera rostochiensis* pathotype Ro1 in the St-Amable regulated area in Quebec, Canada. Plant Disease 94:1510.

Morris, R. 1971. Distribution and biology of the golden nematode *Heterodera rostochiensis* in Newfoundland. Nematologica 26:370–376.

Mugniéry, D. 1978. Vitesse de développement en fonction de la température de *Globodera rostochiensis* et *G. pallida* (Nematoda: Heteroderidae). Revue de Nématologie 1:3–12.

Olsen, O. A., and Mulvey, R. H. 1962. The discovery of golden nematode in Newfoundland. Canadian Plant Disease Survey 42:253.

Orchard, W. R. 1965. Occurrence of the golden nematode on Vancouver Island, British Columbia. Canadian Plant Disease Survey 45:89.

Philis, J. 1980. Life history of the potato cyst nematode *Globodera rostochiensis* in Cyprus. Nematologica 26:295–301.

Renčo, M. 2007. Comparison of the life cycle of potato cyst nematode (*Globodera rostochiensis*) pathotype Ro1 on selected potato cultivars. Biologia 62:195–200.

Robinson, M. P., Atkinson, H. J., and Perry, R. N. 1987. The influence of temperature on the hatching, activity and lipid utilization of second stage juveniles of the potato cyst nematodes *Globodera rostochiensis* and *G. pallida*. Revue de Nématologie 10:349–354.

Scholte, K., and Vos, J. 2000. Effects of potential trap crops and planting date on soil infestation with potato cyst nematodes and root-knot nematodes. Annals of Applied Biology 137:153–164.

Stone, A. R., Thompson, P. R., and Hopper, B. E. 1977. *Globodera pallida* present in Newfoundland. Plant Disease Reporter 61:590–591.

Statistics Canada. 2013. Canadian potato production. Catalogue no. 22-008-X Vol. 10, no. 2.

Sun, F., Miller, S., Wood, S., and Côté, M. J. 2007. Occurrence of potato cyst nematode, *Globodera rostochiensis*, on potato in the St-Amable region, Quebec, Canada. Plant Disease 91:908.

Townshend, J. L. 1963. A modification and evaluation of the apparatus for the Oostenbrink direct cotton-wool filter extraction method. Nematologica 9:106–110.

Turner, S. 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 Evans, K. 1998. The origins, global distribution and biology of potato cyst nematodes (*Globodera rostochiensis* (Woll.) and *Globodera pallida* (Stone). Pp. 7–26 *in* R. Marks and B. Brodie, eds. Potato Cyst Nematodes. Biology, Distribution and Control. Cambridge, UK: University Press.

Webley, D. P., and Jones, F. G. W. 1981. Observations on *Globodera pallida* and *G. rostochiensis* on early potatoes. Plant Pathology 30:217–224.

Whitehead, A. G., and Turner, S. J. 1998. Management and regulatory control strategies for potato cyst nematodes (*Globodera rostochiensis and Globodera pallida*). Pp. 135–152 *in* R. Marks and B. Brodie, eds. Potato Cyst Nematodes, Biology, Distribution and Control. Cambridge, UK: University Press.