

Storage of potato cyst nematodes at -80°C

Rolf T. FOLKERTSMA, Johannes HELDER, Fred J. GOMMERS and Jaap BAKKER

Department of Nematology, Wageningen Agricultural University, Binnenhaven 10,
6709 PD, Wageningen, The Netherlands.

Accepted for publication 2 July 1996.

Summary – Batches of cysts from a population of *Globodera rostochiensis* and a population of *G. pallida* were stored at -80°C and 4°C . Samples of 50 cysts were taken periodically and the hatchability of the eggs was tested at 20°C . Storage for 4 years at 4°C severely reduced the hatchability of both populations. In contrast, the number of juveniles hatching from cysts kept at -80°C did not change over a four-year period.

Résumé – *Conservation des nématodes à kyste de la pomme de terre à -80°C* – Des lots de kystes d'une population de *Globodera rostochiensis* et d'une population de *G. pallida* ont été conservés à -80°C . Des échantillons de 50 kystes ont été prélevés régulièrement et la capacité des œufs à éclore a été testée à 20°C . La conservation pendant 4 ans à 4°C a réduit considérablement la capacité des œufs à éclore dans les deux populations. Par contre, le nombre de juvéniles émergeant des kystes conservés à -80°C n'a pas changé au cours de la période de 4 années.

Key-words : cryopreservation, cyst nematodes, freeze tolerance, *Globodera pallida*, *G. rostochiensis*.

Two species of potato cyst nematodes, *Globodera rostochiensis* and *G. pallida*, have evolved mechanisms to withstand prolonged periods of environmental stress, such as freezing or drought, in the absence of host plants (Perry & Wharton, 1985). Protected by an egg-wall, unhatched second-stage juveniles (J2) are the most stress-tolerant life stage. For research purposes, it can be desirable to store potato cyst nematodes, usually as air-dried eggs encapsulated in cysts, at a moderately low temperature for a prolonged period. This temperature may range from 0°C (Kühn, 1975) to room temperature (Perry *et al.*, 1992). Inevitably, viability decreases during storage at these temperatures (Myagi, 1976) and populations have to be regenerated on susceptible *Solanum tuberosum* cultivars at regular time intervals. The costs and risks involved in regenerating populations could be reduced if loss of viability during storage could be minimized.

Triantaphyllou and McCabe (1989), and more recently Van der Beek *et al.* (1996), described methods for storage of eggs and J2 of several *Heterodera* and *Meloidogyne* species at -196°C in liquid nitrogen. Although these methods may be suitable for long term storage of *G. rostochiensis* and *G. pallida*, they are labor-intensive and risky. We developed a simple and adequate method to preserve populations of *G. rostochiensis* and *G. pallida*: dry storage of cysts at -80°C . Possible mechanisms that underlie the freeze tolerance of these nematode species are briefly discussed.

Materials and methods

In 1988, two populations of potato cyst nematodes, *G. rostochiensis* Ro1 Mierenbos R5-7 and *G. pallida* Pa2

HLP-1 P6-5, were reared on *Solanum tuberosum* ssp. *tuberosum* L. cv. Eigenheimer. Cysts were collected, air-dried and stored at 4°C . From 1991 onwards, two batches from each population were stored either at -80°C or 4°C in closed 5 ml containers. To allow free gas exchange, the lids of these containers were punctured.

Before starting the experiment, the number of J2 hatching from cysts of both species was determined in potato root diffusate (PRD). To prepare PRD, concentrated potato root diffusate (Janssen *et al.*, 1987) was diluted with tap-water (v/v 1:2) and stored at 4°C until use. Subsequently, the number of J2 hatching was determined after 1 day, 1 week, 6 months, 1 year, and 4 years of storage at both temperatures. Hatch of J2 was analyzed by taking two random samples of 50 cysts from each population. These were incubated for 1 week in tap-water at 20°C followed by incubation in PRD. The number of hatched J2 was determined 2 days, 4 days, 1 week, and 2 weeks after incubation in PRD. After the first week, the hatched J2 were removed and fresh PRD was added.

After 4 years of storage at one or the other temperatures, the hatchability of J2 was monitored in more detail. Again, two samples of 50 cysts were taken from each population. The number of J2 hatching from cysts was determined after 2 days, 4 days, 1, 2, 3, 4, 5, 7, 9, and 19 weeks of incubation in PRD. Hatched J2 were removed after each count and the PRD was replaced. After 19 weeks of incubation in PRD, the cysts were crushed and the number of non-hatched J2 was determined. The number of hatched J2 per treatment was expressed as a percentage of the total number of J2 present in 50 cysts.

Results

The number of J2 that hatched from each population within 2 weeks exposure to PRD is presented in Table 1. In contrast to the hatch from cysts of both species stored at 4 °C, there was no detrimental effect of short or long term storage at -80 °C on the viability of air-dried cysts of the populations tested. Differences in the number of hatched J2 between the replicates can be attributed to variation in the number of eggs within the cysts.

After 4 years of storage, the hatchability was studied in more detail over a period of 19 weeks. Fig. 1 presents the percentage of hatched J2 for the populations of *G. rostochiensis* and *G. pallida*. There is a clear difference in hatching pattern of *G. rostochiensis* and *G. pallida* J2, with *G. rostochiensis* J2 hatching gradually over time, whereas a large percentage (approximately 80 %) of the *G. pallida* J2 hatched during the first 4 weeks. The viability of cysts stored at 4 °C is severely reduced after 4 years of storage.

Table 1. Total number of hatched juveniles of *Globodera rostochiensis* and *G. pallida* after incubation of cysts in PRD during 14 days. The cysts were stored at -80 °C or 4 °C for 1 day, 7 days, 6 months and 4 years. (A and B are duplicates; n.d. = not determined).

	Storage at -80 °C				Storage at 4 °C			
	<i>G. pallida</i>		<i>G. rostochiensis</i>		<i>G. pallida</i>		<i>G. rostochiensis</i>	
	A	B	A	B	A	B	A	B
day 0	n.d.	n.d.	n.d.	n.d.	5910	8940	4770	3900
day 1	4461	7290	4080	5820	n.d.	n.d.	n.d.	n.d.
day 7	8580	3750	3414	3330	n.d.	n.d.	n.d.	n.d.
6 months	8250	6030	2640	2010	n.d.	n.d.	n.d.	n.d.
4 years	5007	4254	3841	3669	90	366	0	4

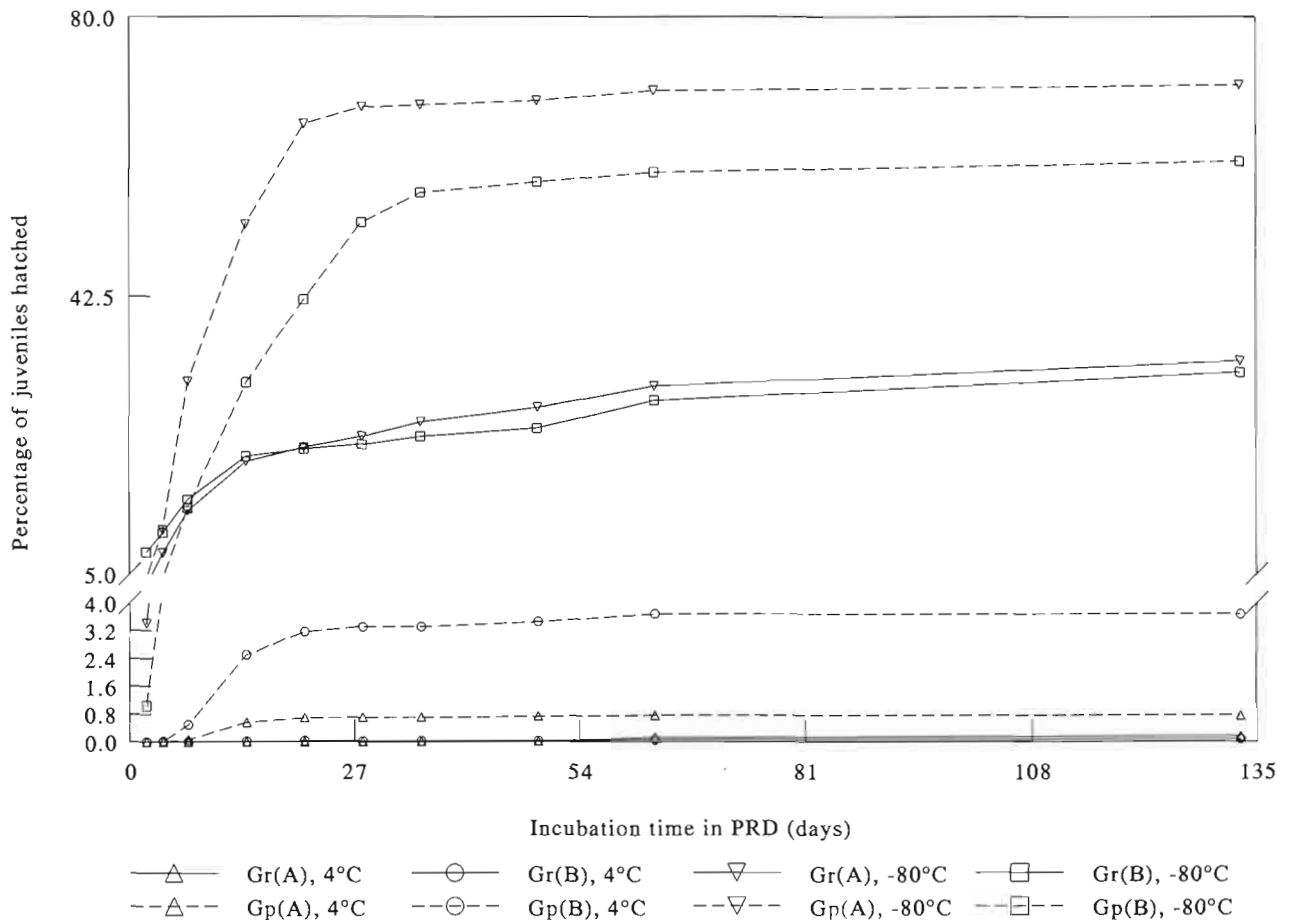


Fig. 1. Percentages of *Globodera rostochiensis* (*Gr*) and *G. pallida* (*Gp*) juveniles hatching from 50 cysts after 4 years of storage at either 4 °C or -80 °C. (A and B are duplicates).

Discussion

In terms of viability, storage of air-dried cysts from *Globodera rostochiensis* and *G. pallida* populations at -80°C was superior to storage at 4°C . Storage at -80°C reduces the efforts that are normally involved in the regeneration of populations and lowers the risk of losing populations.

Both potato cyst nematode species originate from high altitude regions in the Andes in South America (Evans *et al.*, 1975) and evolved mechanisms to withstand sub-zero temperatures (Perry & Wharton, 1985). In the absence of host plants, only unhatched J2 that are encapsulated in eggs, which are in turn contained in a cyst, will survive. The cyst provides, at most, a small contribution to the protection of the J2 against low temperatures (Perry & Wharton 1985). Within the egg, unhatched J2 are surrounded by a perivitelline fluid that contains a high trehalose concentration (Clarke & Hennessy 1976; Clarke *et al.*, 1978). Trehalose is a non-reducing disaccharide that consists of two glucose moieties. In a number of organisms, trehalose was shown to be an efficient cryoprotectant (*e.g.*, Crowe *et al.*, 1984; Crowe *et al.*, 1990). Therefore, this compound could contribute substantially to the ability of potato cyst nematodes to survive exposure to -80°C at certain life stages.

It should be noted that only a few invertebrates possess the ability to survive at -80°C without pretreatment with cryoprotectants. Recently, three Tardigrada species from Antarctica, *Echiniscus jenningsi*, *Macrobiotus furciger* and *Diphyscon chilense*, were shown to have high survival rates after exposure to very low temperatures for *ca* 600 days (Somme & Meier, 1995). Similar characteristics were described for a number of insect species including, *e.g.*, *Polypedilum vanderplanki* and *Pterostichus brevicornis* (Lee, 1991). To our knowledge, no vertebrates are known to survive prolonged exposure to -80°C .

The hatchability of cysts kept for 4 years at 4°C was very low in comparison to cysts kept at -80°C . An explanation could be that J2 kept at 4°C are metabolically still active, whereas the metabolism of J2 at -80°C is negligible. Four years of storage at 4°C would reduce the energy reserve of the J2, ultimately leading to their death. Alternatively, microbial contamination within the cyst samples might be responsible for the reduced survival of cysts stored at 4°C .

Using potato cyst nematode populations from the UK, faster hatching rates were observed for *G. rostochiensis* J2 as compared to *G. pallida* (McKenna & Winslow, 1972, Robinson *et al.*, 1987; Whitehead, 1992). On the other hand, Ellis and Hesling (1975) observed a more rapid emergence of *G. pallida* J2. The hatching patterns presented in Fig. 1 agree with the latter observation. It should be stressed that our observations are limited to two Dutch potato cyst nematode populations,

a hatching temperature of 20°C and a pretreatment of the J2 at -80°C .

The method we describe in this paper is remarkable, both in its simplicity and in its effectiveness. If our findings hold true for potato cyst nematodes populations in general, which is likely, this method could be very useful for all potato cyst nematode researchers.

Acknowledgements

The research is supported by a grant of the Dutch Multi-Year Crop Protection Plan and EC-grant AIR3 CT 92.0062.

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