Effect of storage temperature on survival and infectivity of *Steinernema rarum* (OLI strain) (Rhabditida: Steinernematidae)

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A B S T R A C T

Nematode strains of the entomopathogenic family Steinernematidae differ in their ability to infect insects at different temperatures. Survival and infectivity of infective juveniles (IJs) of *Steinernema rarum* (OLI) were studied after their storage at 23 ± 2 °C and at 5 ± 1 °C. Survival at 23 ± 2 °C was always above 95%. At 5 ± 1 °C, survival decreased at week 5, but infectivity did the same after week 2. Unlike other Steinernematids, both infectivity and survival of IJs would be higher for *S. rarum* (OLI) when stored at 23 ± 2 °C.

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Infected juveniles (IJs) of the family Steinernematidae are effective biological control agents of insect pests in the soil (Grewal et al., 2005). The effect of temperature on infectivity and survival were studied for numerous entomopathogenic nematodes and different isolates of the same species show differential responses to this factor. It's of special interest to characterize nematodes from different places of the world to be used for biocontrol of damaging species from the same geographical region. Just as nematodes are adapted to climatic conditions of each geographical area, so are their hosts (Mráček et al., 1999).

*Steinernema rarum* was isolated from diverse locations of Argentina and recently from the United States of America (Doucet, 1986; Cagnolo et al., 2004; Nguyen et al., 2006). The effect of temperature on infectivity and survival were studied for *S. rarum* Sargento Cabral strain (Koppenhöfer and Kaya, 1999). Other limited laboratory studies have been conducted with *S. rarum* type isolate (Doucet et al., 1992).

Considering intraspecific differences between isolates and that, to the present, from all the isolates of the same genera recorded in Argentina, *S. rarum* (OLI) is the most virulent one (Cagnolo et al., 2004), the aim of this study was to know the influence of storage temperature on IJs’ survival and infectivity.

*Steinernema rarum* (OLI) strain were collected from the locality of Oliva, Córdoba province (Argentina). This locality is characterized by a tempered climate with annual average temperature of 16.9 °C. IJs were cultured in wax moth larvae *Galleria mellonella* (200–300 g weighed). Infections were performed in 1.5 ml Epfen-dorf tubes. Each tube was filled with fine sterilized sand (1.2 g), 40 IJs/insect and 0.2 ml formalin (0.1%), and kept at 23 ± 2 °C. The IJs emerging from infected wax moth larvae were harvested from White traps. IJs collected after 96 h (first emergence from the cadaver) were diluted to a 1000 IJs/ml suspension (Koppenhöfer and Kaya, 1999). Nematodes were stored in 50 plastic boxes (20 ml) stuffed with 2 g of polyether polyurethane. The boxes were kept in two groups at 23 ± 2 °C and 5 ± 1 °C for 12 weeks. There were two replicate boxes for each temperature tested. Survival was determined at 1, 2, 3, 5, 7, 9, 11 and 12 weeks of storage by observing subsamples of approximately 500 IJs under dissecting microscope (2.0×). Immobile IJs were touched with a fine-wire probe and IJs that did not react were recorded as dead (Koppenhöfer and Kaya, 1999). Each subsample extracted was returned to the box, and the procedure was repeated five times to obtain an average of live IJs. The boxes were destructively sampled. Data on total numbers of live nematodes were expressed as the percentage of the nematodes originally placed in each box. Infectivity was measured at the start of the experiment and at 1, 2, 8, 10, 11 and 12 weeks of storage. This attribute was determined as described previously by exposing 12 individual wax moth larvae per box to 4 surviving IJs and determining the number of dead host after 4 days at 25 °C. Infected wax moth larvae were dissected to determine nematode establishment. As a control, 12 wax moth larvae had been exposed to a formalin solution (0.1%) without nematodes.
IJs survival is related to their metabolic rates and their initial reserve levels (Glazer, 2002). Nematode metabolism is reduced at low temperatures; hence, IJs do not use their lipid reserve (Georgis and Manweiler, 1994), which is rapidly used at higher temperatures due to their great mobility. However, for S. rarum (OLI), the proportion of mobile IJs started to noticeably decrease after 7 days at both storage temperatures, which allow them not to use their lipid reserve and maintain their infectivity.

Results of the present study agree with those obtained by Jung (1996) for different isolates of *Heterorhabditis* in which the percentage of living IJs decreased noticeably after 7 weeks of storage at 5 ± 2 °C.

It is probable that the climatic characteristic of the biotope where an isolate is found has an essential impact on their optimum temperature of infectivity (Mráček et al., 1997; Hazir et al., 2001). This work provides data which could optimize storage conditions under which *S. rarum* (OLI) is maintained in laboratory. Unlike other steinernematids, this isolate maintains its infectivity at 23 ± 2 °C. Taking also into account that the limited room temperature shelf life is one of the obstacles for using entomopathogenic nematodes (Georgis et al., 2006), further studies should be performed to know even better this promissory control agent for insect pests in Argentina.

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**References**


Fig. 1. Effect of storage temperature on survival (A) and infectivity, measured as the number of infective juveniles (IJs)/host (B) of *Steinernema rarum* (OLI), at 23 ± 2 °C (●) and at 5 ± 1 °C (□).

Experiments were conducted twice. In all experiments, results in the two trials were similar and combined for analysis. Relationship between storage time and temperature on survival and infectivity was described using an analysis of variance and significant differences among means were separated by Tukey’s test (InfoStat v1.1, 2002).

The percentage of living IJs was significantly different depending on storage temperature used (F = 28.36; p = 0.0001). Survival was always above 95% during 12 weeks, when IJs were stored at 23 ± 2 °C. The group kept at 5 ± 1 °C did not show statistically significant differences during the first three weeks; however, at week 5 a noticeable decrease occurred and the percentage of living IJs remained below 60% since then (Fig. 1A). Temperature–time interaction also revealed significant differences (F = 54.36, p < 0.0001).

At weeks 8, 10 and 12 of storage, infectivity of the group kept at 23 ± 2 °C showed higher values with respect to the other group (Fig. 1B).

Survival of nematodes stored at 23 ± 2 °C remained above 95% during the whole study period. Koppenhöfer and Kaya (1999) observed in another isolate of the same species that optimal storage temperature, which favours survival and infectivity of the 95% IJs, is about 15 °C. However, Schirocchi and Haghe (1995) mentioned that two isolates of *Steinernema feltiae* and *Steinernema carpocapsae* survive perfectly well after being stored at 5 °C for more than 8 weeks.