

# CRYOPRESERVATION OF NEMATODE SPECIMENTS AVOIDING LIQUID NITROGEN

ABI M, Lucerna 23-24 October 2006

## WHAT IS CRYOPRESERVATION ?

A technique for freezing microorganisms, tissue or cells to preserve for use at a later date.

Macroorganisms or tissues are usually stored at ultra-low temperatures below  $-196^{\circ}\text{C}$ , in liquid nitrogen.



# HOW TO GET CRYOPRESERVATION

The technology is based on the induction of cell vitrification during a very fast decrease of temperature.

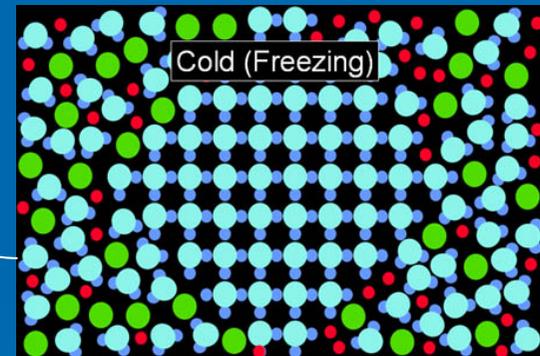
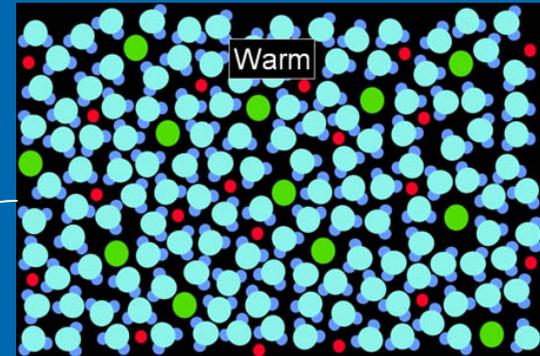


# "VITRIFICATION"

the physical process  
which avoids intracellular-  
ice formation

by

the transition of the  
aqueous solution of the  
cytosol into an amorphous  
glassy state.

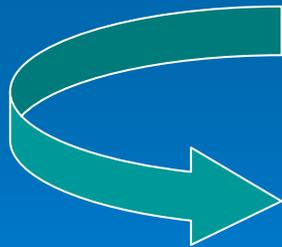


## WHY TO DO IT?

To freeze cultures for storage  
thus “freeing” us from the bimonthly task of  
nematode culture transfers with all of the  
associated risks:

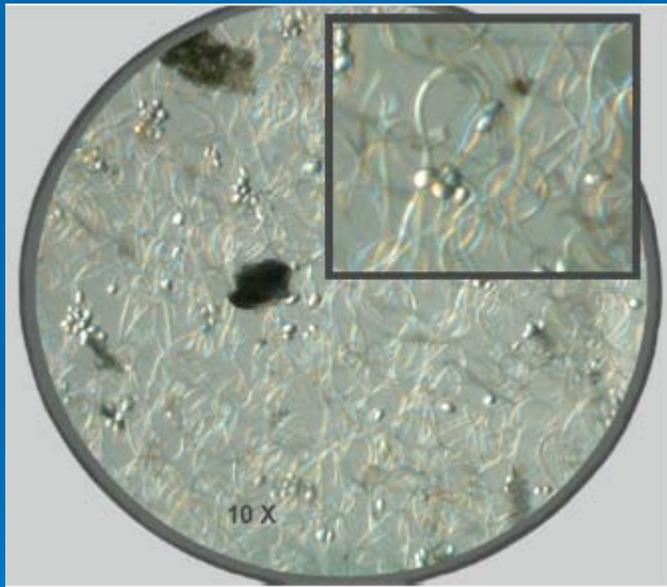


i.e. contamination,  
inadvertent genetic selection  
loss of culture or mislabeling



# EXPERIMENTAL METHOD

ORGANISM USED: nematodes (*Bursaphelenchus*, *Globodera*, *Meloidogyne*, *Heterodera*)



- Equilibrate juveniles (J2) in two steps of cryoprotectant solutions.
- Fast freezing J2 in liquid nitrogen.
- Store in a "dry way", mechanical freezers, at  $-140^{\circ}\text{C}$  for 1-6 months.



IOBC/WPRS Working Group  
"Insect Pathogens and Insect Parasitic Nematodes"  
in cooperation with  
COST ACTIONS 842 and 850  
"Entomophorales" & "Biocontrol Symbiosis"

**10<sup>th</sup> European Meeting**  
*Invertebrate pathogens in biological control:  
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Locorotondo, Bari, Italy, June 10-15, 2005

**Nematode cryopreservation: preliminary reports**  
T. Irdani, B. Carletti, L. Ambrogioni & P. F. Roversi  
CRA-Istituto Sperimentale Zoologia Agraria, Cascine del Riccio, 50125 Firenze.

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**Rapid-cooling and storage of plant nematodes at  $-140^{\circ}\text{C}$  ☆**

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**Abstract**

Low temperatures can assure the long-term or even indefinite preservation of important biological specimens. Nematode cryopreservation allows for the availability of large numbers of living nematodes at any one time, especially for experimental purposes. New isolates of *Bursaphelenchus* have recently been collected, including *Bursaphelenchus crassus* (Rühm) Goodey. This species was identified in north-central Italy on dying oak trees and from the bark beetle *Scolytus intricatus* Ratzeburg as dauer larvae. We therefore, sought to develop a cryopreservation technique for the long-term storage of all available *Bursaphelenchus* spp. The technique consists of a rapid-cooling protocol involving immersion in a liquid nitrogen bath before storage of the frozen samples in a mechanical freezer at  $-140^{\circ}\text{C}$ . The survival of nematodes subjected to this rapid-cooling protocol was higher than previously reported using slow-cooling methods and is suitable for several species of *Bursaphelenchus* and other phytoparasitic nematodes.  
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**Keywords:** Cryopreservation; Cryoprotectant; *Bursaphelenchus* spp.; Long-storage nematode cultures

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**CRYOPRESERVATION OF NEMATODES: AN OPPORTUNITY FOR THE CONSTITUTION  
OF AN EUROPEAN GENETIC BANK**

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Table 1  
Motility and reproduction rate of 25% EG cryopreserved *Bursaphelenchus eremus* 24 h after thawing

Storage at –140 °C (time)	Motility % (mean ± SD) (n = 6)	Reproduction rate ( $R = P_f/P_i$ ) (n = 3)
4 h	84.7 ± 0.06 a	13.6 (4.0–23.3) a
6 days	77.0 ± 11.0 a	14.5 (4.7–24.4) a
1 month	85.6 ± 4.14 a	9.0 (4.8–16.3) a
2 months	76.6 ± 5.73 a	7.2 (4.3–9.0) a
6 months	75.7 ± 1.95 a	13.0 (5.7–19.8) a

Percentages followed by the same letter are not significantly different (Tukey HDS test,  $p < 0.01$ ).

Mean reproduction rate ( $R$ ) for untreated animals = 2.8 (2.6–3.1).

Table 2  
Survival rates of four *Bursaphelenchus* species after a month in –140 °C freezer

Species	Survival % (mean ± SD) (n = 6)
<i>B. mucronatus</i>	77.6 ± 1.95 a
<i>B. thailandae</i>	77.7 ± 1.52 a
<i>B. xylophilus</i>	66.3 ± 3.59 b
<i>B. eremus</i>	85.6 ± 4.14 c

Percentages followed by different letters are significantly different (Tukey HDS test,  $p < 0.01$ ).

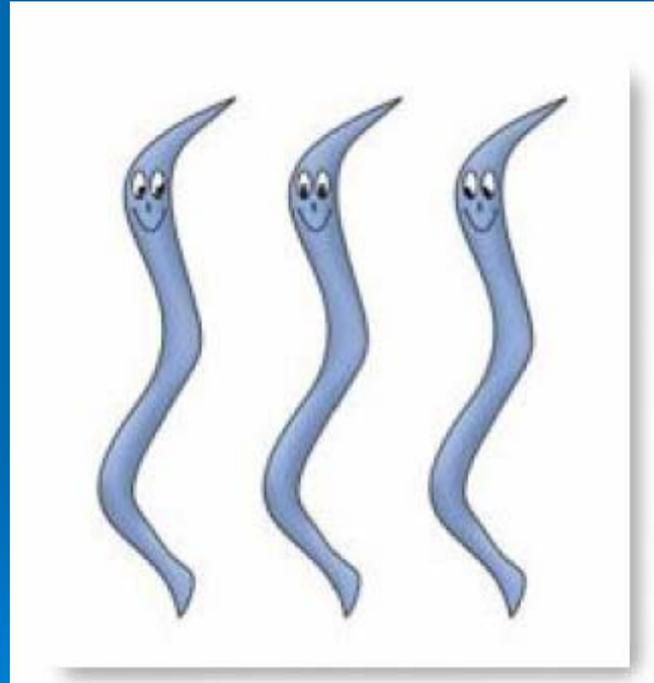


No differences in the cryo-J2 survival or in reproduction rate after different intervals of time



High survivals among different species

MI GHT BE TH I S TECHNI QUE USEFUL ALSO  
FOR OTHER NEMATODE SPECI ES?  
PERHAPS, "BENEFICIAL" ONES ?



# CRYOPRESERVATION WITHOUT LIQUID NITROGEN



A COMFORTABLE METHOD FOR THE OPERATORS  
MORE HANDY EQUIPMENTS  
TEMPERATURE UNIFORMITY COMPARED TO LN<sub>2</sub>  
LESSENERED RISK OF LEAKING VIALS

## IN SUMMARY:



Cryopreservation obviates the high labour and space requirements as well as the need for controlled environmental conditions, all of which are costly.

Efficient cryopreservation protocol, offers the possibility of storage to a great number of nematode populations (living collection).

Maintain viability and genetic stability, ensure full developmental and functional potential.

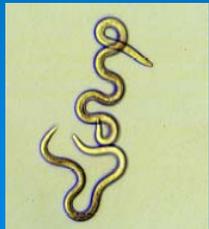
# CRYO-GROUP

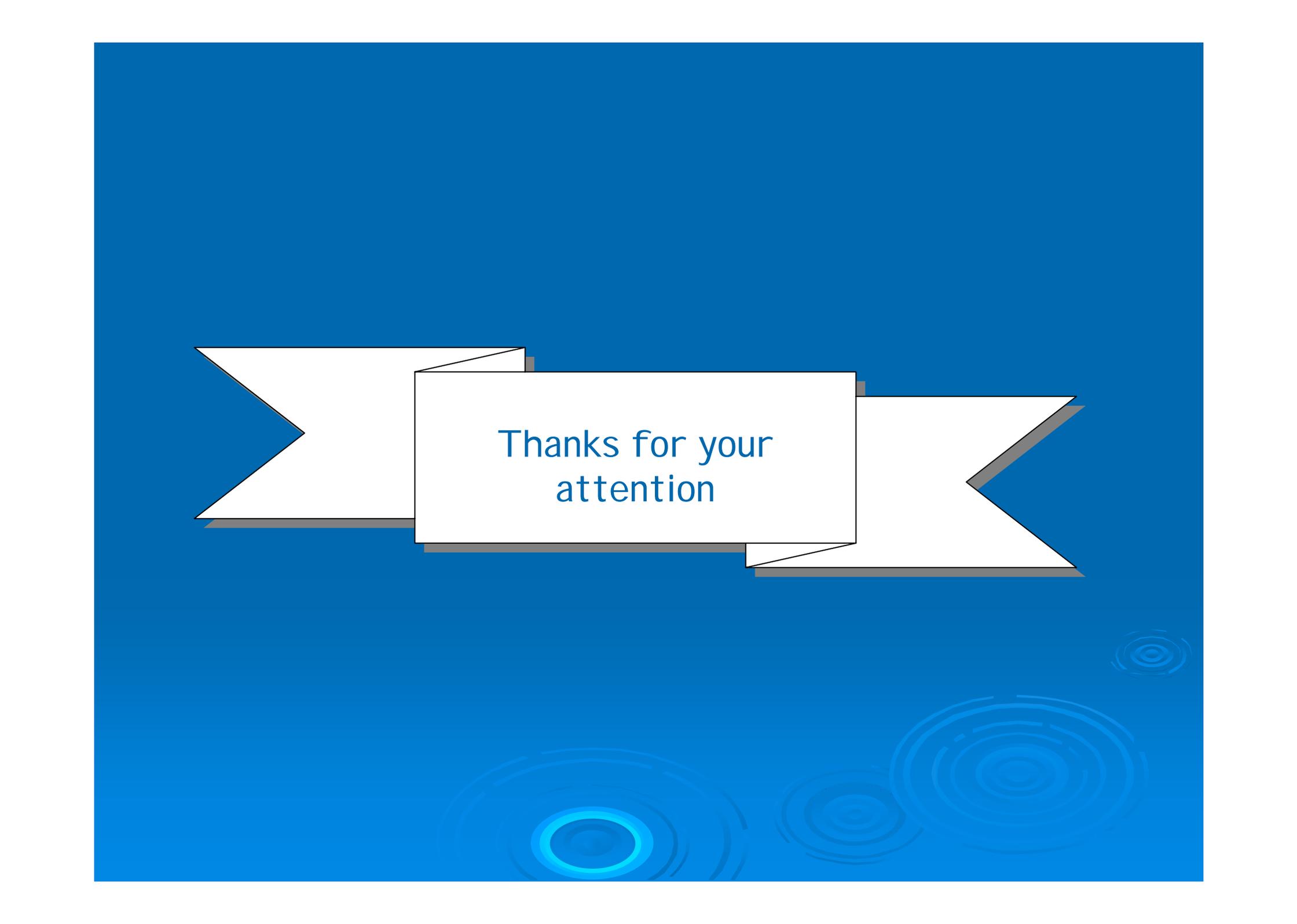
Dr. T. Irdani

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Prof. P. F. Roversi





Thanks for your  
attention