Report Short Term Scientific Mission

COST Action 850

Mª del Mar Martinez de Altube

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Identification and characterization of the heat and desiccation tolerance of four nematode strains from Spain

Objective

The purpose of the visit was to identify and characterize isolates of entomopathogenic nematodes from Spain, regarding heat and desiccation tolerance. Four different strains from Spain were evaluated, three of them *Steinernema carpocapsae,* (1SSA, 3SSA, 18SSA) and another strain probably from the glaseri group, (12SSA). The strains from Spain have been compared with *S. carpocapsae* and *S. feltiae* strains commercially produced by the e-nema GmbH (Germany). The extreme environmental conditions in Spanish fields and greenhouses needs commercial nematodes products, better adapted to heat and desiccation conditions to improve the efficacy.

Bioassays

Strains identification

In order to identify the Spanish strains, characters like body and tail length, the position of the excretory pore and the shape of the spicula of first generation males were determined.

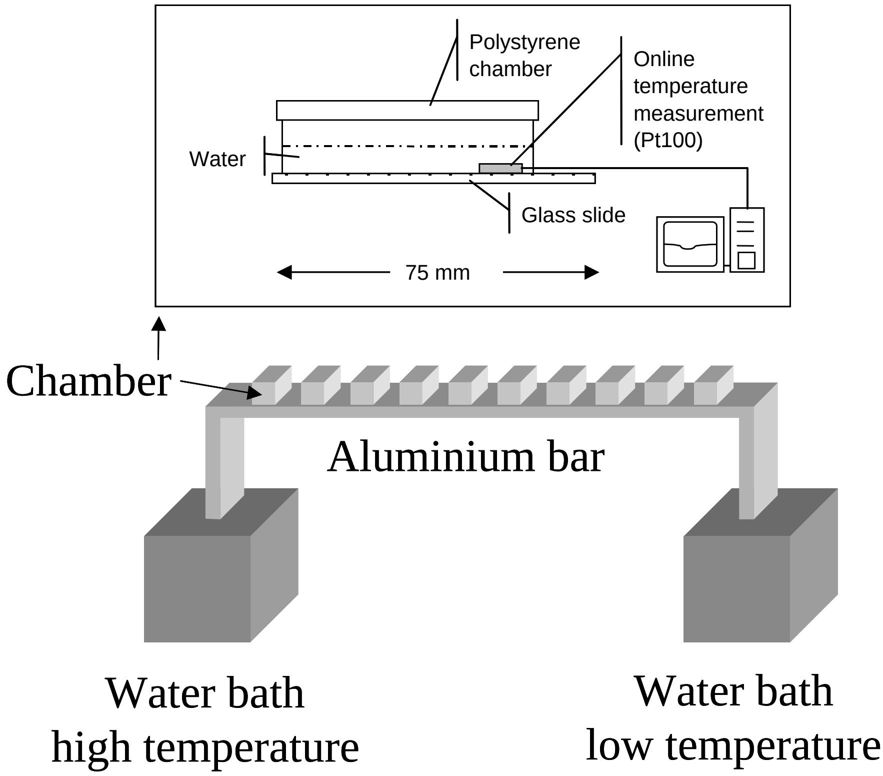
Desiccation tolerance

To remove the water from DJs, the nematodes were suspended in solutions with different water activities (a_w- values) during 24 hours at 25ºC. The a_w-value expresses the relative content of unbound water in aqueous solutions. The different water activities were adjusted by the addition of hygroscopic polyenthyleneglycol (PEG 600). Ten different PEG solutions were created with aw-values from 0.98 to 0.54 for the six different strains. The control was kept in Ringer's solutions at 25ºC. Nematodes from each strain were recovered from infected *Galleria mellonella* larva and kept in 4ºC in tap water, until two hours before the treatments. After two hours at 25ºC a sample with 200±50 DJs was suspended in10µl tap water and added into 1 ml of each of the different a_w-value solutions (all samples were repeated twice). After the exposure of the DJs to the different a_w-values, the nematodes were separated from the PEG600. The nematodes were washed over a 20µm sieve with Ringer's solution. The DJs were kept in the Ringer's solution and the alive and dead were counted 24 hours later.

Heat tolerance

Nematodes emerging from *G. mellonella* larva of each strain were kept at 4ºC in tap water. One hour before the temperature treatment, the nematodes were moved to 25ºC. Each strain was tested to 10 different temperatures between 37 and 42.3, during one hour. 200 ± 50 DJ were placed into cover slide chambers (Fig. 1) with 4 ml tap water. The slide chambers were placed onto a temperature gradient, an aluminium bar with its two ends in water baths adjusted to 37° and to 45°C. The temperature on the bottom of the chambers was recorded by a platinum Pt100 thin layer sensor. After one hour of heat treatment, the nematodes were recovered from the chambers, and live and dead nematodes were counted after 24 hours at 25ºC. The control was kept on a chamber at 25ºC in tap water.

Fig.1



Results

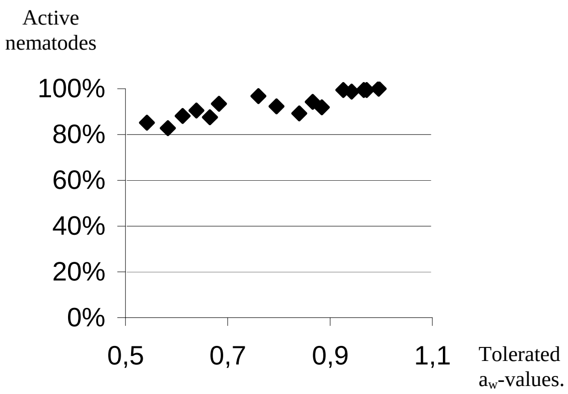
Strains identification.

1SSA, 3SSA, 18 SSA strains were identified as *Steinernema carpocapsae*. It was not possible to give an exact species designation for strain 12SSA. But due to the body dimensions and the shape of the spicula we can conclude that it belongs to the glaseri group.

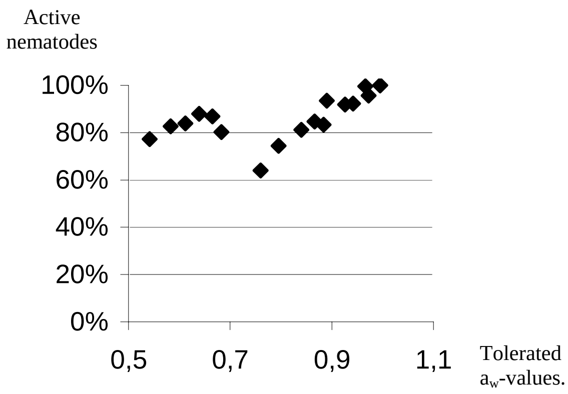
Desiccation tolerance

In the graphics below, for each strain, the survivals at defined a_w-values are presented.

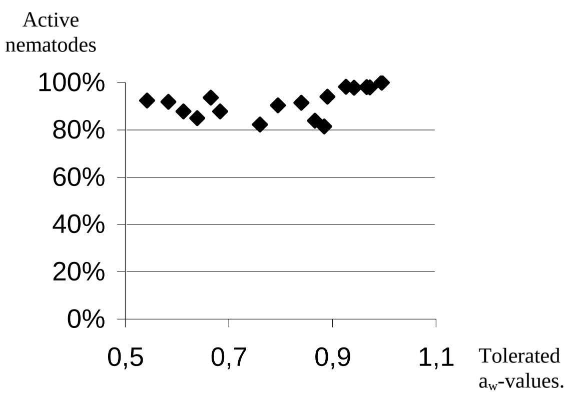
1SSA



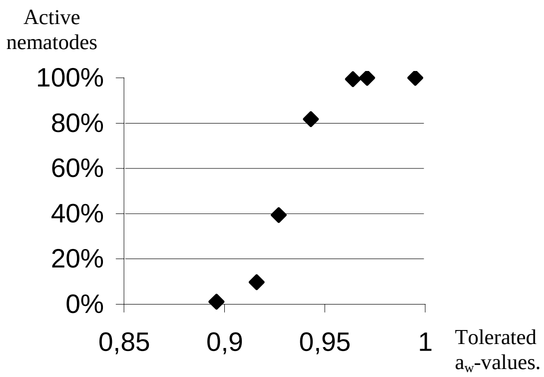
3SSA



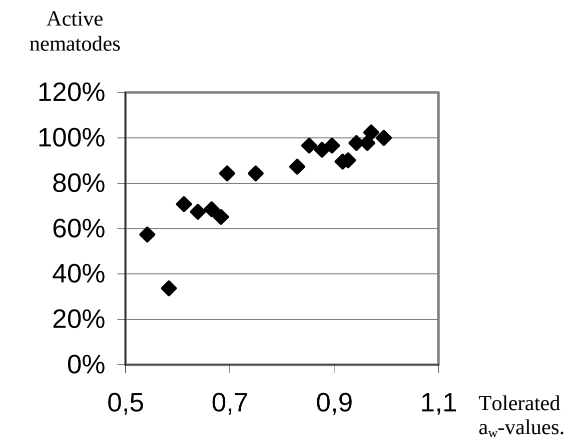
18SSA



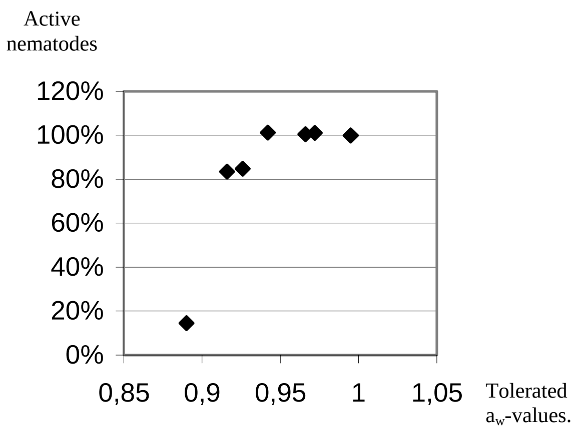
12SSA



S.c e-nema



S.f e-nema



For desiccation stress, two Spanish strains, 1SSA and 18SSA, are nearly not affected by the a_w-values tested, since 90% of the population were still active after the treatment at 0.54 a_w-value. These two strains seem to be better adapted to desiccation than the commercial *S. carpocapsae* strain. All the investigated *S. carpocapsae* strains have a better tolerance for desiccation stress than the *S. feltiae* and the unidentified strain 12SSA, where most of the DJs were already killed at a_w- values between 0.9-0.85.

After the desiccation treatment the tissue of survived nematodes seems to be affected. Atypical vesicles occurred inside the DJs. To estimate the virulence of such nematodes, one-on-one filter paper bioassays (Kaya and Stock, 1997) were realised with DJs of the 18SSA strain treated with different a_w- values. A filter paper was placed in each cell of the 24cell-well plate. From a well-mixed nematode sample, one living DJ was transferred in 30 µl of the Ringer's solution into one of the cell. Afterwards one *G. mellonella* was added to each cell and all the cell-well plate was covered humid sand (80% RH). Two controls were used one from nematodes kept in Ringer´s solution at 25ºC for 24 hours and the other with fresh nematodes preserve at 4ºC.

Table. 1

A_w-values	Number of *Galleria mellonella*	
	Alive	Dead
0,54	20	4
0,61	8	15
0,68	7	16
Control at 25ºC	4	19
Control at 4ºC	6	18

Only the virulence of nematodes recovered from the lowest a_w value (0.54) seem to be affected. (Table 1)

Heat tolerance

To calculate the tolerated temperatures for the nematodes populations a cumulative normal distribution was fitted to the original data (Fig.2). In Fig. 3 the temperature tolerated by 50% and 90% of the population of the different strains are represented.

Fig. 2

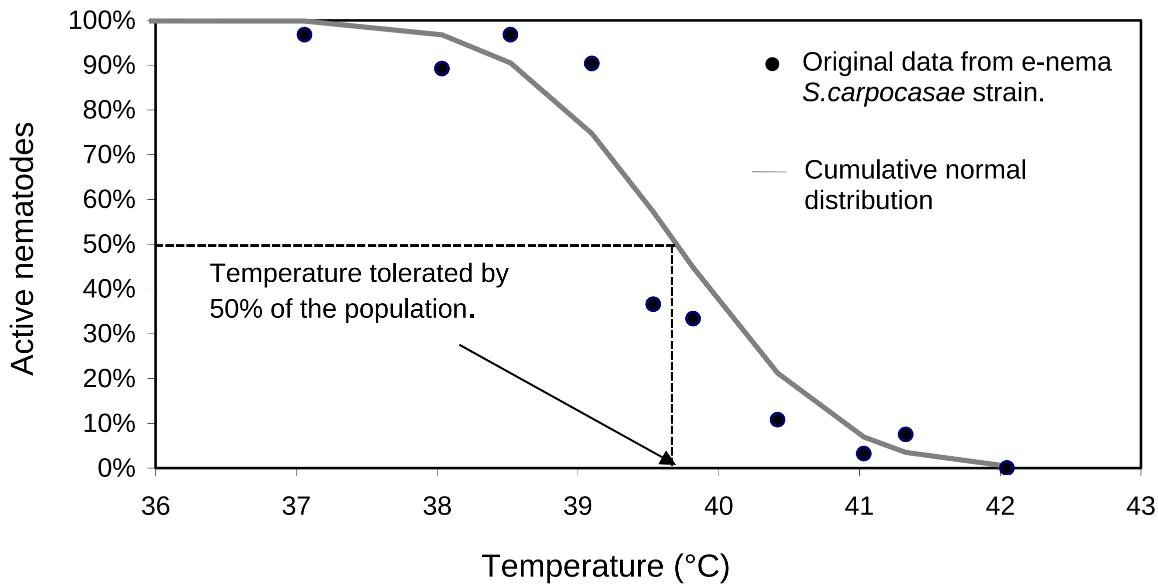
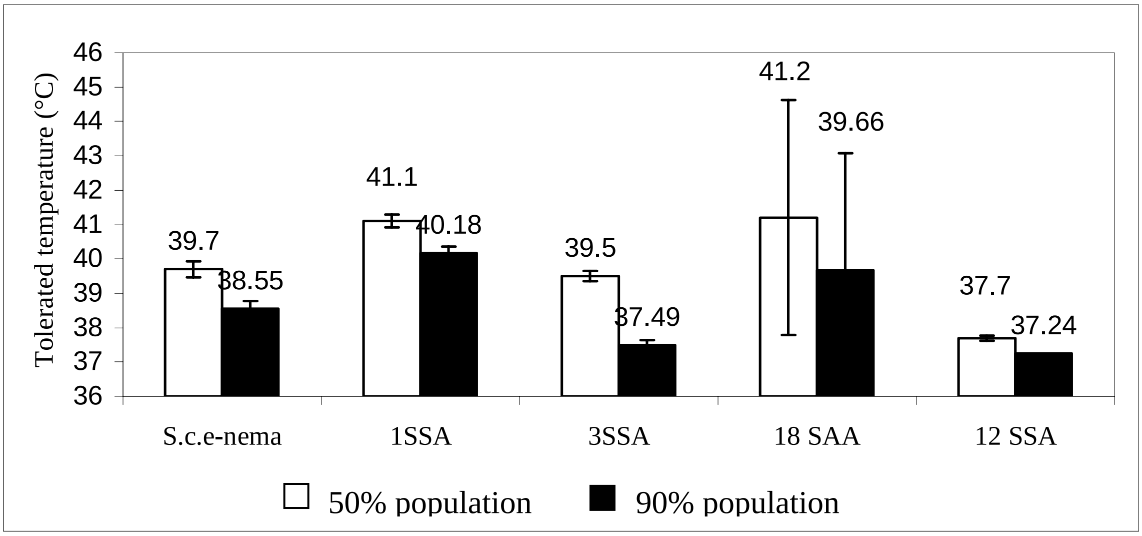


Fig.3



The commercial *S. feltiae* strain is not represented in the graph because at 37.7°C already 100% of the population was dead and the tolerated temperature for 50% and 90% of the population could not be calculated. The 18 SSA strain, at the maximum temperature value 41.3ºC, 44% of the population was still alive. Therefore, only 3 points could be used to fit the normal distribution to the original data. This caused the high variability. The same two Spanish strains, which had the highest desiccation tolerance (1SSA and18SSA), also were most tolerant to heat. 50% of the 1SSA and 18 SSA populations were able to tolerate one degree higher temperature than the other two *S. carpocapsae* strains. The temperature tolerated by 90% of the population was also variable. Almost 3°C of difference were recorded between the most tolerant strain (1SSA) and the least tolerant (3SSA) *S.carpocapsae* strain.

Conclusions

Compared to the *S. feltiae*, *S. carpocapsae* strains can better tolerate heat and desiccation stress. The use of these two strains 1SSA and 18SSA as commercially products could improve shelf-life, quality and field performance, making them better adapted for storage and transport conditions. For the Spanish situation, it would be useful if nematodes better adapted to desiccation and heat tolerance would be available. It would be of interest to study the heritability of the traits in *S. carpocapsae*, in order to crossbreed these characters into other stains already adapted to artificial mass production in liquid culture.

References:

Ehlers, R.-U. , Oestergaard, J., Hollmer, S., Wingen, M. and Strauch, O. (2005): Genetic selection for heat tolerance and low temperature activity of the entomopathogenic nematode-bacterium complex *Heterorhabditis bacteriophora* - *Photorhabdus luminescens*, BioControl 50, 699-716.

Strauch, O., Oestergaard, J., Hollmer, S. and Ehlers, R.-U. (2004): Genetic improvement of desiccation tolerance of the entomopathogenic nematode *Heterorhabditis bacteriophora* through selective breeding. Biological Control 31, 218-226.