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EFFECTS OF CONSTANT VS. FLUCTUATING TEMPERATURE ON THE GROWTH AND INTERACTIONS OF TWO BACTERIVOROUS NEMATODE SPECIES

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ABSTRACT
Temperature influences nematode mobility, reproduction, and development, temperature that is suitable to the development of nematodes, such as hatching, reproduction, movement and growth, are different between species and community. The temperature character of the same species is different if community is different. *Diplolaimelloides meyli* (Monhysteridae) and *Rhabditis (Pellioditis) marina* (Rhabditidae) responded sharply to temperature. Population of *R. (P.) marina* increases at 25^0C and 30^0C were significantly greater than at any other temperature between 10-40^0C. Temperature had a pronounced influence on both nematodes over the entire range studied that is 5^0C to 30^0C. *Diplolaimelloides meyli* still reproduced and matured at temperatures exceeding 30^0C, while *R. (P.) marina* had an upper temperature limit for reproduction of 25^0 C. In the present study, two estuarine bacterivorous nematode, cryptic species of *Rhabditis (P.) marina* I and *Rhabditis (P.) marina* IV were selected, under the classification of Kingdom: Animalia, Phylum: Nematoda, Class: Secernentea, Order: Rhabditida, Suborder: Rhabditina, Superfamily: Rhabditoidea , Family: Rhabditidae, Genus: Rhabditis, Subgenus: *Rhabditis (Pellioditis)*. In this experiment two temperature regimes (fluctuating temperature and constant temperature) were selected *to* find out the influence of this abiotic variable on the reproduction and the competition between these two closely related cryptic species of *Rhabditis (Pellioditis) marina* I and *Rhabditis (Pellioditis) marina IV*. The interaction of temperature and nematode species was also significantly different ($P<0.05$) for both adult and total population. At eleven days, temperature didn't give significant population difference.

KEY WORDS: Nematode, Temperature, *Rhabditidis*, agnotobiotic

INTRODUCTION

Nematodes are three out of five metazoans that are the diverse group of invertebrates. More than 28000 described species probably represent only a small portion of the total member in the phylum Nematoda. The large intraspecific variability within *Rhabditis (Pellioditis) marina* is reflected in the description of a number of varieties, all but one of them later having been considered as synonyms of *R. (P.) marina*. There is evidence for ten sympatrically distributed cryptic species of *R. (P.) marina*s, seven of which shows a strong genetic structuring and were morphologically distinguishable. The Aufwuchs habitats frequented by monhysterid and rhabditid nematodes are highly unstable: in a tidal environment, they are subject to daily fluctuations in salinity and temperature. Temperature influences nematode mobility, reproduction, and development. Temperature that is suitable to the development of nematodes, such as hatching, reproduction, movement and growth, are different between species and community. The temperature character of the same species is different if community is different. *Diplolaimelloides meyli* (Monhysteridae) and *Rhabditis (Pellioditis) marina* (Rhabditidae) responded sharply to temperature. Population of *R. (P.) marina* increases at 25^0C and 30^0C were significantly greater than at any other temperature between 10-40^0C Temperature had a pronounced influence on both nematodes over the entire range studied that is 5^0C to 30^0C. *Diplolaimelloides meyli* still reproduced and matured at temperatures exceeding 30^0C, while *R. (P.) marina* had an upper temperature limit for reproduction of 25^0 C. In the present study, two estuarine bacterivorous nematode, cryptic species of *Rhabditis (P.) marina* I and *Rhabditis (P.) marina* IV were selected, both are under the classification of Kingdom: Animalia, Phylum: Nematoda, Class: Secernentea, Order: Rhabditida, Suborder: Rhabditina, Superfamily: Rhabditoidea , Family: Rhabditidae, Genus: Rhabditis, Subgenus: *Rhabditis (Pellioditis)*.

In this experiment two temperature regimes (fluctuating temperature and constant temperature) were selected to find out the influence of this abiotic variable on the reproduction and the competition between these two closely related cryptic species of *Rhabditis (Pellioditis) marina* I and *Rhabditis (Pellioditis) marina IV*. Though there are some observations on the effects of temperature on this species, no observation till to date found about the competition among the cryptic species of the *Rhabditis marina*. As they are genetically different and do not undergo interbreed, we think that constant and fluctuating temperature have a separate effects on the growth of these cryptic species and also there is competition between them.

MATERIALS & METHODS

Culture of experimental nematodes

Details on the isolation and agnotobiotic cultivation of two cryptic bacterivorous nematode species *Rhabditidis (Pellioditis) marina* I and *Rhabditidis (Pellioditis) marina* IV are given elsewhere (Moens and Vincx, 1998). Briefly, nematodes were isolated from macrophyte detritus and cultivated on a 1% agar prepared with artificial seawater (ASW, Dietrich and Kalle, 1957) with salinity of 25%, and with bacto- and nutrient agar in a weight/weight ratio of 4/1. Above mentioned species were selected for observing the interactions in same Petri dishes because, both the species shown to be similar in their morphological characters. This cryptic speciation, found in a small geographical area of *P. marina*, has strong implications for diversity estimates within the Nematoda, which are mainly based on morphological characteristics. Because *P. marina* has a very short generation time with a high reproductive output (Derycke *et al*., 2005, Inglis & Coles, 1961). For experiment, adults of *Pellioditis* (*Rhabditidis*) *marina* I and *Pellioditis* (*Rhabditidis*) *marina* IV from a culture in exponential growth phase were manually transferred to 1% bacto-agar. Since bacterial-feeding nematodes cannot obtain all necessary sterols from bacteria, and cannot sufficiently synthesize these themselves, cholesterol was added to the medium and pH of the agar was buffered at 7.5-8 with TRIS-HCl at a final concentration of 5 mM. The agar medium was sterilized by autoclaving it for 20 min at 1.1 atm. Food was added as *Escherichia coli* K12; the bacterial suspension was offered with a cell density of ca. 3 x 10^{10} cells ml^{-1}. Six female and four male of each species were transferred in two separate 5 cm diameter petri dishes to see the effect of temperature on the growth of nematode species individually. Another six female and four male of both species were transferred in a 8 cm diameter petri dishes with double amount of food for evaluation of temperature effect on both species either they interact themselves or not. Few drops of artificial sea water were placed at the center of the petri dish before transfer of the nematodes. Then the control treatments *i.e*.,prepared petri dishes contaninig *Rhabditidis (Pellioditis) marina* I and *Rhabditidis (Pellioditis) marina* IV and mixture of both species were subjected to a constant (20^0C) and the another set of same treatments were subjected to a fluctuating temperature between 15-25^0C (3 days/3 days).

Design of Experiment

The experiment was laid out in the completely randomized design (CRD) with four replications. Data on the total number of juveniles and adults per petridish were recorded three times at 3rd, 7th and 11th days after incubation.

Statistical Analysis

Data were analyzed following the statistical program SAS 9.2 and MS-Excel. For mono-specific cultures, the effect of time, species and temperature was analyzed on total juveniles and adult abundances. For mixed culture [*Rhabditidis (Pellioditis) marina* I + *Rhabditidis (Pellioditis) marina* IV], the effect of time, temperature and competition was studied on the nematode populations. Another theoretical control of both species was made in order to elucidate the effect of competitive interactions on the total abundances. Data were analyzed using generalized linear mixed models for repeated measures analysis (GLIMMIX procedure).

RESULTS & DISCUSSION

The two species didn't show statistically significant difference in adult population based on data pooled over culturing time and temperature regime. Though the effect of temperature was not significant, its interaction with time and species found to be significant ($P < 0.05$ Referring to juvenile, temperature, temperature * species, temperature *time and temperature * time * species interactions were statistically significant ($P < 0.05$). The analysis on total number depicted that the effect of species and temperature * time * species interaction were statistically significant ($P < 0.05$). In the mixed culture, the effect of temperature was significant on juvenile number ($P < 0.05$). The effect of the interaction of temperature and time was also significant for the two nematode species.

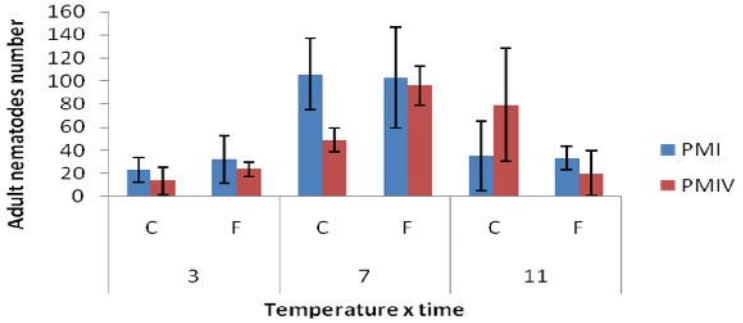

FIGURE 1. Effect of temperature on the relative abundance of adults of two nematode species

Three days after culturing of nematodes, the effect of temperature on both juvenile and adult population was found statistically significant ($P<0.05$). However seven days after culturing, the effect of temperature was found statistically significant on adult and the total nematode population but not for juveniles. More numbers of juveniles were recorded from PMI cultured at constant temperature as compared to the culture at fluctuating temperature after seven and eleven of culturing (Fig.2). Relatively more number of adults was recorded from constant temperature for both *Rhabditis (P.) marina* PMI and *Rhabditis (P.) marina* PMIV at all time series after nematode culturing except less number of PMI was recorded at constant temperature eleven days after

culturing. In general higher numbers of juveniles were recorded from constant temperature at all time series as compared to fluctuating temperature (Fig.2). Peak number of adults and juveniles were recorded from both tested temperature regimes at seven and eleven days of culturing, respectively. Similarity, highest total number of PMI was recorded eleven days after culturing.

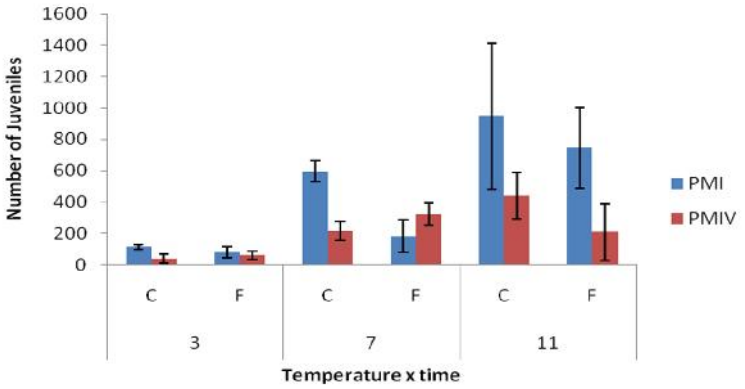

FIGURE 2. Effect of temperature on the relative abundance of juveniles of two nematode species

The two species didn't show statistically significant difference in adult population based on pooled data over culturing time and temperature regime, implying that the species have similar response to the tested temperature. This result is in agreement with (Moens *et al*., 1996), who reported that population of *D. meyli* reached high population density than the *P. marina* at 20 and 25. Referring to juvenile population, temperature, temperature * species, temperature * time and temperature * time * species interactions were statistically significant ($P < 0.05$), indicating that the performance of species varies in time and across temperature regimes. This might be due to the fact that the biological response of two species is different as there might have different temperature requirement.

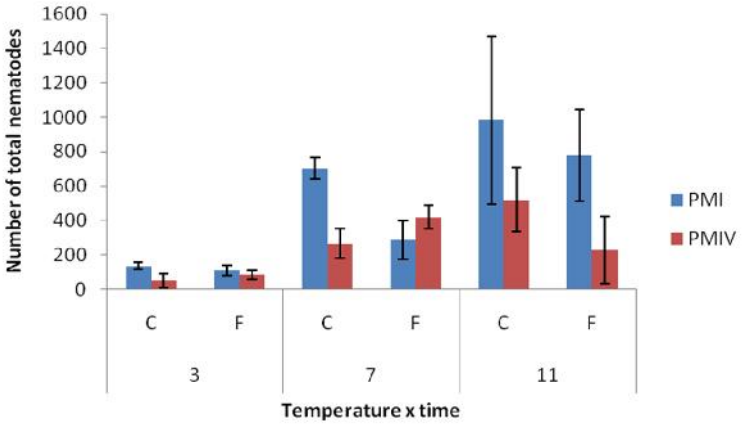

FIGURE 3. Effect of temperature on total abundance of two nematode species

The interaction of temperature and nematode species was also significantly different ($P<0.05$) for both adult and total population. At eleven days, temperature didn't give significant population difference.

Three days after culturing, the effect of temperature on the juvenile and adult population was not statistically significant. This may be attributed to not only the time taken by the inoculated nematodes to adapt to the new culture media but also some of the inoculated females may not be fully matured to mate and give progenies within three days. This is substantially evidenced by a sharp population increase of the two species at both constant and fluctuating temperature at seven days after inoculation. Working on *P. marina* Tom Moens and Magda Vincx (2000) reported a high population increment at 20^0C and 15^0 C. However, a sharp population decrease at eleven days after culturing was mainly due to depletion of food, accumulation of toxics and natural senescence.

Except the juvenile of PMI, all populations of PMI and PMIV didn't show remarkable variation at constant and fluctuating temperature (Fig.4).

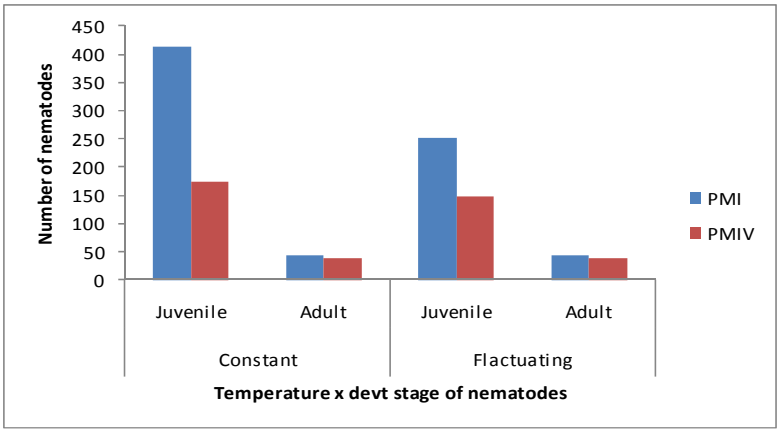

FIGURE 4. Effect of temperature on reproduction of two nematode species

For the mixed species culture, the effect of temperature was found statistically significant (P< 0.05) only on juvenile population at three days after nematode culturing. Though not statistically significant, the actual nematode population is less than the theoretical population at constant temperature whereas the corresponding values are equal at fluctuating temperature (Fig. 5).

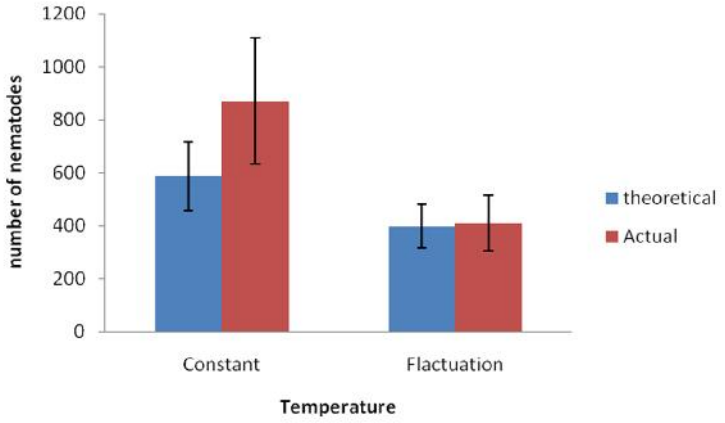

FIGURE 5. Effect of temperature on total number of nematodes in a mixture two nematode species

The higher number of juveniles and adults recorded from a constant temperature culture for both *Rhabditis (P.) marina* PMI and *Rhabditis (P.) marina* PMIV species, suggesting that this temperature may be an optimum for the growth of this species as compared to fluctuating temperature. The highest adult number at seven days after culturing subsequently resulted in the highest number of juveniles at eleven days. Statistical analysis using data from mixed species gave significantly high abundance of juvenile populations from the constant temperature at three days culturing as compared to fluctuating temperature. Similar trend was observed both for the adults and total population though the difference was not statistically significant. However, the result from the monospecific culture indicated that the juvenile population tends to increase for PMIV and decrease for PMI whereas the adult population was higher in fluctuating as compared to constant temperature. There was no significant difference between the actual mixed species population and the theoretical population. However, the genetic analysis result indicated that Rhabtis (P) merina PMIV had 79.83±7.96 % dominance over Rhabtis (P) merina PMIV. The result is supported by Gibson (1981), who reported that the duration of the *Ostertagia ostertagi* larval stages from hatching up to the third stage showed a decline with increasing constant temperature up to 25^0 C. The actual nematode population is by far less than the theoretical population at constant temperature whereas the corresponding values are equal at fluctuating temperature. This may be due to inhibitory action of one species over the other

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