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## OBSERVATIONS ON THE BIOLOGY OF A PREDATORY NEMATODE BELONGING TO DIPLOGASTERIDAE

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Colagiero M., Rosso L.C., Ciancio A., Murga-Gutierrez S.N. – Observations on the biology of a predatory nematode belonging to Diplogasteridae.

The use of predatory nematodes as biological control agents of phytoparasitic nematodes has been re-evaluated only recently. To be effective, predatory nematodes should be easy to grow, cheap, and available on a commercial scale. In addition, they must show a reproductive rate sufficient to sustain a high population density and a significant longevity and stability for storage. The predators are not phytopathogenic nematodes, and among the positive outcomes of their introduction are remarkable their environment compatibility and safety for other non-target organisms, as well as the ability to search for prey. These features are present in Diplogasteridae. Their adaptability enables them to withstand changing climatic conditions, as well as the temporal variability between predator and prey. It should also be emphasized the ability to disperse, persist and reproduce in the absence of prey, the spectrum of action, all factors ideal for the management of phytoparasitic species. Adverse factor is cannibalism, due the lack of preys, that can reduce their biological control potential. We herein present preliminary data on occurrence, reproduction and intestine bacterial flora of the predatory nematode, *Koerneria sudhausi*.

KEY WORDS: nematodes, predation, biocontrol.

### INTRODUCTION

The damages caused by phytoparasitic nematodes are considerable, and in mediterranean environments many vegetable, pulse and fruit productions are under pressure due to their attacks. In recent years the expansion of organic croppings promoted the development of new agricultural inputs, including products and protocols based on biological control agents as possible alternatives to pesticides.

The use of nematodes as biological control agents of other phytoparasitic nematodes dates back several decades, since first proposal by COBB (1917), but only recently it was re-evaluated by studies on the role of a number of predators (BILGRAMI, 2009). To act as effective biological control agents, predators must meet a number of specific requirements including ease of cultivation and low cost commercial production, together with a high reproduction rate allowing high population densities. Furthermore, they must have significant longevity and stability, in order to be preserved. To achieve success in their use also important are their safety and compatibility with other non-target soil organisms.

The ability to seek prey is an important factor that a predator should also possess, and some groups, i.e., Diplogasteridae, can efficiently perform this task (BILGRAMI & JAIRAJPURI, 1988). Diplogasteridae are bacterial feeders which can also prey on nematodes. Due to their biology they can be multiplied *in vitro*, feeding on bacterial cells (BILGRAMI, 2009).

In this study, specimens of a population of *Koerneria sudhausi*, a member of the Diplogasteridae (VON LIEVEN, 2008), were studied in detail. This species is known to act as an efficient predator of root-knot nematodes (BAR-EYAL *et*

*al.*, 2008). Preliminary data on its occurrence, reproduction and intestine bacterial flora, useful for multiplication in controlled conditions, are herein provided.

### MATERIALS AND METHODS

For collections, standard nematological techniques were used. Soil and root samples were collected from farms specialized in *Asparagus* productions at Virú district (La Libertad, Peru). A few g of soil were collected on filter paper and inoculated on 1% water agar (WA) in Petri plates, to allow multiplication of bacteria and of bacteria feeding nematodes. The free living and predatory species were identified in temporary water mounts with a Leitz Orthoplan light microscope at 200-400 $\times$ . Eggs or adults were hand-picked and placed on 1% water agar and stored at 26°C, for subsequent observations.

Studies on the biology of *K. sudhausi* were carried out *in vitro*. Since the diplogasterid can also feed on bacteria, we studied the intestine bacterial flora. For identification of the bacterial species from the intestine of *K. sudhausi*, nematodes were picked up from the WA plates and surface sterilized by immersion for 3 min in 0.5% NaOCl, followed by incubation with antibiotic solution (0.1% ampicillin, streptomycin and chloramphenicol) for 5 min. The nematodes were later rinsed twice in sterile distilled water (SDW), followed by the rupture of their bodies in 10  $\mu$ l of SDW on a glass slide, with a sterile blade. The bacterial cells thus released were smeared on Luria-Bertani (LB) agar medium with a sterile loop. Subsequently, the plates were incubated at 26°C for 48 hours, and the bacterial colonies were isolated in pure cultures on LB medium.

For DNA extraction, the bacterial cells were recovered from the plates with a sterile spatula. The cells were suspended in 350 µl of STET buffer (8% sucrose; 20 mM Tris HCl pH 8; 50 mM EDTA; 0.5% Triton.) and 300 µg of lysisyme. The cells were then crushed by incubation at 95°C for 40 sec and immediately cooled on ice. An amount of 175 µl of phenol and 175 µl chloroform/iso-propanol (24:1) was added to the suspension, vortexed and centrifuged at 12000 rpm for 10 min. DNA was precipitated from the aqueous layer by adding 1/10 v/v of 5M ammonium acetate and 2 volume of cold ethanol. After centrifugation, pellets were washed with 70% ethanol, dried under vacuum, and re-suspended in 50 µl sterile-distilled water.

For species identification, PCR amplification of the 16S rDNA regions was performed using the primers 27F and 1492R (LANE *et al.*, 1985), on a thermocycler (iCycler, BiRad) with the following cycles: a first cycle at 95°C for 7 min; 35 denaturation cycles (30 sec at 94°C), annealing (30 sec at 50°C) and extension (1 min at 72°C), and a single final extension cycle at 72°C for 7 min. Amplified products were examined on 1.5% agarose gels, purified using a commercial GenElute PCR Clean-up kit (SIGMA) and directly sequenced through an available commercial service (Eurofins MWG Synthesis GmbH, Germany). The sequences produced were then assigned to their corresponding species by means of BLAST analyses (ALTSCHUL *et al.*, 1997) in the sequence databases available at NCBI (<http://www.ncbi.nlm.nih.gov/>).

## RESULTS AND DISCUSSION

Adults of *K. sudhausi* were observed on WA petri plates. The nematodes were identified according to the original species description and were multiplied on WA with their original bacterial microflora for several weeks. Adult females produced 6-8 eggs per day on WA, at 26°C. The eggs irregular external formations and swellings, characteristic of this species, persisted during the devel-

opment of the embryo until hatching, and were gradually covered by a dense layer of bacterial cells (fig. I). Although the function of the surface eggs formations is not known, freshly hatched juveniles were often observed moving around the eggs and touching the shell irregularities with their lips, during feeding on bacteria. One generation was completed within 4-7 days, at 26°C.

A total of 7 bacterial culture isolates were obtained from the *K. sudhausi* intestine content. The PCR amplification of the 16S gene fragments yielded several amplicons of the expected range size. BLAST analyses showed a 99% homology with the following species: *Pseudomonas putida* (fragment length: 780 bp, accession n. AM942549), *Pigmentiphaga kullaiae* (860 bp, NR\_025112), *Acidovorax avenae* (954 bp, AY512827), *Bosea thioxidans* (804 bp, FJ581444), *Xenophilus* sp. (950 bp, GQ246689) and *Bradyrhizobium* sp. (900 bp, DQ520809).

Predatory nematodes like *K. sudhausi* show a potential in biocontrol, as their biology allows the development and use as possible, innovative bio-nematocides. Their multiplication *in vitro* on bacteria allows production in large numbers, facilitating applications as massive inocula that may have a significant impact on phytoparasitic nematodes (YEATES, 1969). Diplogasteridae show selectivity in predation (CHITAMBAR & NOFFSINGER, 1989). A prey preference, including endoparasitic nematodes, was reported for *Mononchoides fortidens*, *M. longicaudatus* and *M. gaugleri* (BILGRAMI & JAIRAJPURI, 1988; 1989; BILGRAMI *et al.*, 2005). Studies on predation also showed a chemoattraction towards prey (BILGRAMI, 2009). These properties of Diplogasteridae are useful in biological control, increasing their regulation efficiency. Treatments with Diplogasteridae reduced densities of the root-knot nematodes *Meloidogyne javanica* and *M. arenaria* or the citrus nematode *Tylenchulus semipenetrans* in pots (BILGRAMI, 2009). Assays carried out in controlled conditions with *K. sudhausi* confirmed the assumptions about its potentials, and showed promising results against *M. javanica* (BAR-EYAL *et al.*, 2008).

As candidate biological control agents of phytoparasitic

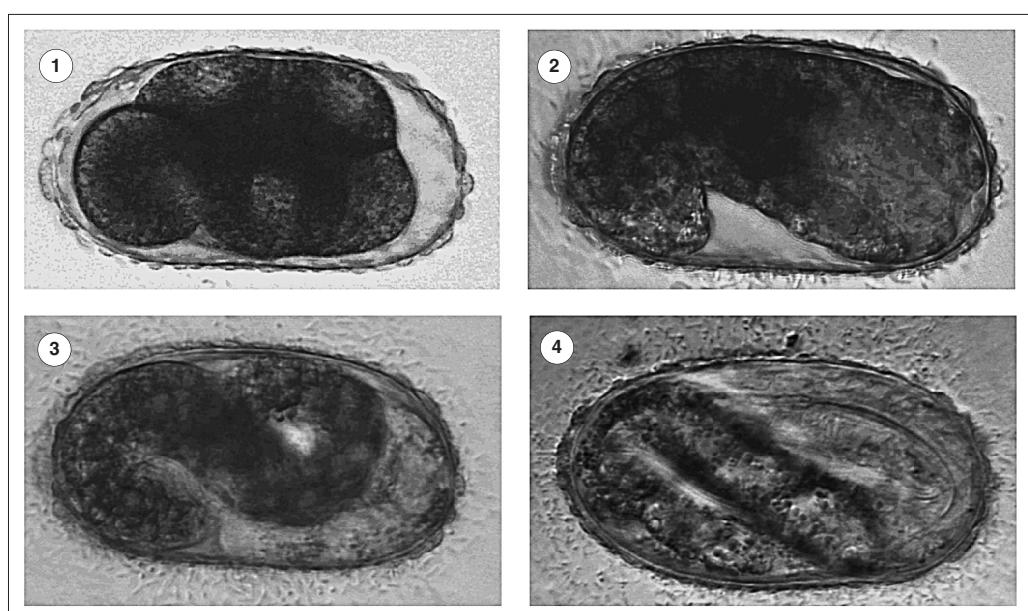


Figure I – Developmental stages of *Koerneria sudhausi* eggs on WA, showing the typical surface irregular swellings and the increasing density of surrounding bacterial cells, from early (1, 2) to the final pre-hatching stages (3, 4).

nematodes, the adaptability of Diplogasteridae allows them to tolerate changing climatic conditions, as well as to display a stable, temporal compatibility with preys (BILGRAMI, 2009). The capacities to disperse, persist, survive and reproduce, even in absence of prey (GROOTAERT *et al.*, 1977), and the presence of a broad spectrum of action, are ideal traits for the biological management of phytoparasitic nematodes with such predators. The only drawbacks are represented by the occurrence of pathogens or parasites, as well as by hyperparasitism that, in absence of prey can lead to cannibalism.

The possibility to introduce useful bacteria (i.e. *Bradyrhizobium*), represents a favourable trait of *K. sudhausi* and needs to be further checked also for other Diplogasteridae species. The range of Diplogasteridae interactions with bacteria appears wide, as i.e. *Pristionchus pacificus* showed dissemination, reduction in brood size, chemoattraction to particular species and also avoidance and mortality. In controlled assays this species avoided *Serratia marcescens*, *Bacillus thuringiensis* and other insect pathogenic bacteria, because of their pathogenicity (RAE *et al.*, 2008).

For practical applications, i.e. introduction in soil or inundative treatments, parameters on conservation, transport, formulation as well as doses of application of Diplogasteridae appear crucial. Practical beneficial potentialities for exploitation of predatory nematodes also include the absence of a registration procedure and their very low environmental impact. Finally, further benefits of a management approach based on Diplogasteridae include the possibility to maintain and apply the inocula *in situ*, in simple organic compost or production systems, as well as the possibility of inundative applications, in a classical biocontrol approach.

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#### RIASSUNTO

#### OSSEVAZIONI SULLA BIOLOGIA DI UN NEMATODE PREDATORE APPERTENENTE AI DIPLOGASTERIDAE

L'impiego di nematodi predatori come agenti di controllo biologico contro nematodi fitoparassiti è stato rivalutato solo recentemente. Per essere efficaci, i nematodi predatori devono essere facili da allevare e a buon mercato per essere disponibili su scala commerciale. Inoltre, essi devono mostrare un tasso di riproduzione sufficiente a sostenere un'elevata densità di popolazione e una notevole longevità e stabilità per la loro conservazione. I predatori non sono nematodi fitopatogeni e, tra i risultati positivi della loro introduzione, sono la compatibilità ambientale e la sicurezza per gli altri organismi non bersaglio così come la capacità di ricerca delle prede. Queste caratteristiche sono presenti nei Diplogasteridae. La capacità di adattamento consente loro di resistere a condizioni climatiche variabili così come alla variabilità temporale tra predatore e preda. Si sottolineano anche la capacità di disperdersi, persistere e riprodursi in assenza di prede, lo spettro d'azione, fattori ideali per la gestione delle specie di

fitoparassiti. Fattore negativo è il cannibalismo, a causa della mancanza di prede, che può ridurre il potenziale biologico di controllo. Vengono presentati dati preliminari sulla presenza, riproduzione e flora batterica dell'intestino di un nematode predatore, *Koerneria sudhausi*.

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