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Interactions between earthworms and plant-parasitic nematodes

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ABSTRACT

Earthworms can ingest large amounts of soil and litter and thus many nematodes. Several studies have actually shown a decrease in nematode populations in the presence of earthworms. We studied interactions between earthworms (the tropical peregrine *Pontoscolex corethrurus*) and plant-parasitic nematodes (*Heterodera sacchari* and *Pratylenchus zeae*) in the laboratory.

In the presence of earthworms, we observed a significant decrease in populations of *P. zeae* in the rhizosphere of *Oryza sativa* after 6 weeks (1st generation), and 12 weeks (2nd generation), respectively, in comparison to a control with no earthworms. A parallel experiment with *H. sacchari* and *P. corethrurus* showed that *H. sacchari* cysts were ingested by earthworms with the soil, and the interaction between the gut and the gut contents of *P. corethrurus* and *H. sacchari* showed that i) populations of cysts had decreased 5 weeks (1st generation) after the start of the experiment, and ii) the total number of eggs and infesting larvae (J2 alive) had decreased.

1. Introduction

Earthworm biomass accounts for a major share of the soil fauna in many tropical humid natural ecosystems [16]. Geophagic worms consume large amounts of soil organic matter and passively digest nematodes at the same time. It is now well known that earthworms can reduce free soilborne nematode populations as well as plant parasitic nematodes [1,3,6,28,31,32]. The impact of earthworms on nematodes can also depend on the situation, sometimes leading to contrary results. In *Festuca rubra* monocultures, a reduction in nematodes in the presence of *Aporrectodea caliginosa* has been noted, but with a concomitant increase in the abundance of nematodes in a mixture of plants including eight grass species [12]. Other studies have shown better resistance/tolerance of plants against plant parasitic nematodes in the presence of earthworms, without any modification in the nematode abundance [2,13].

The ingestion of soil organic matter by endogeic earthworms has an impact over a broad time scale. Intestinal digestion, which takes 2–4 h, is accompanied by significant mineralization of phosphorus and nitrogen in particular. Inside newly deposited casts, the fresh organic matter is progressively reorganised over the following days or weeks [30]. Over a longer period, the confinement of organic matter within the compact structure of the cast blocks the mineralization process for as long as the cast lasts [5,14,15].

Numerous studies have clearly illustrated the positive relationship between the abundance of earthworms and increased productivity of certain cultivated plants [4,13,25]. This favourable effect is generally attributed to chemical (availability of mineral elements) and physical (structure, porosity) earthworm-induced modifications in the soil [15,18,29]. Considering the possible effects of earthworms on nematodes, i.e. by passive ingestion or by physical, chemical or microbiological modification of their environment, the observed improvement in plant productivity during these experiments could partly be due to the antagonistic effect of earthworms on plant parasitic nematodes.

We therefore conducted laboratory experiments to assess the possibility of such an effect by studying interactions between the earthworm *Pontoscolex corethrurus* and overall populations of two plant parasitic nematodes (*Pratylenchus zeae* and *Heterodera sacchari*).

2. Materials and methods

The earthworms, *P. corethrurus* (commonly found in tropical soils) used in this study were from the island of Reunion (France),

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and were maintained in the laboratory in their native soil, i.e. an Andisol (Dystrandept) [19]. The main chemical characteristics were: $pH_{H2O} = 5.8$; CEC = 16.3 mmol; organic carbon = 8.3%; total nitrogen = 7.5%, total phosphorus = 596 ppm. The nematodes were from Chad (*P. zeae*), which is migratory endoparasitic nematode (nematode which may feed on external surfaces of roots but generally burrow into the root to feed on internal root cells), and the Republic of the Congo (*H. sacchari*, a sedentary endoparasitic nematode which invades root tissues soon after hatching and then establish settles in a permanent, stationary feeding location, where the females remain settled do not move from that site for the rest of their life), and were reared on rice (*Oryza sativa*, cv Moroberekan) grown on sand with a mineral nutrient solution [22]. *P. zeae* and *H. sacchari* are often found in upland rice in tropical areas.

2.1. Interactions between P. corethrurus and P. zeae

Interactions between *P. corethrurus* and *P. zeae* were studied in plastic cylindrical pots (180 ml) closed at the bottom with a stainless steel screen (mesh 0.25 mm) in order to retain and drain the soil. The pots contained 200 g of soil (Andisol), which was twice sterilised by freezing to ensure the elimination of soil nematodes. To do this, the soil was wetted to field capacity, left to dry for 24 h at room temperature and then placed in the freezer at −20 °C for 3 days. It was then left to thaw at room temperature for 3 days before being re-frozen at −20 °C for another 3 days. Following sterilisation, the soil was watered with a nematode-free aqueous extract from the same soil. This double-freezing sterilisation eliminates plant parasitic nematodes, as well as practically all free-living nematodes, while at the same time preserving the organic matter in a satisfactory state to ensure nutrition of the earthworms. The efficiency of this double-freezing sterilization was made using the two-flask technique [27].

A 5–7 day old rice seedling (*O. sativa*, cv Moroberekan) was planted in each pot and the pots were then placed together in a plant growth room at a temperature of 29 °C +/− 1 °C. The room was artificially lit with an ultraviolet lighting unit which gave a lighting intensity of around 4000 lux, 12 h/day. Each pot was inoculated (by a hole made in the soil) with 100 *P. zeae* females (initial population: Pi) 3 days after the rice was planted. The earthworms were added 2 days after nematode inoculation. Two earthworms were placed in each pot for the short-term experiment (6 weeks) whereas for the long-term (12 weeks) experiment three earthworms were placed in each pot to ensure that no earthworms would be lost (escape via the surface of the pots). A total of 40 pots were prepared, 10 of which contained two juvenile earthworms each (mean weight 0.26 g/worm), 10 contained three juvenile earthworms (mean weight 0.30 g/worm) and the other 20 contained no earthworms (controls). The physiological characteristics of the plant (rice) were not considered in this experiment since it was simply designed to check for possible interactions between the earthworms and the studied nematodes. The plant simply served as nematodes hosts.

The first batch of 10 pots with two worms per pot (assays) and 10 pots without worms (controls) was harvested 6 weeks after nematode inoculation (1st generation nematodes). The second batch, consisting of the 10 remaining pots with three worms per pot (assays) and 10 pots without worms (controls), was harvested 12 weeks after nematode inoculation (2nd generation nematodes).

Following each harvest, the roots were placed in a mistifier for 4 weeks to extract root nematodes [26], and then they were dried and weighed. Soil nematodes from each pot were then extracted from the soil using the two-flask technique [27] and sieved for 10 days. The final population (Pf) per pot represented the total population extracted from both the roots and soil.

2.2. Interactions between P. corethrurus and H. sacchari

Two types of interaction were studied. The first concerned interactions between the earthworms (*P. corethrurus*) and *H. sacchari* cysts, while the second involved direct interactions between the earthworms (especially their guts) and the infesting nematodes larvae (J2 stage). Due to problems encountered during the laboratory rearing of *P. zeae*, it was not possible to study interactions between *P. corethrurus* gut and *P. zeae* nematodes.

The first step consisted of verifying whether the nematodes (*H. sacchari)* had been ingested by the earthworms. Cysts of *H. sacharri* were placed in a container with soil (Andisol) and earthworms. Another (control) container was also filled with the same soil containing cysts but without earthworms. Twenty-four hours later, casts found on the soil surface were collected and cysts found within these casts were individually placed in water-filled test tubes so that the hatching of second-stage juveniles (infesting larvae, J2) could be studied over a 5-week period. At the end of the 5-week period, each cyst was opened up and the contents were placed in potassium permanganate ($KMnO_4$, 4 mM) for 24 h. This substance stimulates artificial hatching of viable *H. sacchari* eggs within 24 h [21]. Finally, after 5 weeks, J2 larvae (dead and alive), dead eggs and empty egg shells were counted and J2 larvae that had emerged before 5 weeks (in the containers with soil or in casts) were determined by calculating the difference with the total number of analysed eggs.

The second step consisted of verifying the impact of the *P. corethrurus* gut on J2 *H. sacchari*. To do this, J2 larvae were placed in contact with:

1. Extracts of the earthworm gut alone (wall). The worms were starved for 2 or 3 days in cotton soaked in physiological saline along with antibiotics and fungicide. They were then dissected and the empty digestive tube alone was removed, crushed, and centrifuged in phosphate buffer. The supernatant was recovered (gut).
2. Extracts of the earthworm gut along with associated contents (ingested soil). Earthworms were removed from their native soil. Dissected earthworm guts and contents were crushed and

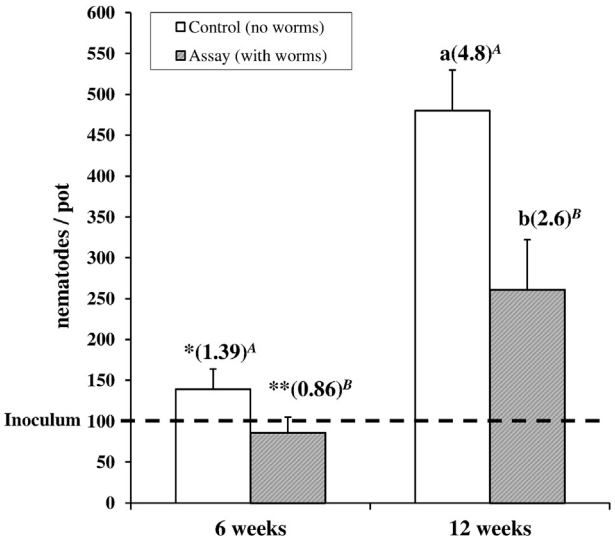

Fig. 1. Numbers of plant-parasitic nematodes (*Pratylenchus zeae*) per pot with earthworms (*Pontoscolex corethrurus*) and without earthworms after week 6 and 12. Inoculum of 100 females. At week 6, different symbol indicate that the difference is marginally significant (PLSD, $p = 0.09$). At week 12, different small letter indicate that the difference is significant (*U*-test, $p \leq 0.01$). The vertical bar shows the standard error (mean of 10 replicates). The multiplication factor (final population/initial population) is in parenthesis and the different capital letter in exponent indicates that the difference is significant (*U*-test, $p \leq 0.05$).