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Development of the earthworm *Pontoscolex corethrurus* in soils amended with a peat-based plant growing medium

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ABSTRACT

An experiment was set in laboratory conditions to determine the capacity of *Pontoscolex corethrurus* to develop in a substrate rich in organic matter and to define the percentage of organic matter where development was best as it is an endogeic geophagous earthworm that typically occurs in mineral-rich rather than organically rich soils. Five treatments were established in soil (S) as the control and then this soil was enriched with 25% (S25), 50% (S50), 75% (S75) and 100% (OM) of a commercial substrate composed mainly of peat moss (*Sphagnum*). The physico-chemical parameters of the mixed substrates and cast were determined. The growth of the earthworms was measured every 15 days during 90 days, as well as cocoon and cast production and mortality. The results show that *P. corethrurus* is unable to grow in a substrate of 75 to a 100% organic matter where its mortality was seen to be more than 56–100%. Its optimal development was in S and in S25 reaching an individual weight of 0.6 g and a cocoon production of 44 ind.$^{-1}$ y^{-1}. Its substrate consumption was shown to be less when the soil was richer in organic matter and the cast production varied from 0.33 to 0.047 g ind.$^{-1}$ per day^{-1}. From a comparison of the composition of substrates and cast physico-chemical parameters, we observed that *P. corethrurus* chooses its food, concentrating on organic matter but breaks down fibers of *Sphagnum*, particularly hemicellulose fibers by up to 17%.

1. Introduction

The soil offers a variety of resources that differ in their physical and chemical availability to earthworms. The content of soil organic matter can be considered as a low-quality resource for worms, as it is dispersed in the mineral soil matrix and includes more than 75% of long, complex humic molecules which are associated with clay particles (Stout et al., 1981).

All worms are influenced by the environment in which they live and factors such as temperature, humidity, pH, organic matter content and quality and texture determine their activity. The functional group to which they belong is defined by their habitat and their feeding habits (Bouché, 1972; Lavelle, 1981). Thus, they have different environmental requirements for their optimal growth. The earthworm diet is mainly organic matter in different states of decomposition (Curry and Schmidt, 2007). Some authors have suggested that they feed on the microorganisms that are within this organic matter such as the protozoa (Rouelle, 1983), bacteria or fungi, although up to now it has not been demonstrated what they exactly eat. Besides, it has been shown that earthworms do not have sufficient digestive enzymes to digest the soil organic matter (Lattaud et al., 1998) and they have a strong mutual relationship with microorganisms in digestion (Drake and Horn, 2007; Lavelle et al., 1995).

The majority of species consume mineral fractions of soil to a greater or lesser extent depending on their functional group. They seem to prefer a mixture of organic and mineral material rather than a purely organic diet (Doube et al., 1997). The consumption rate of soil is also variable, reflecting the feeding habits of earthworms and organic content and soil quality (Curry and Schmidt, 2007).

The assimilation of organic matter by geophagous tropical earthworms appears to be high (3–19% of ingested organic matter), which reflects a high activity of the microflora present in the gut (Barois and Lavelle, 1986; Lavelle and Spain, 2001).

Endogeic polyhumic species live in the soil, the quality of their food is less than that of the epigeic species, but they feed on soil rich in organic matter, thus these earthworms gather near the surface or and in the rhizosphere where the organic matter is concentrated and available; they select their particular food in relation to their digestive capacity (Lavelle, 1983).

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The geophagous earthworm *Pontoscolex corethrurus* (Rhinodrilidae James, 2012) is a peregrine pantropical species. It belongs to the polyhumic endogeic ecological category, inhabiting the first 30 cm of soil. It can be found over a wide range of soil pH, organic matter content and texture. It has been collected in soils ranging from 95% sand to 80% clay (Buch et al., 2011), with a pH of 4–8, organic matter content of 1.0–12.6% and a temperature range from 15 to 35 °C (Lavelle et al., 1987). Because of its large capacity to adapt, *P. corethrurus* is being produced to restore soil fertility and enhance productivity with the bio-organic fertilization technique (FBO, Senapati et al., 1999) and for eco-toxicological tests (Buch et al., 2011). In this intensive production, *P. corethrurus* is grown in soils enriched with horse or cow manure and sawdust in a volume proportion of 3:1 (Pashanasi, 2007). However, we do not know *P. corethrurus*'s tolerance to high amounts of organic matter and exactly what percentage is required for its development, thus the present study aimed to determine what percentage of organic matter in the soil is optimal for *P. corethrurus* development. This point was raised in order to test whether *P. corethrurus* can be used in experiments with seedlings that generally are produced in substrates which are made exclusively of organic matter.

At present, substrates for seedlings or plants growing in greenhouses have specific properties and peat moss (*Sphagnum*) fulfills most of them. For this reason *Sphagnum* is one of the most common components used in the formulation of growing media in tree nurseries, either alone or as a component of a growth substrate both in temperate regions as well in tropical regions to increase porosity and water retention (Cattivello, 1991; Sambo et al., 2008). This is why we used a commercial *Sphagnum* peat as the source of organic matter for the experiment. However, *Sphagnum* is nutritionally very poor, it contains mainly fibers which are not easily assimilable, so the response of earthworms to different amounts of peat in the soil cannot be understood strictly as an organic matter enrichment of soil as it is for manure. Therefore, we intended also to look into the matter of whether *P. corethrurus* can digest part of the *Sphagnum*. That is an interesting point in endogeic earthworm nutrition to know, since up to now there is little information about what geophagous earthworms eat (Curry and Schmidt, 2007).

2. Materials and methods

Soil between 0 and 20 cm deep was collected from a coffee farm located in the municipality of Coatepec, in the State of Veracruz, Mexico. It is a clayey soil with 24% of sand, 28% of silt and 48% of clay. It has a pH of 6.7 and an organic matter content of 8.31%. The soil was brought to the laboratory where it was sieved at 2 mm in order to obtain a homogeneous texture and to be able to recognize the earthworm casts and group them to estimate the cast production and measure some of their bio- and chemical contents. This method, established by Lavelle (1975), is very accurate for mineral substrates but less so in organic ones. The organic substrate was a commercial brand COSMOPET, a substrate based on *Sphagnum* peat moss as mentioned above. It comes from Canada, has a pH of 5.7 and an organic matter content of 82% (Table 3).

P. corethrurus samples were collected at the same site as where we took the soil from, in the stratum 10–30 cm deep. We gathered a total of 250 worms and cocoons in various states of maturity and they were placed in 2 large plastic containers (60 × 30 × 35 cm), where they remained until the experiment was set up, they were fed with the soil and cow manure in a proportion of 3:1 (Senapati et al., 1999; Pashanasi, 2007), to keep them in optimal condition and then juvenile earthworms of similar weight were selected for the experiment.

2.1. Experimental design

The experiment was conducted under laboratory conditions to determine the optimal organic matter content in the substrate for the development of *P. corethrurus*. Thus, five treatments were set up with the addition of organic matter content to the soil where the earthworms lived and fed. The original soil was enriched with the commercial substrate (by volume), giving the following treatments:

1. 100% original soil (S).
2. 75% original soil mixed with 25% of the organic substrate (S25).
3. 50% original soil mixed with 50% of the organic substrate (S50).
4. 25% original soil mixed with 75% of the organic substrate (S75).
5. 100% organic substrate (OM)

The experimental units were plastic containers of 10 × 10 × 5 cm with 250 ml of soil or a mixture with organic matter depending on the treatment. Each of them was inoculated with three juvenile worms (without clitellum). The worms had a mean weight of 0.41 ± 0.017 g.

There were six replicates making thus 30 experimental units, arranged randomly in a rack at a controlled temperature (25 °C) and the substrates were at their field capacity. They were renewed completely every fifteen days, to provide fresh food for the worms and to remove the casts and cocoons from the non ingested substrate (NIS). This procedure was repeated during the 90 days of the experiment.

2.2. Data collection

Every fifteen days, when the substrates were renewed, the following data were taken: the weight of the worms, cast production, mortality, cocoon production and hatchings. The casts were dried in an oven at 105 °C for 24 h and weight assessed. The NIS and casts were analyzed to get to know some of their chemical properties and fiber content in order to determine how the earthworms transformed them. The organic matter content and total carbon content were obtained using the Walkley–Black method and total N with the micro-Kejeldahl method. Available phosphorus was measured with Bray's method. Cation Exchange Capacity (CEC) and exchangeable cations (Na, K, Mg, Ca) were measured by extraction with ammonium acetate 1 N at pH 7, followed by quantification using atomic absorption (for Ca and Mg) and flame spectrophotometer (for K and Na). The pH was measured with a potentiometer (ratio 1:2 soil:water) and electric conductivity (EC) with a conductimeter. Texture was determined from particle size analysis using the Bouyucos hydrometer method (Page et al., 1982), moisture content by the gravimetric method. The measurements were made according to standardized Mexican protocols NOM-021-RECNAT-2000 (Semarnat-2000, 2002). The fiber content: hemicellulose, cellulose and "lignin-like" components were obtained using the Ankon Fiber Analyzer according to Van Soest's method (1963) where the different fibers are evaluated by successive hydrolysis. Regarding "lignin-like" content, *Sphagnum* is a moss which is a primitive plant and is supposed not to have lignin in its cell wall (Mues, 2000). For many years there has been a discussion regarding whether or not the mosses have lignin. Many analyses have been carried out and the literature available suggests that at least there are some "lignin-like" components in the mosses cell-wall including the *Sphagnum* genus (Logan and Thomas, 1985; Edelmann et al., 1998; Tsuneda et al., 2001). Thus, our fiber analysis may have obtained lignin results due to its "lignin-like" component as the Van Soest method cannot differentiate between lignin and "lignin-like" components. In

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