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Earthworm mediated dispersal of baculovirus occlusion bodies: Experimental evidence from a model system

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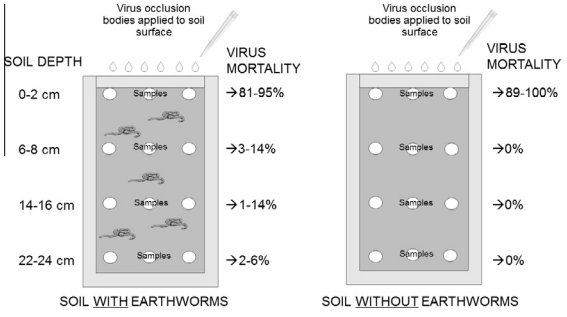
HIGHLIGHTS

- We demonstrate earthworm mediated transport of baculovirus occlusion bodies (OBs).
- *Eisenia fetida* transported 10^4–10^5 OBs/g to depths of 6–24 cm.
- Incubation of soil with earthworms was not detrimental to OB virulence.
- The earthworm intestine was found to be slightly acidic.

GRAPHICAL ABSTRACT



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ABSTRACT

The soil is the most important reservoir of baculovirus occlusion bodies (OBs) in the environment. The ability of the earthworm *Eisenia fetida* to transport OBs of *Spodoptera frugiperda* multiple nucleopolyhedrovirus was examined in laboratory terraria filled with an artificial soil. OBs were detected in soil samples using a soil-diet incorporation bioassay, for which the 50% lethal concentration was estimated at 2.7×10^6 OBs/g soil in *S. frugiperda* second instars. Incubation of earthworms in soil containing 10^9 OBs for 7 days did not result in a significant loss of OB virulence compared to soil without earthworms. The earthworm intestine was found to be slightly acidic, with acid-base indicators applied to lengths of dissected intestine suggesting a pH of 6.0–6.3. Despite their epigean habits, *E. fetida* individuals were observed to form burrows up to 22.5 cm deep in laboratory terraria. Soil-diet bioassays indicated the presence of OBs at depths of 6–8, 14–16 and 22–24 cm in samples taken at 1, 7 and 14 days following the application of 10^9 OBs to the surface of terraria containing earthworms. In contrast, OBs were only detected in samples from the soil surface in terraria without earthworms. We conclude that earthworms likely affect the distribution and dynamics of OB populations in soil habitats.

1. Introduction

The baculovirus occlusion body (OB) is the key structure that allows these viruses to persist in an infective state outside of the insect host. The OB is also the unit of transmission of baculoviruses, as susceptible insect larvae must consume at least one, and possibly many OBs to acquire an infection (Harrison and Hoover, 2012). Once released from the cadaver of an infected insect, OBs contaminate plant foliage and ultimately arrive at the soil surface, mainly by the action of rainfall (D'Amico and Elkinton, 1995; Fuxa and Richter, 1996). OBs in the environment can be dispersed by abiotic

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factors, particularly rain and wind blown dust (Fuxa and Richter, 2001; Olofsson, 1988; Young and Yearian, 1986). Biotic factors can also be involved in OB dispersal. Predatory arthropods and birds that consume infected cadavers can excrete viable OBs in their feces over considerable distances (Entwistle et al., 1993; Lee and Fuxa, 2000a). This is because the acidic pH of the gut of these organisms does not degrade the occlusion matrix of the OB, so that occlusion derived virions (ODVs) remain viable following passage through the gut (Entwistle et al., 1978; Lee and Fuxa, 2000b).

Considering that the soil is the most important reservoir of OBs in the environment, the number and breadth of studies of OB populations in the soil are relatively sparse (reviewed by Fuxa, 2004). Early studies underlined the longevity of OBs in the soil (Fuxa and Geaghan, 1983; Hukuhara and Namura, 1972; Jaques, 1967; Thompson et al., 1981), and the importance of factors such as soil pH (Thomas et al., 1973), whereas later studies were able to quantify the movement of OBs from the soil on to surfaces of field crops (Fuxa, 2008; Fuxa and Richter, 2001, 2007). However, apart from some studies on predatory carabids (Capinera and Barbosa, 1975; Vasconcelos et al., 1996), the role of the soil fauna in the dispersal of OBs has been largely overlooked.

Given the paucity of studies on the role of soil invertebrates in baculovirus dispersal, the present study aimed to examine the potential of earthworms in the dispersal of OBs in the soil ecosystem. Ever since the pioneering studies by Darwin (1881), the role of earthworms in the breakdown of organic matter, nutrient cycling and the redistribution of soil particles has been recognized as a key contribution to defining soil texture, aeration, drainage, microbiota and soil fertility (Satchell, 1983). The part of the soil ecosystem that is influenced by earthworm activities is known as the drilosphere. One of the most studied species of earthworm is *Eisenia fetida*, a European species that is now distributed worldwide and commonly used for vermicomposting (Edwards et al., 2010). It is an epigean species that usually lives at the interface between the soil and the leaf litter or compost. It is readily cultivated in a diversity of organic wastes and is amenable to laboratory rearing using established techniques (Edwards and Bohlen, 1996).

To evaluate the ability of earthworms to transport OBs we used a model system consisting of *E. fetida* individuals from a vermicompost colony that were allowed to inhabit an artificial soil developed for testing the toxicity of pesticides to earthworm populations (OECD, 1984). The use of an artificial soil allowed us to overcome repeatability issues arising from the enormous variability in the composition and physico-chemical characteristics of natural soils (Edwards and Bohlen, 1992). The soil was contaminated with *Spodoptera frugiperda* multiple nucleopolyhedrovirus (SfMNPV) OBs. The fall armyworm, *S. frugiperda* (Lepidoptera: Noctuidae), is a major pest of maize from the southern United States to northern Argentina. SfMNPV OBs can be readily isolated from the soil of maize fields in the United States and Mexico (Fuxa et al., 1985; Rios-Velasco et al., 2011), and this virus has attracted the attention of biocontrol researchers as a potential biological insecticide in this region (Barrera et al., 2011; Williams et al., 1999). Laboratory studies were used to examine the effects of earthworm activity on the viability and vertical distribution of OBs.

2. Methods

2.1. Insects, earthworms, virus and soil

Larvae of *S. frugiperda* were obtained from a laboratory colony reared on a semisynthetic diet based on soya flour, wheat germ, yeast, agar, and vitamins (adapted from Greene et al., 1976). The colony was started using insects from a colony reared in the Universidad Michoacana de San Nicolás de Hidalgo, Michoacán, Mexico. Our colony was reared in an insectary at 25 ± 2 °C, 70–90% relative humidity and 16:8 h L:D photoperiod.

Specimens of *E. fetida* were obtained from a small scale vermicomposting plant used for processing coffee berry pulp in the Instituto de Ecología AC, Xalapa, Mexico. Earthworms were maintained in the insectary in plastic containers with ~50% coffee berry pulp supplemented with ~50% cow manure for 3–4 weeks prior to use in experiments.

A Nicaraguan wild-type isolate of SfMNPV (Simón et al., 2004, 2008) was amplified in *S. frugiperda* by the diet surface contamination technique. For this, fourth instar larvae were individually placed in 15 ml plastic cups and allowed to feed on a 2 g cube of diet contaminated with 5×10^8 OBs, at 25 ± 2 °C, 70–90% relative humidity and 16:8 h L:D photoperiod. The diet was replaced with non-contaminated diet after 4 days and virus-induced mortality was checked daily for the following 6 days. Virus killed insects were triturated in 0.1% (vol./vol.) Tween 80, filtered through a 80 μm pore nylon mesh, centrifuged at 2300*g* for 5 min and washed three times in sterile distilled water. The resulting OB suspension was counted in triplicate using a Neubauer chamber under a phase contrast microscope and stored at 4 °C for up to one month prior to use in experiments, without significant loss of OB activity.

An artificial soil was prepared according to the OECD pesticide testing protocol (OECD, 1984). Briefly, the dry components (70% wt/wt washed sand, 20% wt/wt kaolin, 10% wt/wt sphagnum peat) were mixed thoroughly using a kitchen spatula in a bucket and passed through a 2 mm pore sieve. The pH was measured and found to be within recommended limits (pH 7.0 ± 0.5). The soil was stored dry in the laboratory for up to one month until required in experiments.

2.2. Calibration of the OB detection technique

The presence of OBs in soil was detected using the soil-diet incorporation technique in which samples are mixed with insect diet and fed to early instar larvae that may become infected if OBs are present (Richards and Christian, 1999). To estimate the sensitivity of the OB detection technique, 10 g samples of soil were mixed with 1 ml of one of the following concentrations of SfMNPV OBs: 1×10^2, 1×10^3, 1×10^4, 1×10^5, 1×10^6, 1×10^7, 1×10^8, 1×10^9 OBs/ml, resulting in a range of concentrations of 10^1–10^8 OBs/g of soil.

Semisynthetic diet was prepared and when cooled to 45 °C each 10 g soil sample was incorporated into a 10 ml volume of diet. Each mixture was divided among 30 *S. frugiperda* second instars from the laboratory colony. After feeding individually on contaminated diet for 4 days, larvae were transferred to non-contaminated diet, reared individually and checked daily for virus-induced mortality until death or pupation. The procedure was performed on six occasions for all concentrations. Polyhedrosis disease deaths were confirmed by observation of Giemsa-stained smears using a phase contrast microscope (Lacey, 1997). The OB concentration-mortality response was estimated by logit regression in GLIM 4 (Numerical Algorithms Group, 1993).

2.3. Estimation of earthworm intestinal pH

As OBs are sensitive to alkaline conditions, the pH of earthworm gut was estimated using the following acid-base indicators: 0.1% methyl orange (pH 2.5–4.4), 0.5% wt/vol Congo red (pH 3.0–5.0), 0.1% bromocresol green (pH 4.5–5.5), 0.1% methyl red (pH 4.8–6.0), 0.1% bromothymol blue (6.0–7.6), 0.1% indigo carmine (pH 11.4–13.0). Earthworms were incubated in Petri dishes with moist filter paper discs for 24 h prior to evacuate most of the intestinal tract. Each earthworm was then sacrificed by immersion for 5 s in hot water and a section comprising the foregut (posterior to