EFFICIENCY OF *HETERORHABDITIS BACTERIOPHORA* (NEMATODA: HETERORHABDITIDAE) ON *ANASTREPHA SERPENTINA* (DIPTERA: TEPHRITIDAE) LARVAE UNDER LABORATORY CONDITIONS

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The sapote fruit fly, *Anastrepha serpentina* (Wiedemann), sometimes called the tropical fruit fly, is an important species in Mexico because its larvae infest sapote (*Calocarpum* spp.), mammee [*Pouteria sapota* (Jacq.) Moore & Stearn], sapodilla (*Achras zapota* L.), willowleaf lucuma (*Lucuma salicifolia* Hbk.) and related fruits (Aluja 1994). Infestations in tree-ripe fruits frequently are so high that in parts of the country where these fruits are grown, especially in Veracruz, the growers do not allow them to mature on the trees, but pick them green and ripen them artificially to avoid infestation. Mammee tree is native to Central America and southern Mexico and it is becoming important as an exotic fruit in international commerce. For this reason, the sapote fruit fly is part of the pest management program of the National Campaign Against Fruit Flies (CNCMF, after its Spanish acronym) (Reyes et al. 2000). Unfortunately, its control is mostly based on the use of chemical insecticides, applied either on the foliage to control adults or on the soil to control larvae or newly emerged adults. Consequently, new control alternatives are being explored, such as natural products and biological control agents, which may at least partially substitute for the chemical insecticides. This is an important strategy due to the growing interest in organic agriculture.

The entomopathogenic nematode *Heterorhabditis bacteriophora* (Poinar) is a natural soil dweller that parasitizes a number of insect species. Infection occurs through the insect's natural apertures such as the mouth, spiracles, or anus (Woodring & Kaya 1988). Once in the host hemocoel, the nematode releases its symbiotic bacterium *Photorhabdus* spp., which causes a rapid and lethal septicemia. This allows the growth and reproduction of the nematode for one or more generations. Due to its lethal efficiency, *H. bacteriophora* may become an important regulation factor for several insect populations whose larvae co-exist within the soil. This includes several species of fruit fly larvae (Tephritidae) whose susceptibility to nematode infection has been demonstrated previously (Beavers & Calkins 1984; Lindegren & Vail 1986; Lindegren et al. 1990; Lezama-Gutiérrez et al. 1996; Gazit et al. 2000; Toledo et al. 2001, 2005, 2006). In this report we present evidence on the infectivity of *H. bacteriophora* to third instars of *A. serpentina* under laboratory conditions.

Sapote fruit fly larvae were obtained from the mass rearing facility at Moscafrut Plant (SAGARPA-IICA), located in Metapa de Domínguez, Chiapas, México. They were reared on artificial diet, following the procedure and conditions described by Domínguez et al. (2000). The nematode was originally collected in Costa Rica with wax moth (*Galleria mellonella* L.) soil traps from a warm, rainy region, described by Castillo & Marbán-Mendoza (1996). The nematode was reared by infecting wax moth larvae, and infective juveniles (IJ) were collected in White traps (Woodring & Kaya 1988). IJs were quantified and working concentrations were adjusted to 800 IJ/mL in sterile, distilled water. Suspensions were stored at 10 ± 2°C until further use (Woodring & Kaya 1988).

Bioassays were performed on late, mature third instars of the sapote fruit fly with infection units made from PVC pipes 5 cm long and 5 cm in diameter (19.63 cm^2 surface). Each unit was filled with 70 g of sandy soil (96% sand, 3% clay, 1% lime, 0.18% organic matter, and adjusted to 6.6 pH), previously sieved (mesh 18), autoclaved, and adjusted to 15% mixture (weight/volume). A total of 25 larvae was added to each unit. Larvae immediately crawled into the soil (<10 min). The nematode IJ concentrations tested were 0, 6, 13, 25, 51, 76, 102, 127, and 178 IJ/cm^2 soil, added in 1 mL suspension and uniformly distributed on the soil surface. Infection units were incubated at 26 ± 1°C, 70 ± 5% RH, and L12:D12 photoperiod for 7 d. After this period, soil was sieved to separate larvae and pupae, and mortality was quantified under a dissecting microscope to verify nematode infection. To estimate an LC_{50}, a total of five replicates was performed and data were subjected to Probit analysis (SAS Institute 1992), in which statistical requirements were fulfilled as described by Ibarra & Federici 1987.

Once an LC_{50} was estimated, a simple test on the dispersion of mortality was performed by testing the LC_{50} and three times the LC_{50}, under the

same bioassay conditions. A total of five replicates was carried out and statistical difference was analyzed by Student's *t* test (Steel & Torrie 1993).

The LC_{50} of *H. bacteriophora* infective juveniles tested on late third instars of the sapote fruit fly was estimated at 36.0 ± 5.4 IJ/cm^2 (n = 491; $\chi^2 = 3.6$; Y = 3.00 + 1.28 X), within highly precise fiducial limits (26.7-46.4). The LC_{95} was estimated at 686 IJ/cm^2. The negative control never showed infection and the natural mortality was always around 2%, with >90% adult emergence. In the dispersion of mortality test, although mortality caused by the LC_{50} and three times the LC_{50} (108 IJ/cm^2) showed a statistically significant difference (t = -3.5; df = 4; P = 0.001), actual mortality barely increased, ranging only from 42.4 ± 2.0% at LC_{50} to 54.5 ± 2.7% at three times the LC_{50}. These results indicate that, in spite of the low number of IJs required to kill 50% of the larval population, a much larger number of nematodes is required to kill a significant proportion of the insect population. According to these results, approximately 700 IJ/cm^2 are necessary to obtain significant control levels, which is close to the estimated LC_{95}.

This is the first report on the susceptibility of sapote fruit fly larvae to *H. bacteriophora* under laboratory conditions. Based on our finding this nematode can be considered a potential biological control agent for this pest, and the results should be corroborated under field conditions. The test was conducted on third instars because it is the only larval stage that may be in contact with the soil under natural conditions. The highest tolerance to nematode infection occurs in the 3rd instar, as observed in other *Anastrepha* species (Toledo et al. 2005). However, the invasive ability of the nematode varies not only among the different species but also between strains of the same species, as observed when different species and strains of nematodes were tested against *A. suspensa* (Beavers & Calkins 1984).

Laboratory tests on the infectivity of nematodes are important because they are performed under controlled, optimum conditions. The interaction host/parasite is tested without the influence of other factors that may be found in the field. It is known that *H. bacteriophora* moves easily in the soil, showing a high ability to find hosts at different soil depths (Campbell et al. 1996). However, its performance can be severely hampered by some soil factors such as texture, pH, humidity, and possibly other factors. In general, these factors influence the failure or success of these control agents when tested under field conditions (Portillo-Aguilar et al. 1999). Fruit fly larvae also are influenced by these factors (Eskafi & Fernández 1990; Jackson et al. 1998; Alyokhin et al. 2001), and especially the soil compactness (Aluja 1994), which can influence nematode infectivity (Portillo-Aguilar et al. 1999).

Moisture is another important factor. IJs of *H. bacteriophora* are more infective in sandy-clay soils with 15% moisture (Toledo et al. 2006), while in sandy soils the optimum is at 10% moisture (Toledo et al. 2005). Slightly higher or lower moisture levels drastically decrease its infective efficiency. High moisture content in a sandy soil may slow down the IJ's movement, due to an excess of water between the particles, while a low moisture content may limit the search for hosts. The effect of humidity may vary among the nematode species. The nematode *Steinernema riobrave* kept its infectivity to the Mediterranean fruit fly larvae in a sandy-clay soil at humidity levels ranging from 3 to 20% (Gazit et al. 2000).

Heterorhabditis bacteriophora has shown its potential as a biological control agent in the field, against other fruit fly larvae (Toledo et al. 2006). A field test on the sapote fruit fly is feasible, and should be followed by an analysis of economical and practical viability.

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Summary

The infectivity of the entomopathogenic nematode *Heterorhabditis bacteriophora* was tested on third instars of the tropical fruit fly, *Anastrepha serpentina*, under laboratory conditions. An LC_{50} was estimated at 36.0 ± 5.4 IJ /cm^2 of sandy soil, adjusted to 15% humidity, with 5-cm-deep infectivity units. Significant amounts of nematodes are required to obtain satisfactory control levels, as shown by 3× the LC_{50} value. This is the first report on the susceptibility of the tropical fruit fly larvae to *H. bacteriophora*. Potential of this nematode as a biological control agent of this pest should be corroborated under field conditions.

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