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POTENTIAL OF *STEINERNEMA CARPOCAPSAE* (RHABDITIDA: STEINERNEMATIDAE) AGAINST *HYPOTHENEMUS HAMPEI* (COLEOPTERA: CURCULIONIDAE) IN HAWAII

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Hypothenemus hampei (Ferrari), the coffee berry borer (CBB), is the world's most devastating insect pest of coffee (Vega et al. 2009; Vera et al. 2011). Entomopathogenic nematodes (EPNs) are a type of natural enemy, which has potential as a commercial biopesticide against CBB (Fig. 1). For control of CBB, EPNs could either be sprayed onto coffee berries while they are still on the tree, or onto berries that have fallen to the ground (Lara et al. 2004). The latter has been studied more intensively in EPNs because the natural habitat of these nematodes is on or in sand or soil. Nematodes in the genera *Heterorhabditis* and *Steinernema* have proven effective against CBB in several laboratory studies using infested berries held over sand (Allard & Moore 1989; Castillo & Marbán-Mendoza 1996; Molina & López 2002). Success rates were variable, likely owing to differences in experimental design. For example, mortality rates differed greatly depending on type of coffee used (parchment versus fresh), and the holding method following application (over sand on a petri dish versus over filter paper versus held outside under trees).

In Hawai'i, importation restrictions prohibit the field use of all commercially available insect-killing nematode species except *Steinernema*

carpocapsae (Weiser). Due to this restriction, it is important to establish the potential of *S. carpocapsae* as a biopesticide for CBB, because this potentially devastating pest was recently detected in Hawai'i (Burbano et al. 2011). Castillo & Marbán-Mendoza (1996) showed that *S. carpocapsae* could kill up to 90% of CBB adults when parchment coffee was held in Petri dishes, but no significant CBB mortality occurred in fresh coffee. Here, we present evidence that *S. carpocapsae* has the ability to both enter fresh coffee berries and to cause significant mortality to multiple life stages of CBB in the field.

To evaluate CBB mortality, laboratory and field tests were carried out in which CBB-infested berries picked from trees were sprayed with nematodes and held on soil. In the laboratory test, carried out in Mar 2012, infested coffee berries from Kona, Hawai'i (coordinates N 19.589954° W -155.952217°) were held in 2-L, round plastic containers (4 per treatment group) that held 250 g of soil (37% moisture, collected from a coffee farm at Captain Cook, Hawaii). Soil was placed on top of a 2.5 cm thick plaster of Paris/charcoal brick (10:1 ratio) (Premachandra et al. 2005) to buffer humidity changes within each container. Infested coffee berries were placed on top of the soil in a single layer. *Steinernema carpocapsae* (Millenium®, Becker-Underwood, Ames, Iowa) were applied to the coffee berries using hand sprayers to the point of runoff with 4,600 infectious juveniles (IJ)/mL in test 1. In each container, 20 mL of fluid was sprayed over an area of approximately 133 cm². Containers were fitted with a solid lid for the first 24 h following application, and a ventilated lid thereafter. Containers were held in an environmental chamber (70% RH, 12:12 h L:D cycle) for a period of no less than 8 d at 20 °C. Humidity was continuously measured using HOBO Pro V2 data loggers (Onset Corp., Cape Cod, Massachusetts).

In the field test, carried out in May 2012, mature infested coffee berries were picked from trees then placed as a single layer on the soil surface in 3 adjacent areas encircling the base of each of 4 selected coffee trees. Beneath each tree, in 2 of the single-layer piles, coffee berries were covered with approximately 1 in (2.54 cm) of leaf litter. In

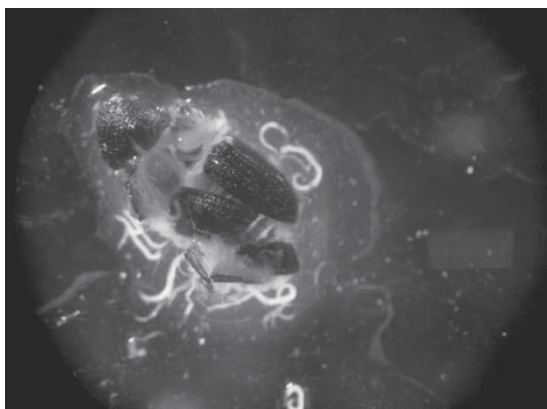


Fig. 1. Dead *Hypothenemus hampei* adult torn open in a small amount of water to release living *Steinernema carpocapsae* nematodes within insect's body.

a third pile, leaf litter was omitted. One of the 2 piles with leaf litter under each tree was sprayed with 500 mL of *Steinernema carpocapsae*. The other pile with leaf litter was sprayed with a matching volume of water. The remaining pile with no leaf litter was also treated with 500 mL of nematodes. Each pile was estimated to cover a similar surface area as the laboratory test, (133 cm²). Coffee berries were left to sit in the field for 7 d. During this time HOBO Pro V2 data loggers (Onset Corp., Cape Cod, MA, USA) were used to record temperature and RH. An RS-232 RainLog Annual Rain Data Logger (Rainwise, Inc., Bar Harbor, Maine) was used to record rainfall. After coffee berries were collected from the field, they were held in ventilated containers with the same conditions described for the laboratory test (4 containers for each of the 3 treatments over a saturated 2.5 cm thick plaster of Paris/charcoal brick (10:1 ratio) (Premachandra et al. 2005). Environmental chambers were held at 70% RH (12:12 h L:D cycle), at 20 °C until the time of dissection. For both experiments, dissections were completed during d 8-14, and number of living, dead, and nematode-killed insects were tallied for each life stage. Nematode-killed insects were identified by tearing apart dead insects in a small volume of water and observing the nematodes released. Occasionally positive diagnoses were based on signs only (hollow empty adult bodies with slime residue or yellow/brown deflated larvae).

For purposes of statistical analysis, each ventilated container holding infested coffee berries was considered an experimental replicate, and the percentage of nematode-caused mortality was used as the dependent variable after arcsin square-root transformation (Steel & Torrie 1980).

In laboratory and field experiments where *S. carpocapsae* was applied to infested coffee berries, there was evidence that infective juveniles of the species were capable of entering the coffee berries and killing insects of all mobile life stages. In the laboratory trial, CBB inside coffee berries treated directly with nematodes had an average nematode-caused CBB mortality of 26.6% in adults and 23.7% in larvae (Fig. 2). There was no nematode-caused mortality in the controls, and

mortality from other causes averaged 1.6% percent and 0% for adults and larvae, respectively. A t-test confirmed that nematode-caused mortality was significantly greater in the nematode-treated coffee berries ($t = -14.5$; $P = 0.0004$, $df = 3$ (SAS Institute 2010). Results presented here for our lab trial were consistent with results obtained in several preliminary bioassays in which *S. carpocapsae* was sprayed on coffee cherries held at high humidity in ventilated containers at 20 °C for 9-10 d. These bioassays resulted in > 20% mortality of larvae, whereas controls sprayed with water were unaffected (data not shown).

In the field trial, nematode-caused mortality of CBB in berries treated directly with the nematode solution averaged 4.7% in adults and 17.1% for larvae (Fig. 2). Mortality of CBB adults and larvae from other causes averaged 2% and 1.7%, respectively. Addition of mulch as a humidity buffer prior to application of the nematode solution had relatively little effect, as nematode-caused adult and larval mortality under these conditions averaged 7.4% and 12.8%, respectively. Mortality from other causes averaged 4.7% and 6.2% for adults and larvae, respectively. There was no nematode-caused mortality in the berries treated with water only (Fig. 2). Mortality from other causes averaged 3.8% and 2.8% for adults and larvae, respectively. Using Dunnett's Method for treatment versus control comparisons with an α of 0.05, nematode-caused mortality of both adults and larvae dissected from berries used in the leaf mulch/nematode treatment was significantly greater than mortality of insects dissected from berries treated with water only (SAS Institute 2010). For the nematode only treatment (no leaf litter), nematode-caused mortality of adults did not differ significantly from the water control, but nematode-caused mortality of larvae was significantly higher (SAS Institute 2010). In a small percentage of cases, nematodes appeared to complete their life cycle within the host. In these cases, dozens of IJ spilled out of the body of a single adult or larvae when it was torn open within a few drops of water.

In the laboratory trial, humidity stayed at 100% throughout the test within containers. In the field test, RH in the coffee field fluctuated between 50%-100%, and temperature fluctuated daily between 18-28.5 °C, with an average of low of 18 °C and an average high of 26.5 °C. During this week-long period the field received 0.66-cm of rainfall.

In separate Petri dish trials, the lot of commercial nematodes used in both lab and field experiments were tested for viability and to gain a first look at how frequently *S. carpocapsae* reproduces in CBB. In this bioassay, we exposed CBB larvae and adults to *S. carpocapsae* on filter paper treated with 1 mL of water containing 2500 IJ per mL within 10-cm diam Petri dishes.

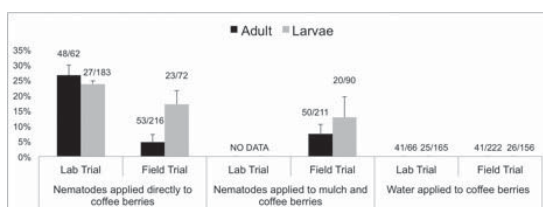


Fig. 2. Percent nematode-caused mortality (No. berries/No. insects) of *Hypothenemus hampei* in 2 experiments in which *Steinernema carpocapsae* IJs were applied to infested coffee berries.

After 3 d, all CBB larvae were dead while 55% of adults were still alive. Dead insects were transferred to white traps and examined 6-7 d later. While nematode recovery from most insects was poor, one adult beetle and one larva were found to contain more than 60 and 50 living IJ, respectively.

Our data shows the potential of *S. carpocapsae* as a control for CBB in fallen coffee berries in coffee fields. We found all life stages of CBB being killed by *S. carpocapsae*, with highest mortality in larvae. Low mortality in controls supports that nematode infections were, in fact, the cause of death in treated coffee berries. The commercial availability of *Steinernema carpocapsae* in Hawai'i, combined with laboratory and field evidence that the nematode can kill CBB, suggest new avenues for research. These preliminary observations show that *S. carpocapsae* has potential to reduce CBB populations in coffee farms where berries have fallen to the ground during picking, provided large numbers of nematodes are applied in generous volumes of carrier.

Manual removal of over-ripe and dried coffee, coupled with sanitation of dropped cherries have proven to be very effective in Columbia (Aristizábal et al. 2011). However, in Hawaii labor prices are high, and good sanitation practices may be impractical on some farms, such as those using mechanical harvesting or those which have very rocky soil. Given the costs of picking up coffee from the ground on such farms, there is a greater need to find alternative methods for controlling CBB in fallen coffee, such as the, use of EPN's.

In the future, we plan to explore various adjuvants in an attempt to enhance the efficacy of the nematodes. We also would like to understand if there are any synergistic effects when *S. carpocapsae* is combined with another entomopathogen, *Beauveria bassiana* (Bálsamo) Vuillemin. Although the application of nematodes alone might be insufficient as a control measure, they could be incorporated into an integrated pest management (IPM) plan in Hawai'i, and may partially substitute for sanitation practices where labor costs are expensive.

SUMMARY

Evidence is provided here that *Steinernema carpocapsae*, an entomopathogenic nematode, has the ability to both enter fresh coffee berries, and kill different life stages of the coffee berry borer (*Hypothenemus hampei*) both in the laboratory and field. In Hawai'i, CBB was reported in 2010, and currently importation of all commercial nematode species are banned with the exception of *S. carpocapsae*. These data indicate entomopathogenic nematodes deserve more research as a potential biopesticide against CBB in Hawai'i.

RESUMEN

La evidencia del nemátodo entomopatogénico *Steinernema carpocapsae* ha sido provada. Este nemátodo tiene la habilidad de entrar y de matar los diferentes estados biológicos de la broca del café (*Hypothenemus hampei*) tanto en laboratorio como en condiciones de campo. En Hawaii, la broca del café fue reportada en 2010 y actualmente la importación de todas las especies de nemátodos comerciales son prohibidas, excepto para *S. carpocapsae*. Los datos indican que los nemátodos entomopatogénicos requieren mas investigación como potenciales agentes biopesticidas contra la broca del café en Hawaii.

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