BIOCONTROL SCIENCE AND TECHNOLOGY
2019, VOL. 29, NO. 8, 786–795
https://doi.org/10.1080/09583157.2019.1597336

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

Sterility of the coffee berry borer, *Hypothenemus hampei* (Coleoptera: Curculionidae), caused by the nematode *Metaparasitylenchus hypothenemi* (Tylenchidae: Allantonematidae)

Alfredo Castillo[a], Félix Martínez[a], Jaime Gómez[a], Juan Cisneros[a] and Fernando E. Vega [b]

[a]Ecología de Artrópodos y Manejo de Plagas, El Colegio de la Frontera Sur (ECOSUR), Tapachula México; [b]Sustainable Perennial Crops Laboratory, United States Department of Agriculture, Agricultural Research Service, Beltsville, MD, USA

ABSTRACT

Metaparasitylenchus hypothenemi is an endoparasitic nematode that causes partial or total sterility of coffee berry borer (*Hypothenemus hampei*) females, although the causes are unknown. Fecundity and the average size of the common and lateral oviduct, vitellarium, and germarium in the four ovarioles (I, II, III and IV) were compared between parasitised and non-parasitised insects to determine the causes of sterility. The nematode significantly lowers the number of oocytes and 86% of parasitised insects (24 out of 28 insects) were sterile, while fecundity in the remaining 13% was non-significantly different to that in non-parasitised insects. No significant differences were recorded in the size of the common oviduct, lateral oviduct, vitellarium, and germarium between parasitised and non-parasitised insects and the nematode does not cause any apparent damage on the surface of the ovary.

ARTICLE HISTORY
Received 2 February 2019
Accepted 10 March 2019

KEYWORDS
Endoparasite; potential fecundity; realised fecundity; egg fertility; oocytes

Introduction

Metaparasitylenchus hypothenemi (Tylenchida: Allantonematidae) is the only nematode reported in the Americas that naturally parasitises the coffee berry borer (*Hypothenemus hampei* (Ferrari); Coleoptera: Curculionidae: Scolytinae), the most important insect pest of coffee worldwide (Vega, Infante, & Johnson, 2015). This obligate endoparasite was first discovered affecting the reproduction of coffee berry borer females in the laboratory (Castillo & Barrera, 1998), and subsequently it was found in the field in various areas of the Soconusco region in the state of Chiapas, Mexico (Castillo, Infante, Barrera, Carta, & Vega, 2002; Pérez, Infante, Poinar, Castillo, & Vega, 2015 Poinar, Vega, Castillo, Chavéz, & Infante, 2004;). Several entomopathogenic nematodes have been tested against the coffee berry borer and results have been reviewed by Vega et al. (2015). Parasitic nematodes of insects have been associated with many species of bark beetles

CONTACT Alfredo Castillo acastill@ecosur.mx Ecología de Artrópodos y Manejo de Plagas, El Colegio de la Frontera Sur (ECOSUR), Carretera Antiguo Aeropuerto Km. 2.5, Tapachula, Chiapas, 30700 México

(Wegensteiner, Wermelinger, & Herrmann, 2015) and their effects on its hosts have been reviewed by Hofstetter, Dinkins-Bookwalter, Davis, and Klepzig (2015).

Metaparasitylenchus hypothenemi causes partial or total sterility of coffee berry borer females (Castillo et al., 2002), although the biology of this endoparasite and its interaction with its host has not been studied in detail. The nematode has the potential to cause physical or mechanical damage to internal tissues because its developmental stages occur within the host (Poinar et al., 2004). However, it is not known whether nematode parasitism of the coffee berry borer causes damage to the reproductive organs. *Parasitaphelenchus papillatus* (Aphelenchida: Aphelenchidae) causes a reduction in the size of the seminal vesicles of the pine shoot beetle, *Tomicus piniperda* (L.) (Coleoptera: Curculionidae: Scolytinae; Tomalak, Michalsky, & Grocholsky, 1984). Significant damage to the genital structures has been reported for *Tomicus minor* (Hartig) parasitised by species of *Allantonema*, *Parasitaphelenchus*, and *Parasitylenchus*, whose juveniles pierce the ovaries, causing partial or total destruction (Gurando & Tsarichkova, 1974).

We compared various parameters in parasitised and non-parasitised coffee berry borers, including fecundity, average size (μm ± S.E.) of the common and lateral oviduct, vitellarium, and germarium in the four ovarioles. Scanning electron microscopy (SEM) was used to visually assess if the nematode had caused any damage to the ovary. The results are a contribution towards a better understanding of the interaction of *M. hypothenemi* with the coffee berry borer.

Materials and methods

Biological material

Coffee berry borers were obtained from infested robusta coffee berries (*Coffea canephora* Pierre ex. A. Froehner; Rubiaceae) collected in three commercial coffee plantations in the municipality of Cacahoatán, Chiapas, México: (1) La Unidad (15°01' N, 92°17'W; 612 masl); (2) San Antonio (15°05N, 92°15'W; 556 masl); and (3) La Alianza (15°44'N, 92° 18'W; 673 masl). The berries were kept in the laboratory (28 ± 2°C; 60–70% RH) in 3.5 L ventilated plastic containers to obtain the insects used in the experiments described below.

Fecundity

Fecundity was assessed in two cohorts of insects. The first cohort consisted of females emerging from the coffee berries collected in the field. The assessment was based on determining the number of oocytes. A total of 42 parasitised and 42 non-parasitised insects collected from infested coffee berries were dissected using the technique described in Poinar et al. (2004). The entire ovary was removed using entomological pins and potential fecundity was based on the presence of oocytes in the vitellar body. Nematode parasitism was determined by dissecting each insect on a drop of Hartmann's solution (HT PISA®, Laboratorios PISA, Guadalajara, México) placed on an excavated slide.

The second cohort consisted of females reared on artificial diet and was based on the number of eggs that were oviposited. The insects were obtained from coffee berries

collected in a plantation with previous records of nematode parasitism (Pérez et al., 2015). Insects ($n = 118$) were dissected from the berries and disinfected in a 3% sodium hypochlorite solution for 3 min followed by washing with distilled water before being placed individually in a 1.5 × 7.5 cm glass vial containing ca. 4.5 ml of a meridic diet (Villacorta & Barrera, 1993). Each vial was covered with cotton fibre and kept in the laboratory (28 ± 2°C; 60–70% RH) for 40 days, at which time the progeny produced by each female and the presence or absence of parasitism by the nematode were recorded. Nematode parasitism was determined as described above. Insect fertility was based on the number of viable eggs (resulting in larval and pupal development) compared to total number of eggs.

Size of ovary

The size of the ovary was measured in parasitised ($n = 12$) and non-parasitised ($n = 13$) insects. Insects were individually dissected under the stereoscope on a drop of Hartmann's solution to extract and measure each segment of the ovary: length of the lateral oviduct (LLO), common oviduct (LCO), germarium (LG), and vitellarium (LV), as well as the width of germarium (WG) and vitellarium (WV) in the four ovarioles (I, II, III, IV) (Figure 1). All measurements were done using a compound microscope (Model CX31, Olympus®, Tokyo, Japan) connected to a digital ruler (10X) and a digital camera (Jenoptik ProgRes® CT5, Optical Systems, Goeschwitzer, Germany).

Observations of ovarian surface

The surface of ovaries from parasitised ($n = 3$) and non-parasitised ($n = 3$) borers was examined using SEM and prepared as follows: the ovaries were individually placed in 3% glutaraldehyde and a 0.1 M, pH 7 buffer phosphate solution for 18 h. They were then dehydrated in 30%, 50%, 70%, 90%, and 100% ethanol for 1 h followed by critical point drying (SPI-Dry Critical Point Dryer, SPI Supplies, Pasadena, CA, USA). They were then individually placed on slides and coated with gold palladium using a sputter coater (Desk II TSC, Denton Vacuum LLC, Moorestown, NJ, USA). SEM observations were done using a Topcon SM-510 SEM (Tokyo, Japan) operating at 10 kV. Images were digitised using Image Tool for Windows, Version 3.0 (Wilcox, Dove, McDavid, & Greer, 2002).

Statistical analysis

A logistic regression model with a binomial response was used to compare the fecundity between parasitised and non-parasitised females (presence of oocytes in the vitellarium and females that laid eggs), while the data on the progeny size were analysed using a logistic regression model with a negative binomial response (Venables & Ripley, 2002). The data on the size of the reproductive structures were analysed using Student's t-test; females with oocytes were not included to reduce standard deviations. Statistical analyses were conducted using R version 3.4.3 (R Core Team, 2017).

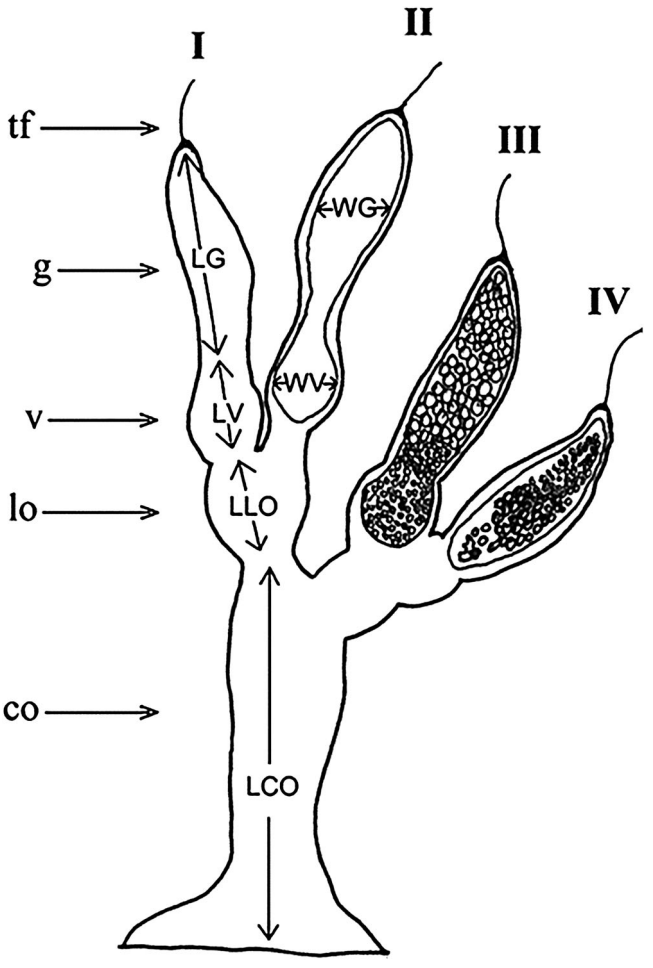

Figure 1. Diagrammatic representation of the coffee berry borer ovary showing terminal filament (tf), germarium (g), vitellarium (v), lateral oviduct (lo), common oviduct (co) and four ovarioles (I, II, III and IV). Arrows inside ovary show length of germarium (LG), length of vitelarium (LV), length of lateral oviduct (LLO), length of common oviduct (LCO), width of germarium (WG), and width of vitelarium (WV).

Results

Fecundity

The number of parasitised and non-parasitised coloniser females, with and without oocytes in the vitellarium was significantly different ($\chi^2 = 22.1$, $df = 1$, $P = 0.000003$) (Figure 2). There were no oocytes inside the vitellarium in 59.5% (i.e. 25 of 42 insects) of the parasitised insects, while in non-parasitised females 88.1% (i.e. 37 of 42 insects; Figure 2) had oocytes inside the vitellarium. These results suggest that the reduction in oocytes was caused by nematode parasitism.

Nematode parasitism was 23.7% (i.e. 28 of 118 borers), with an average of 81.2 ± 7.1 infective juveniles per female with progeny ($n = 4$) and 131.9 ± 1.9 infective juveniles per female without progeny ($n = 24$). The number of non-parasitised females that laid eggs

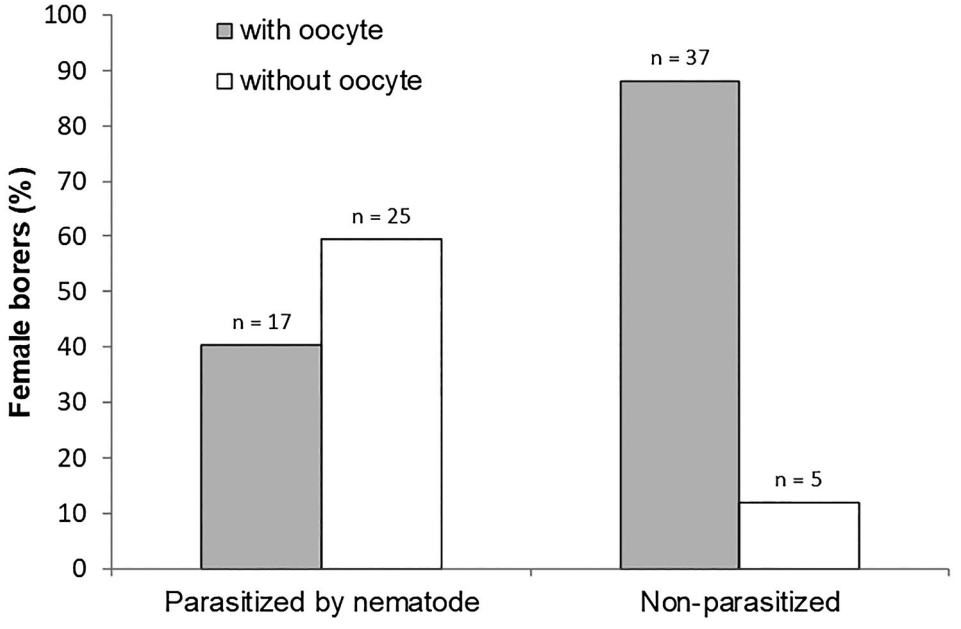

Figure 2. Percentages of nematode parasitised and non-parasitised coffee berry borers with and without oocytes in the vitellarium. The number of parasitised and non-parasitised insects with and without oocytes was significantly different ($\chi^2 = 22.14$, d$f = 1$, $P = 0.000002$).

(47.8%; i.e. 43 of 90) was significantly higher than in parasitised females (14.3%; i.e. 4 of 28 insects) ($\chi^2 = 11.1$, d$f = 1$, $P = 0.0008$). More than half of the non-parasitised insects (52.2%; i.e. 47 of 90) did not lay eggs, which might be a result of non-mating. Therefore, nematode parasitism was partially responsible for 85.7% (i.e. 24 of 28 insects) sterility of females, with the possibility of non-mating by some females also being a contributor factor.

Parasitism did not affect fecundity in the four females that oviposited based on an average progeny per insect (6.5 ± 0.9) non-significantly different to non-parasitised insect (9.8 ± 0.8) ($\chi^2 = 1.5$, d$f = 1$, $P = 0.215$). In this experiment, 60.2% (i.e. 71 of 118 borers) of the insects did not oviposit and only two non-parasitised females laid eggs that did not hatch.

Size of ovary

There were no significant differences in the average length of the four areas of the ovarioles between parasitised and non-parasitised insects (Table 1): length of the common oviduct ($t = 0.52$, d$f = 23$, $P = 0.61$); lateral oviduct ($t = 0.57$, d$f = 23$, $P = 0.57$); vitellarium ($t = 0.04$, d$f = 23$, $P = 0.97$); and germarium ($t = 0.77$, d$f = 23$, $P = 0.45$), as well as the width of vitellarium ($t = 0.42$, d$f = 23$, $P = 0.67$), and germarium ($t = 0.41$, d$f = 23$, $P = 0.68$).

Observations of ovarian surface

There was no evidence of damage on the surface of the ovary (Figure 3) in parasitised or non-parasitised insects. During the dissections, nematodes were observed within the alimentary canal and were never observed piercing or within of ovaries.

Table 1. Average size (μm ± S.E.) of common oviduct, lateral oviduct, vitellarium and germarium in the four ovarioles (I, II, III and IV) of the coffee berry borer (parasitised by *M. hypothenemi* and non-parasitised). There were no significant differences in any of the variables tested.

Segment	Parasitised ($n = 12$)	Non-parasitised ($n = 13$)	P
Length of common oviduct	401.6 (±13.1)	417.6 (±20.4)	0.611
Length of lateral oviduct I	26.9 (±1.9)	32.3 (±2.1)	0.157
Length of lateral oviduct II	25.4 (±1.9)	28.6 (±1.7)	0.331
Length of lateral oviduct III	33.4 (±2.3)	28.7 (±1.8)	0.232
Length of lateral oviduct IV	27.3 (±1.8)	29.5 (±1.3)	0.695
Length of vitellarium I	84.5 (±5.3)	99.8 (±5.6)	0.138
Length of vitellarium II	99.0 (±9.2)	104.0 (±13.4)	0.811
Length of vitellarium III	93.9 (±5.0)	84.7 (±5.3)	0.342
Length of vitellarium IV	109.9 (±14.5)	96.7 (±9.2)	0.563
Width of vitellarium I	57.6 (±4.7)	70.9 (±5.5)	0.184
Width of vitellarium II	59.6 (±4.9)	60.9 (±4.9)	0.878
Width of vitellarium III	57.7 (±4.3)	57.6 (±2.6)	0.978
Width of vitellarium IV	68.8 (±9.7)	69.0 (±6.5)	0.987
Length of germarium I	209.1 (±8.7)	216.0 (±15.9)	0.978
Length of germarium II	233.9 (±13.6)	201.8 (±15.6)	0.257
Length of germarium III	224.8 (±11.6)	208.6 (±9.6)	0.419
Length of germarium IV	239.8 (±11.0)	223.4 (±11.6)	0.433
Width of germarium I	60.2 (±2.3)	57.6 (±2.8)	0.580
Width of germarium II	61.8 (±2.9)	59.5 (±2.8)	0.668
Width of germarium III	66.9 (±2. 5)	66.6 (±2.72)	0.952
Width of germarium IV	64.6 (±2.5)	63.0 (±4.02)	0.800

Discussion

The parasitic stages of allantonematids absorb nutrients through their body wall from the host's hemocoel. The stylet and associated penetration glands of the nematodes are only used to perforate the cuticle of the host for entrance into the hemocoel and not for withdrawing nutrients. Allantonematids usually partially sterilise their hosts and may also reduce the host's lifespan (Poinar, 1983). The present study shows that not just partial sterility but sometimes complete sterility of coffee berry borer females are brought about by the endoparasitic nematode *M. hypothenemi*.

Our findings indicate that the reduction in oocytes was caused by nematode parasitism. The suppression of oocyte development caused by parasitic nematodes in some species of scolytids has also been previously reported (Pouvreau, 1962; Thong & Webster, 1975a) and has been attributed to several causes. In *Scolytus ventralis*, the inhibition of the development of oocytes could be due to a secretion of toxins by the larvae of the nematode *Sulphuretylenchus elongatus* (Nematoda: Sphaerulariidae) or may result from nematodes feeding on gonadal tissue (Ashraf & Berryman, 1970a). Lower egg production by *C. brevicomi*-parasitised *D. pseudotsugae* may be due to depletion of hemolymphatic proteins (Thong & Webster, 1975b). Pouvreau (1962) concluded that toxins produced by a parasitic sphaerulariid nematode seriously affected the corpora allata and the vitellogenesis on *Bombus* spp (Hymenoptera: Apidae). The inhibition of the development of oocytes suggests a physiological response involved in the loss of fertility in coffee berry borer females, possibly as a consequence of nutritional and/or metabolic stress. However, further studies are needed to determine if the sterility caused by nematode parasitism in the coffee berry borer is a consequence of the decrease and/or reabsorption of the nutrients of the oocytes for formation of the vitellarium.

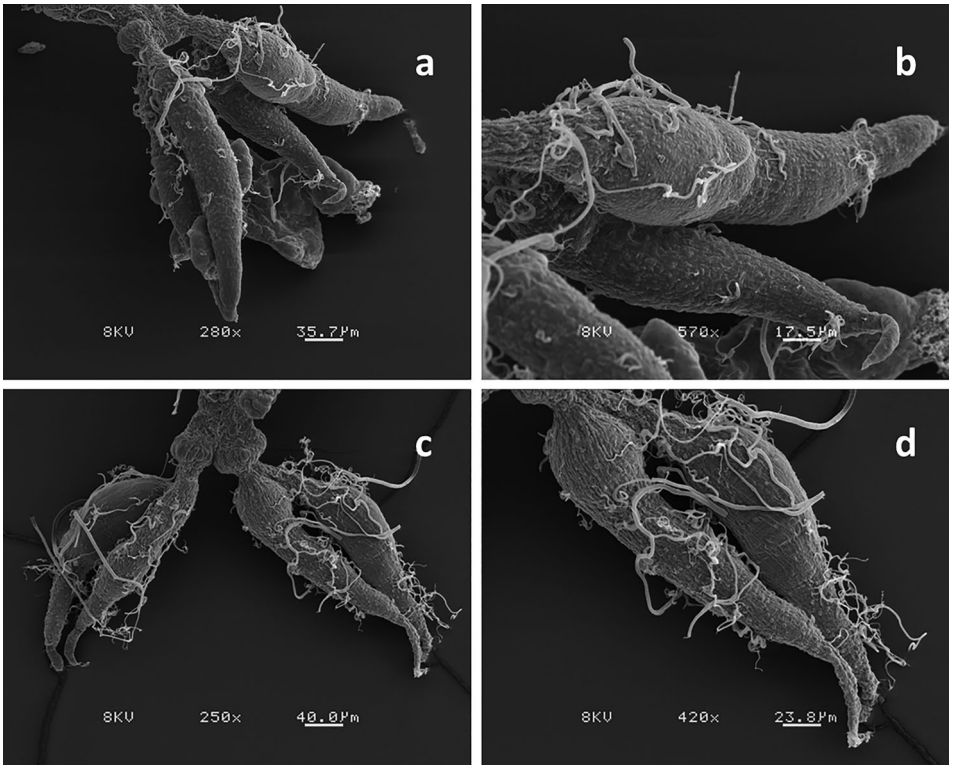

Figure 3. Scanning electron microscopy images of ovaries in non-parasitised (a, b) and nematode parasitised coffee berry borers (c, d). There was no evidence of nematode-inflicted damage.

The coffee berry borer reproduces sexually (Bergamin, 1943; Vega et al., 2015), and López and Frérot (1993) reported that 37% of female adults leaving the berry were not fertile because they had not mated. It is possible that the 86% sterility observed in the parasitised insects (i.e. 24 of 28 insects) in the present study, could be the result of an additive effect caused by the lack of mating and by nematode parasitism. Non-mating could also explain the absence of progeny in non-parasitised insects (i.e. 52% of the insects did not oviposit).

Thong and Webster (1975b) and MacGuidwin, Smart, Wilkinson, and Allen (1980) have reported lower fecundity in females of *Dendroctonus frontalis* Zimmerman and *D. pseudotsugae* Hopkins (Coleoptera: Curculionidae: Scolytinae) parasitised by *Contortylenchus brevicomi* (Nematoda: Sphaerulariidae); the nematode also causes partial sterility in males of *D. frontalis*. In the present work, the reproductive activity of coffee berry borer males was not analysed.

Parasitic nematodes can also cause direct damage when they feed on the reproductive tissue of its host (Hocking, 1967; Tomalak et al., 1984), as well atrophied reproductive structures (Hocking, 1967 Oldham, 1930;). We did not observe a variation in size, possible damage, or any deformation in the ovaries. However, the size of the ovaries was smaller than reported by Rubio, Bustillo, Vallejo, Benavides, and Acuña (2007), probably because we only included ovaries without oocytes in the analysis. We never observed nematode juveniles perforating the reproductive system or within any reproductive

structure. Saunders and Norris (1961) reported little or no damage caused by parasitic nematodes to the reproductive tissue of the elm bark beetle (*Scolytus multistriatus* (Marsham); Coleoptera: Curculionidae: Scolytinae). Thus, it appears that the sterility observed in nematode-parasitised female coffee berry borers is not due to damage caused by the nematode to the reproductive system.

Ashraf and Berryman (1970b) attribute the degree of *S. ventralis* sterility to the intensity of nematode infection, which appeared to be associated with larval hatching, based on the number of larvae inside the host. However, the sterility observed in the coffee berry borer is not gradual and does not seem to be associated with the number of nematodes observed within the sterile insects, perhaps because *M. hypothenemi* females are ovoviviparous (larvae hatch inside the female).

The reduction of the reproductive activity in a host has been considered as an evolutionary strategy in the parasites, since they increase the available energy for themselves, lengthening the survival and increasing the growth of the host (Baudoin, 1974). This sterility caused by the nematode on the coffee berry borer could be seen as a strategy of the parasite to route resources for its own development versus that of the insect eggs. However, this would need to be experimentally determined.

Coffee berry borer females infected with the nematode did not transmit the infection to its own progeny within the diet, as has been reported to happen with the progeny of females of *Ips* species infected with endoparasitic nematodes (Hoffard & Coster, 1976). It is possible that *M. hypothenemi* might be causing alterations in the behaviour of the coffee berry borer since the parasitised fertile females laid their eggs on the surface of the diet, while the non-parasitised insects, even without progeny, constructed normal galleries in the meridic diet. Although this behavioural change in other species of Scolytinae parasitised by nematodes had also been reported (Thong & Webster, 1975b), these preliminary observations suggest that more research on this interaction is required for the coffee berry borer.

Overall, at this point we cannot state that *M. hypothenemi* has a strong potential to reduce coffee berry borer populations in the field based on the fact that only 24 out of 118 insects (20%) parasitised by the nematode were sterile, and that non-mating likely contributed to this sterility.

Acknowledgments

We thank Marina Simota Ruíz for technical assistance, Javier Valle Mora for valuable statistical support, and Roxana Bautista Arredondo for assistance with scanning electron microscopy. Comments by two anonymous reviewers are greatly appreciated.

Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Fernando E. Vega http://orcid.org/0000-0001-8103-5640

References

Ashraf, M., & Berryman, A. A. (1970a). Biology of *Sulphuretylenchus elongatus* (Nematoda: Sphaerulariidae), and its effect on its host, *Scolytus ventralis* (Coleoptera: Scolytidae). *Canadian Entomologist*, *102*, 197–213.

Ashraf, M., & Berryman, A. A. (1970b). Histopathology of *Scolytus ventralis* (Coleoptera: Scolytidae) infected by *Sulphuretylenchus elongatus* (Nematoda: Sphaerulariidae). *Annals of the Entomological Society of America*, *63*, 924–930.

Baudoin, M. (1974). Host castration as a parasitic strategy. *Evolution*, *29*, 335–352.

Bergamin, J. (1943). Contribuição para o conhecimento da biologia da broca do café "*Hypothenemus hampei* (Ferrari, 1867)" (Col. Ipidae). *Arquivos do Instituto Biológico*, *14*, 31–72.

Castillo, A., & Barrera, J. F. (1998). Primer registro de nematodos parasitando a la broca del café en cafetales de México, Memorias de II Reunión Intercontinental sobre Broca del Café, Tapachula, Chiapas, México, pp. 47.

Castillo, A., Infante, F., Barrera, J. F., Carta, L., & Vega, F. E. (2002). First field report of a nematode (Tylenchida: Sphaerularioidea) attacking the coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae) in the Americas. *Journal of Invertebrate Pathology*, *79*, 199–202.

Gurando, O. V., & Tsarichkova, D. B. (1974). Changes in the sex apparatus of females of the small pine bark-beetle caused by parasitic nematodes. *Zakhist Roslin*, *20*, 35–40.

Hocking, H. (1967). A nematode (*Deladenus* sp.: Neotylenchidae) associated with *Rhyssa* spp. (Hymenoptera: Ichneumonidae), parasites of siricid woodwasps. *Australian Journal of Entomology*, *6*, 52–56.

Hoffard, W. H., & Coster, J. E. (1976). Endoparasitic nematodes of *Ips* bark beetles in Eastern Texas. *Environmental Entomology*, *5*, 128–132.

Hofstetter, R., Dinkins-Bookwalter, J., Davis, T., & Klepzig, K. (2015). Symbiotic associations of bark beetles. In F. E. Vega, & R. W. Hofstetter (Eds.), *Bark beetles: Biology and Ecology of native and invasive species* (pp. 209–245). San Diego, CA: Academic Press.

López, A., & Frérot, B. (1993). Statut physiologique des femelles colonisatrices de *Hypothenemus hampei* (Ferr.). 15^{éme} Colloque Scientifique International sur le Café, Montpellier, France, pp. 831–832.

MacGuidwin, A. E., Smart, G. C., Jr., Wilkinson, R. C., & Allen, G. E. (1980). Effect of the nematode *Contortylenchus brevicomi* on gallery construction and fecundity of the southern pine beetle. *Journal of Nematology*, *12*, 278–282.

Oldham, J. N. (1930). On the infestation of elm bark beetles (Scolytidae) by a nematode, *Parasitylenchus scolyti* n. Sp. *Journal of Helminthology*, *8*, 239–248.

Pérez, J., Infante, F., Poinar, G., Jr., Castillo, A., & Vega, F. E. (2015). Natural parasitism of *Metaparasitylenchus hypothenemi* (Tylenchida: Allantonematidae) on the coffee berry borer, *Hypothenemus hampei*, in Chiapas, méxico. *Biocontrol Science and Technology*, *25*, 608–612.

Poinar, G. O., Jr. (1983). *The Natural History of nematodes*. New Jersey: Prentice Hall.

Poinar, G., Jr., Vega, F. E., Castillo, A., Chavéz, I. E., & Infante, F. (2004). *Metaparasitylenchus hypothenemi* n. Sp. (Nematoda: Allantonematidae), a parasite of the coffee berry borer, *Hypothenemus hampei* (Curculionidae: Scolytinae). *Journal of Parasitology*, *90*, 1106–1110.

Pouvreau, A. (1962). Contribution a l'étude de *Sphaerularia bombi* (Nematoda, Tylenchida), parasite des reines de bourdons. *Annales de l'Abeille*, *5*, 181–199.

R Core Team. (2017). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from https://www.R-project.org/

Rubio, J. D., Bustillo, A. E., Vallejo, L. F., Benavides, P., & Acuña, J. R. (2007). Morfología del sistema reproductor femenino y masculino de *Hypothenemus hampei* (Ferrari). *Cenicafé*, *58*, 75–82.

Saunders, J. L., & Norris, D. M., Jr. (1961). Nematode parasites and associates of the smaller European elm bark beetle, *Scolytus multistriatus* (Marsham). *Annals of the Entomological Society of America*, *54*, 792–798.

Thong, C. H. S., & Webster, J. M. (1975a). Effects of *Contortylenchns reversus* (Nematoda: Sphaerulariidae) on hemolymph composition and oocyte development in the beetle *Dendroctonus pseudotsugae* (Coleoptera: Scolytidae). *Journal of Invertebrate Pathology*, *26*, 91–98.

Thong, C. H. S., & Webster, J. M. (1975b). Effects of the bark beetle nematode, *Contortylenchus reversus*, on gallery construction, fecundity, and egg viability of the Douglas fir beetle, *Dendroctonus pseudotsugae* (Coleoptera: Scolytidae). *Journal of Invertebrate Pathology*, *26*, 235–238.

Tomalak, M., Michalsky, J., & Grocholsky, J. (1984). The influence of nematodes on the structure of genitalia of *Tomicus piniperda* (Coleoptera:Scolytidae). *Journal of Invertebrate Pathology*, *43*, 358–362.

Vega, F. E., Infante, F., & Johnson, A. J. (2015). The genus *Hypothenemus*, with emphasis on *H. hampei*, the coffee berry borer. In F. E. Vega, & R. W. Hofstetter (Eds.), *Bark beetles: Biology and ecology of native and invasive species* (pp. 427–494). San Diego, CA: Academic Press.

Venables, W. N., & Ripley, B. D. (2002). *Modern applied statistics with S*. New York, NY: Springer.

Villacorta, A., & Barrera, J. F. (1993). Nova dieta merídica para criação de *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae). *Anais da Sociedade Entomológica do Brasil*, *22*, 405–409.

Wegensteiner, R., Wermelinger, B., & Herrmann, M. (2015). Natural enemies of bark beetles: Predators, parasitoids, pathogens and nematodes. In F. E. Vega, & R. W. Hofstetter (Eds.), *Bark beetles: Biology and ecology of native and invasive species* (pp. 247–304). San Diego, CA: Academic Press.

Wilcox, D., Dove, B., McDavid, D., & Greer, D. (2002). UTHSCSA ImageTool for Windows (Version 3.0). San Antonio: The University of Texas, Health Science Center.