Biological Control 67 (2013) 446–450

Contents lists available at ScienceDirect

Biological Control

journal homepage: www.elsevier.com/locate/ybcon

Field-cage evaluation of the parasitoid *Phymastichus coffea* as a natural enemy of the coffee berry borer, *Hypothenemus hampei*

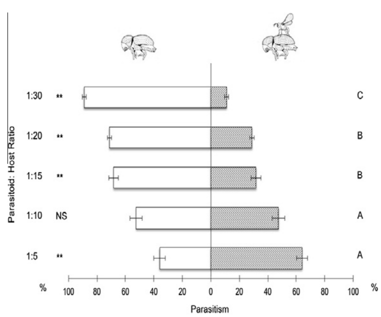
Francisco Infante [a,*], Alfredo Castillo [a], Jeanneth Pérez [a], Fernando E. Vega [b]

[a] El Colegio de la Frontera Sur (ECOSUR), Carretera Antiguo Aeropuerto km 2.5, Tapachula 30700, Chiapas, Mexico
[b] Sustainable Perennial Crops Laboratory, United States Department of Agriculture, Agricultural Research Service, Bldg. 001, Beltsville, MD 20705-2350, USA

HIGHLIGHTS

- Field-cage experiments were conducted to assess the potential of *Phymastichus coffea*.
- The 1:5 parasitoid:borer ratio resulted in highest parasitism.
- Treatments that received parasitoids had a higher seed weight than the control.
- It is concluded that *P. coffea* has a significant potential against this pest.
- Phymastichus coffea is an endoparasitoid of the coffee berry borer.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 28 June 2013
Accepted 29 September 2013
Available online 8 October 2013

Keywords:
Hypothenemus
Coffee berry borer
Phymastichus
Eulophidae
Inclusion cages
Biological control
Mexico

ABSTRACT

Phymastichus coffea LaSalle (Hymenoptera: Eulophidae) is an African parasitoid that has been imported to Mexico and other Latin American countries for the biological control of the coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytinae). As a part of the evaluation of this natural enemy in Mexico, we conducted a series of parasitoid inclusion cage experiments to evaluate parasitism rates under different parasitoid:borer ratios (1:5, 1:10, 1:15, 1:20 and 1:30) using entomological sleeves. The presence of *P. coffea* inside the sleeves did not affect avoid the perforation of coffee berries by the borers, but damages to berries were significantly diminished. Borers that did not enter coffee berries were more susceptible to be parasitized by *P. coffea* than borers that entered inside berries (i.e., borers that perforated the endosperm). The treatment resulting in the highest level of parasitism was the 1:5 parasitoid:borer ratio, which had 79% parasitism when borers where outside berries. In general, the highest percentage of parasitism occurred when the highest proportion of parasitoids was used. The 1:5 and 1:10 parasitoid:borer ratio resulted in the highest parasitism. The use of *P. coffea* resulted in a 2.2–3.1 fold lower coffee berry borer damage to the seeds weight, showing the beneficial effect of this natural enemy. The weight of coffee seeds significantly decreased in treatments where no parasitoids were used (control) and in treatments with the highest number of borers. All treatments that received parasitoids to control the coffee berry borer had a higher seed weight than the control. Our studies indicate that *P. coffea* has a strong potential to become an effective biological control agent against the coffee berry borer.

1. Introduction

The endoparasitic wasp *Phymastichus coffea* LaSalle (Hymenoptera: Eulophidae) is indigenous to Africa, and appears to be present in most coffee producing countries in that continent (Borbón-Martínez, 1989; López-Vaamonde and Moore, 1998). This parasitoid was first noticed attacking adults of the coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytinae) in Togo in 1987 (Borbón-Martínez, 1989), and was subsequently described as a new species (LaSalle, 1990). A preliminary assessment by López-Vaamonde and Moore (1998) suggested that

* Corresponding author.
E-mail address: finfante@ecosur.mx (F. Infante).

1049-9644/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.biocontrol.2013.09.019

P. coffea could be a useful biological control agent against the coffee berry borer. Consequently, the wasp was imported to most coffee producing countries of Latin America to mitigate the damage caused by the coffee berry borer, the most important insect pest of coffee worldwide (Le Pelley, 1968; Murphy and Moore, 1990; Jaramillo et al., 2006a; Vega et al., 2009). Adult females of this pest bore into coffee berries and deposit their eggs within galleries. Larvae feed on seeds inside the berries, greatly reducing yields and quality (Vega et al., 2009). Previous estimates of US $500 million in yearly losses worldwide (Vega et al., 2002) appear to be conservative based on a recent report for Brazil, where yearly losses have been estimated at US $215–358 million (Oliveira et al., 2013).

Phymastichus coffea has been described as a primary, gregarious, idiobiont endoparasitoid of coffee berry borer adults (Feldhege, 1992; Infante et al., 1994; López-Vaamonde and Moore, 1998; Castillo et al., 2004). Parasitoid females are approximately 1 mm long, with males half that size (LaSalle, 1990). Adult females start to search for hosts immediately after emergence. There is no preoviposition time and parasitization of hosts can occur within the first hours after the parasitoid reaches the adult stage (Infante et al., 1994). Females oviposit in the coffee berry borer abdomen, allocating two eggs per host, usually one female and one male. Both larvae feed inside the abdomen, but towards the end of the larval stage, the male larva migrates to feed from the tissues in the prothorax, where it pupates and completes its development (Espinoza et al., 2009). Parasitized hosts do not reproduce because they die a few days after being attacked (Feldhege, 1992; Infante et al., 1994). In the laboratory, the life-cycle from egg to adult of *P. coffea* takes about 35 days at 26 °C (Feldhege, 1992), while under field conditions the cycle is completed in approximately 47 days (Vergara et al., 2001a; Espinoza et al., 2009). Longevity of adults is limited to three days (Feldhege, 1992; Infante et al., 1994). The parasitism of *P. coffea* is strongly influenced by several factors, such as the availability of coffee berry borer adults, infested berries, and berry development, among others (Jaramillo et al., 2005). It has been suggested that the attack by *P. coffea* occurs just when the coffee berry borer initiates berry perforation (Borbón-Martínez, 1989; Feldhege, 1992; López-Vaamonde and Moore, 1998), but more detailed studies reported that this wasp is also able to attack borers that have already colonized coffee berries (Echeverry, 1999; Vergara et al., 2001a; Jaramillo et al., 2006b). Espinoza et al. (2009) confirmed that releases of wasps seven days after berries colonization by the coffee berry borer resulted in parasitism by *P. coffea*.

As a part of the evaluation program of this natural enemy in Mexico, we conducted a series of parasitoid inclusion field-cages experiments, to investigate the potential of *P. coffea* in biological control programs. As the field cages provide favorable trials of natural enemies on small-scale conditions, we designed an experiment to investigate the parasitism of *P. coffea* on the coffee berry borer using different parasitoid:borer ratios.

2. Materials and methods

The study was conducted at Finca Alianza, in the municipality of Cacahoatán, Chiapas (N 15°02′27″, W 92°10′22″; 700 masl). This coffee plantation has a density of approximately 3000 plants per hectare and an age over 25 years old. Average annual rainfall is 2900 mm and the mean annual temperature is 21 °C. We selected a ca. 0.5 ha plot planted with *Coffea arabica* L. Most of the plants had plenty of berries that were at least four months old, i.e., suitable for coffee berry borer infestation (Barrera, 1994; Bustillo et al., 1998). Coffee branches with a variable number of non-infested berries were randomly selected along the experimental plot. Each branch was enclosed with a 30 cm diam and 70 cm long entomological sleeve (Fig. 1). Immediately afterwards, coffee berries were exposed to coffee berry borers females at a ratio of 1.5 borers per berry. Two hours after coffee berry borer infestation, adult females of *P. coffea*, recently emerged from hosts reared in the laboratory, were introduced inside the entomological sleeves at five parasitoid:borer ratios: 1:5, 1:10, 1:15, 1:20 and 1:30. In total there were six treatments, including the control (branches with coffee berry borer, but no parasitoids), with seven replicates each. A detailed description of the treatments, including mean number of berries per branch, and borers and parasitoids per branch is presented in Table 1. Taking into account that the *P. coffea* adult longevity is about three days only (Feldhege, 1992; Infante et al., 1994), sleeves were removed four days after initiation of the experiments.

Fig. 1. A coffee branch enclosed by an entomological sleeve after releasing the parasitoid *Phymastichus coffea*.

The coffee berry borer females that did not bore the berries inside sleeves, were placed in artificial diet (Villacorta and Barrera, 1993), and left hanging from the branch. After 30 days, coffee berries and borers placed in artificial diet were taken to the laboratory to determine the degree of parasitism. We assessed three variables: (i) percentage of berry infestation by the coffee berry borer, (ii) parasitism of *P. coffea* on the coffee berry borer, both inside and outside the berries, and (iii) dry coffee seed weight. Coffee berry borer-infested berries were easily detected by observing the typical hole on or near the disc made by the adult females when entering the berry (Vega et al., 2009). Parasitism by *P. coffea* was visually determined through coffee berry borer dissections under the stereomicroscope (Espinoza et al., 2009). Parasitism rate at each density was calculated dividing the number of parasitized borers by the total number of borers X 100. To determine the impact of the coffee berry borer infestation on seed weight, seeds were removed from the berry and dried for 48 h in an oven.

Table 1
Description of treatments used to evaluate the impact of the parasitoid *Phymastichus coffea* on the coffee berry borer.

Parasitoid:borer ratio	Berries/ branch[a]	Borers/ branch	Parasitoids/ branch
1:5	141 ± 3.1	211	42
1:10	138 ± 1.8	207	21
1:15	122 ± 3.6	183	12
1:20	126 ± 4.3	189	9
1:30	130 ± 3.4	195	7
Control	132 ± 1.9	198	0

[a] Mean for seven replicates ± SE.

Data of percentage of parasitism by *P. coffea* were analyzed using a log-linear model with a binomial response (Zar, 1999). An analysis of variance (ANOVA) was performed to detect differences in the weight of coffee seeds due to coffee berry borer damage. Post hoc comparisons were made using orthogonal contrasts to establish differences among treatments. A one-way analysis of covariance (ANCOVA) using the total berries bored by the coffee berry borer in a branch as a covariate, was performed to detect differences in the total weight of treatments. In all tests values of $P \leqslant 0.05$ were considered significant.

3. Results

The highest berry infestation occurred in the control, followed by treatments with the highest parasitoid:borer ratios (i.e., 1:5, 1:10, 1:15; Table 2). Significantly lower infestation rates ($F_{5,30} = 7.46$; $P < 0.001$) were found in treatments with the lowest parasitoid:borer ratios (i.e., 1:20 and 1:30). There were significant differences in parasitism by *P. coffea* among treatments in coffee berry borers that remained outside the berries (i.e., borers that did not penetrate the berry) ($\chi^2 = 131.3$; d.f. = 4; $P < 0.001$), and when they went inside the berries (i.e. borers that penetrated the endosperm) ($\chi^2 = 143.5$; d.f. = 4; $P < 0.001$). In general, the highest percentage of parasitism occurred when the highest proportion of parasitoids was used. Coffee berry borers that did not enter berries were more likely to be parasitized by *P. coffea* than those that entered the berries. The highest level of parasitism occurred at the highest parasitoid:borer ratio (1:5), which had 79.4% parasitism when borers where outside the berries (Fig. 2).

We compared for each treatment the combined parasitism of coffee berry borers that were outside and inside berries, in order to analyze the total parasitism caused by *P. coffea* (Fig. 3). The 1:5 and 1:10 parasitoid:borer ratios resulted in the highest percentage of parasitism ($\chi^2 = 242.7$; d.f. = 4; $P < 0.001$). A comparison of treatments that contained parasitized coffee berry borers (right side of Fig. 2) *versus* non-parasitized coffee berry borers (left side of Fig. 2) indicates significant differences in all treatments, except at the 1:10 parasitoid:borer ratio. The confidence intervals for these comparisons are presented in Table 3.

The weight of coffee seeds significantly decreased in treatments with the highest parasitoid:borer ratio (1:15, 1:20, 1:30) and in the control. The seed weight per branch ($F = 22.2$; d.f. = 5, 35; $P < 0.001$) and per berry ($F = 17.0$; d.f. = 5, 36; $P < 0.001$) in the 1:5 and 1:10 parasitoid:borer ratio, were significantly higher. The control (no parasitoids) had the lowest coffee seed weight (Table 2).

4. Discussion

Assessing the effectiveness of introduced natural enemies in the target area is probably the most important step in classical biological control programs. Usually the main question to be addressed in this kind of evaluation is whether the natural enemy is capable of reducing the average density of the pest (Van Driesche and Bellows, 2001). In this context, cage exclusion/inclusion techniques are especially valuable because they provide a preliminary assessment of the impact of natural enemies upon pest populations, and also give quantitative information that can be used to understand the insect population dynamics (Luck et al., 1999; Kidd and Jervis, 2005).

Through the use of parasitoid inclusion field-cages experiments we have shown that *P. coffea* has a significant impact on the coffee berry borer population when released at a 1:5 or 1:10 parasitoid:borer ratio. As would be expected, at these densities, the coffee berry borer populations were drastically reduced and therefore the damages of the adult borers to the coffee berries were significantly diminished. Our studies demonstrate that the use of *P. coffea* results in a 2.2–3.1 fold lower coffee berry borer damage to the seeds weight. Another indirect effect of this parasitoid on the coffee berry borer is related to the fact that females that have been already parasitized do not reproduce (Feldhege, 1992; Infante et al., 1994), thus fewer individuals are expected in the next generation. Furthermore, when using the parasitoid at higher densities of borers (e.g., 1:30), the parasitism was diluted and only about 10% of the coffee berry borers were parasitized. Clearly, at this ratio, the impact of the parasitoid is very limited and therefore does not impact the damage caused by the coffee berry borer to the coffee seeds.

The results indicate that is possible to achieve >60% parasitism at the 1:5 and 1:10 parasitoid:borer ratio. Considering that the rearing technique for this wasp has been improved in the laboratory, producing more than one-million of wasps per month (Baker, 1999; Orozco, 2002), the ratio of 1 parasitoid per 5 borers is feasible to achieve under large-scale conditions. Eventually, these

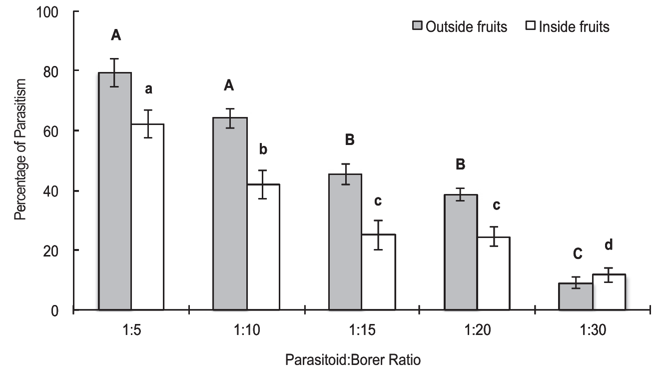


Fig. 2. Mean parasitism (±SE) of *Phymastichus coffea* on the coffee berry borer in treatments with different parasitoid:borer ratio. Upper case letters compare treatments of borers that were outside fruits (i.e. borers that did not penetrate the berry) and lower case letters compare treatments of borers that entered the fruit (i.e., borers that penetrated the endosperm).

Table 2
Percentage coffee berry borer-infested berries when testing different parasitoid:borer ratios and mean seed dry weight (grams) per branch an per berry one-month after exposure to the borers.

Parasitoid:borer ratio	Coffee berry borer infested berries (%)	Mean seed dry weight	
		Per branch*	Per berry*
1:5	64.3 ± 3.97 a	10.2 ± 0.54 a	0.18 ± 0.013 a
1:10	61.3 ± 3.52 a	9.9 ± 0.53 a	0.18 ± 0.006 a
1:15	55.9 ± 4.75 ab	7.2 ± 0.60 b	0.13 ± 0.011 b
1:20	39.1 ± 4.17 b	6.9 ± 0.53 b	0.14 ± 0.014 b
1:30	40.7 ± 3.77 b	6.4 ± 0.56 b	0.12 ± 0.005 bc
Control	65.4 ± 4.43 b	3.2 ± 0.66 c	0.08 ± 0.006 c

* Mean ± SE. Different letters in columns represent significant differences between treatments ($P < 0.05$).

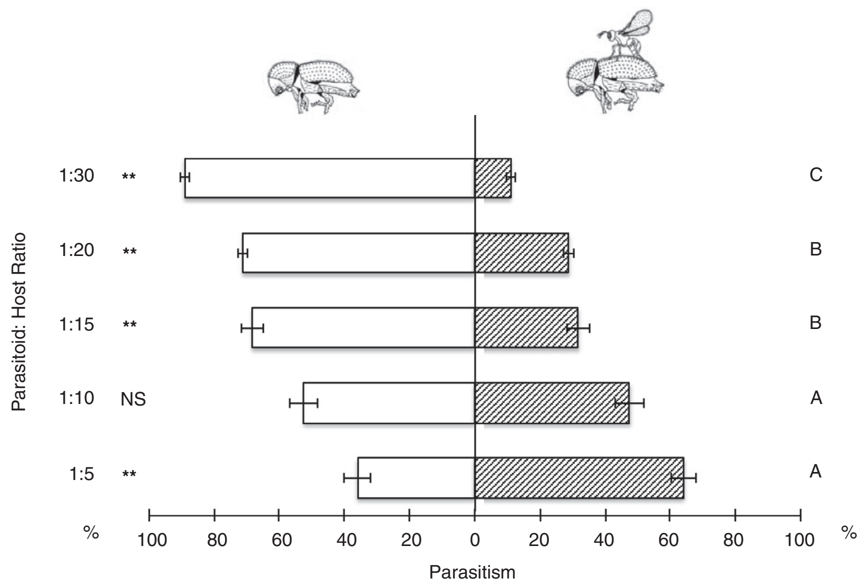


Fig. 3. Comparisons of the parasitism (±SE) by *Phymastichus coffea* on the coffee berry borer in treatments with different parasitoid:borer ratio. On the right side upper case letters compare treatments of parasitized borers (inside and outside coffee berries). On the left side asterisks indicate the significant differences between parasitized and unparasitized borers within the same parasitoid:borer ratio (see Table 3 for confidence intervals for these values).

Table 3
Confidence intervals for paired comparisons made from treatments shown in Fig. 3, between parasitized and non-parasitized coffee berry borers.

Parasitoid:borer ratio	Confidence intervals	
	Upper	Lower
1:5	0.60	0.67*
1:10	0.42	0.50 NS
1:15	0.27	0.34*
1:20	0.24	0.33*
1:30	0.09	0.13*

* Significant differences ($P < 0.05$).

releases could have more impact if parasitoids were released in the period of the year where the coffee berry borer population is at its lower level. This normally occurs approximately three months after plants are blooming, when borers leave the old coffee berries, where they spent the intercropping season, to infest the new coffee berries, which at this time are at a suitable stage to be infested by the coffee berry borer (Barrera, 1994; Bustillo et al., 1998).

The parasitism levels reported in the present study are within the range reported by others. For instance, Echeverry (1999) evaluated releases of *P. coffea* in coffee farms of Colombia at different densities in experimental plots without entomological sleeves. He found that parasitism varied from 8–49% when releasing a 1:13 and 1:1 parasitoids:borers ratio, respectively. In another experiment Echeverry (1999) released parasitoids and borers at a 1:1 ratio in entomological sleeves, but the parasitoids were released five days after the borers, resulting in 83% parasitism. In a field study, Jaramillo et al. (2005) reported a maximum parasitism of 75–85% when *P. coffea* was released one day after the coffee berry borer infestation, at a 1:1 parasitoid:borer ratio. Finally, Vergara et al. (2001b) reported on an augmentative release of *P. coffea* adults in a 70 × 130 m experimental plot containing plenty of berries and borers, followed by the release of 30,000 wasps in the center of the plot. Samples taken at different distances from the center of the experimental plot 25 days after the release, revealed an average parasitism of 61% in a distance range of 0–10 m and 27% parasitism 40–54 m from the release point.

Ultimately, the geographic dissemination of the coffee berry borer throughout most coffee producing countries over the last 100 years has resulted in increasing research efforts to study all aspects of biology, ecology and control methods of the pest (Infante et al., submitted). Although results of classical biological control using the bethylid parasitoids *Cephalonomia stephanoderis* and *Prorops nasuta* in Latin America have not been successful (Infante et al., 2001; Damon, 2000; Jaramillo et al., 2006), there is some potential for using these parasitoids through a farmer participatory integrated pest management program (Aristizábal et al., 2011, 2012). In this context, the use of *P. coffea* has been always visualized not as a single method of control, but as an important component of an integrated pest management strategy (Bustillo, 2006; Espinoza et al., 2009). After all, this species has been reported as the most predominant among the parasitoids of the coffee berry borer in West Africa (Feldhege, 1992). The results obtained in the present study provide some guidance concerning the potential for *P. coffea* as a biological control agent of the coffee berry borer in coffee agroecosystems of Latin America.

Acknowledgments

We are very grateful to the owner and workers of Finca Alianza for the facilities offered and kind support. We thank F. Nucamendi, J. Espinoza and V.H. Galindo for technical assistance. Special thanks to Javier Valle-Mora (ECOSUR) for statistical advice. Financial support by El Consejo Nacional de Ciencia y Tecnología (CONACYT) of Mexico (grant 37335-B) is gratefully acknowledged.

References

Aristizábal, L.F., Jiménez, M., Bustillo, A.E., Arthurs, S.P., 2011. Introduction of parasitoids of *Hypothenemus hampei* (Coleoptera: Curculionidae: Scolytinae) on small coffee plantations in Colombia through farmer participatory methods development. Fla. Entomol. 94, 690–693.

Aristizábal, L.F., Salazar, H.M., Mejía, C.G., Jiménez, M., Bustillo, A.E., Arthurs, S.P., 2012. Establishment of exotic parasitoids of the coffee berry borer *Hypothenemus hampei* (Coleoptera: Curculionidae: Scolytinae) in Colombia through farmer participatory research. Int. J. Trop. Insect Sci. 32, 24–31.

Baker, P.S., 1999. The coffee berry borer in Colombia. Final Report of the IPM for Coffee Project. DFID-Cenicafé-CABI Bioscience, pp. 148.

Barrera, J.F., 1994. Dynamique des populations du scolyte des fruits du caféier, *Hypothenemus hampei* (Coleoptera: Scolytidae), et lutte biologique avec le parasitoide *Cephalonomia stephanoderis* (Hymenoptera: Bethylidae), au Chiapas, Mexique. Ph. D. Thesis. Université Paul Sabatier, Toulouse III, France, pp. 301.

Borbón-Martínez, O., 1989. Bioécologie d́un ravageur des baies de caféier, *Hypothenemus hampei* Ferr. (Coleoptera: Scolytidae) et de ses parasitoides au Togo. Ph. D. Thesis. Université Paul-Sabatier. Toulouse, France, pp. 185.

Bustillo, A.E., 2006. Una revisión sobre la broca del café, *Hypothenemus hampei* (Coleoptera: Curculionidae: Scolytinae) en Colombia. Rev. Colomb. Entomol. 32, 101–116.

Bustillo, A., Cárdenas, R., Villalba, D.A., Benavides, P., Orozco, J., Posada, F.J., 1998. Manejo integrado de la broca del café *Hypothenemus hampei* (Ferrari) en Colombia. CENICAFÉ, Chinchiná, Colombia, 134 pp.

Castillo, A., Infante, F., Vera-Graziano, J., Trujillo, J., 2004. Host-discrimination by *Phymastichus coffea*, a parasitoid of the coffee berry borer (Coleoptera: Scolytidae). BioControl 49, 655–663.

Damon, A., 2000. A review of the biology and control of the coffee berry borer *Hypothenemus hampei* (Coleoptera: Scolytidae). Bull. Entomol. Res. 90, 453–465.

Echeverry, A.O.A., 1999. Determinación del impacto de Phymastichus coffea LaSalle (Hymenoptera: Eulophidae) sobre poblaciones de broca del café Hypothenemus hampei (Ferrari) (Coleoptera: Scolytidae) en la zona cafetera. B. S. Thesis. Universidad Nacional de Colombia, pp. 94.

Espinoza, J.C., Infante, F., Castillo, A., Pérez, J., Nieto, G., Pinson, E.P., Vega, F.E., 2009. The biology of *Phymastichus coffea* LaSalle (Hymenoptera: Eulophidae) under field conditions. Biol. Control 49, 227–233.

Feldhege, M.R., 1992. Rearing techniques and aspects of biology of *Phymastichus coffea* (Hymenoptera: Eulophidae), a recently described endoparasitoid of the coffee berry borer, *Hypothenemus hampei* (Coleoptera: Scolytidae). Café Cacao Thé 36, 45–54.

Infante, F., Mumford, J., Mendez, I., 2001. Non-recovery of *Prorops nasuta* (Hymenoptera: Bethylidae), an imported parasitoid of the coffee berry borer *Hypothenemus hampei* (Coleoptera: Scolytidae) in Mexico. Southwest. Entomol. 26, 159–163.

Infante, F., Murphy, S.T., Barrera, J.F., Gómez, J., de la Rosa, W., Damon, A., 1994. Cría de *Phymastichus coffea*, parasitoide de la broca del café y algunas notas sobre su historia de vida. Southwest. Entomol. 19, 313–315.

Infante, F., Pérez, J., Vega, F.E. The centenary of a biological invasion. Braz. J. Biol., submitted.

Jaramillo, J., Borgemeister, C., Baker, P., 2006a. Coffee berry borer *Hypothenemus hampei* (Coleoptera: Curculionidae): searching for sustainable control strategies. Bull. Entomol. Res. 96, 223–233.

Jaramillo, J., Borgemeister, C., Setamou, M., 2006b. Field superparasitism by *Phymastichus coffea*, a parasitoid of adult coffee berry borer, *Hypothenemus hampei*. Entomol. Exp. Appl. 119, 231–237.

Jaramillo, J., Bustillo, A.E., Montoya, E.C., Borgemeister, C., 2005. Biological control of the coffee berry borer *Hypothenemus hampei* (Coleoptera: Curculionidae) by *Phymastichus coffea* (Hymenoptera: Eulophidae) in Colombia. Bull. Entomol. Res. 95, 1–6.

Kidd, N.A.C., Jervis, M.A., 2005. Population dynamics. In: Jervis, M.A. (Ed.), Insects As Natural Enemies: A Practical Perspective. Springer, Dordrecht, pp. 435–523.

LaSalle, J., 1990. A new genus and species of Tetrastichinae (Hymenoptera: Eulophidae) parasitic on the coffee berry borer, *Hypothenemus hampei* (Ferr.) (Coleoptera: Scolytidae). Bull. Entomol. Res. 80, 7–10.

Le Pelley, R.H., 1968. Pests of Coffee. Longmans Green & Co., Ltd., London.

Lopez-Vaamonde, C., Moore, D., 1998. Developing methods for testing host specificity of *Phymastichus coffea* LaSalle (Hymenoptera: Tetrastichinae), a potential biological control agent of *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae) in Colombia. Biocontrol Sci. Technol. 8, 397–411.

Luck, R.F., Merle-Shepard, B., Kenmore, P.E., 1999. Evaluation of biological control with experimental methods. In: Bellows, T.S., Fisher, T.W. (Eds.), Handbook of Biological Control. Academic Press, San Diego, pp. 225–242.

Murphy, S.T., Moore, D., 1990. Biological control of the coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae): previous programmes and possibilities for the future. Biocontrol News Inf. 11, 107–117.

Oliveira, C.M., Auad, A.M., Mendes, S.M., Frizzas, M.R., 2013. Economic impact of exotic insect pests in Brazilian agriculture. J. Appl. Entomol. 137, 1–15.

Orozco, H.J., 2002. Guía para la producción del parasitoide Phymastichus coffea para el control de la broca del café. Cenicafé, Chinchiná, Colombia, 19 pp.

Van Driesche, R.G., Bellows Jr., T.S., 2001. Biological Control. Kluwer Academic, Dordrecht, 539 pp.

Vega, F.E., Franqui, R.A., Benavides, P., 2002. The presence of the coffee berry borer, *Hypothenemus hampei*, in Puerto Rico: fact or fiction? J. Insect Sci. 2, 13.

Vega, F.E., Infante, F., Castillo, A., Jaramillo, J., 2009. The coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae): a short review, with recent findings and future research directions. Terr. Arthropod Rev. 2, 129–147.

Vergara, J.D., Orozco, J., Bustillo, A.E., Chaves, B., 2001a. Biología de *Phymastichus coffea* en condiciones de campo. Cenicafé 52, 97–103.

Vergara, J.D., Orozco, J., Bustillo, A.E., Chaves, B., 2001b. Dispersión de *Phymastichus coffea* en un lote de café infestado de *Hypothenemus hampei*. Cenicafé 52, 104–110.

Villacorta, A., Barrera, J.F., 1993. Nova dieta merídica para criacão de *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae). An. Soc. Entomol. Bras. 22, 405–409.

Zar, J.H., 1999. Biostatistical Analysis, 4th ed. Prentice Hall, New Jersey.