The Coffee Berry Borer (Coleoptera: Curculionidae): How Many Instars are There?

JAIME GÓMEZ,[1,2] BRENDA Y. CHÁVEZ,[1] ALFREDO CASTILLO,[1] FRANCISCO J. VALLE,[1] AND FERNANDO E. VEGA[3]

Ann. Entomol. Soc. Am. 108(3): 311–315 (2015); DOI: 10.1093/aesa/sav009

ABSTRACT After more than a century since the description of the coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae), and dozens of scientific articles on the basic biology of the insect, there is still debate on the number of female larval instars. This article analyzes the metamorphosis of *H. hampei* females through direct observations during its entire biological cycle in the laboratory, together with scanning electron microscope photos. Also, the size of the head capsule of wild larvae and prepupae was analyzed with Dyar's rule and a discriminant analysis was conducted. Only two instars were observed during *H. hampei* metamorphosis up to the adult stage. Contrasting morphological changes in the larvae occurred when they transformed into prepupae, with no previous ecdysis. The statistical analysis revealed that the width of the cephalic masses form two significantly distinct groups before transformation into pupa, confirming that the prepupal stage forms part of the second larval instar.

KEY WORDS bark beetle, biology, broca del café, *Hypothenemus*, instar

The coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae), is the most important insect pest affecting coffee production worldwide (Le Pelley 1968; Baker 1999; Barrera et al. 2007; Vega et al. 2009, 2015). This minute bark beetle (size in mm: ♂ 0.99–1.3; ♀ 1.6–1.9) lives inside the coffee berry, and the damage caused by the colonizing female and its progeny reduces yield and the quality of the seed in the two commercially traded coffee species, *Coffea arabica* L. and *Coffea canephora* Pierre ex A. Froehner (Vega et al. 2015)

More than a century after the initial description of the coffee berry borer by Ferrari (1867), dozens of important scientific contributions have been published on the basic biology of the beetle, including landmark articles by Hargreaves (1926), Oliveira Filho (1927), Corbett (1933), and Bergamin (1943). Nevertheless, the controversy over the number of female larval instars the species undergoes has not been resolved, whereas it has never been disputed that males only exhibit one instar (Bergamin, 1943).

Some authors mention that female coffee berry borers undergo two larval instars, but make no reference to the prepupa (Morallo-Rejesus and Baldos 1979). Others indicate that the species has two larval instars and consider the prepupa as the second instar (Fernández and Cordero 2007). A third point of view states that the species has two larval instars and the prepupa forms part of the second instar (Bergamin 1943, Costa and Villacorta 1989, Ruiz et al. 1996). Finally, others simply state that the larvae molt twice without mentioning the number of instars (Decazy 1993).

This study was conducted to settle the controversy over the number of larval instars of female coffee berry borers. Females are the dispersal unit in the species, with males never leaving the coffee berry. Therefore, knowing the exact number of female larval instars is an important consideration not only for understanding the basic biology of the insect but also when developing pest management programs that use biological control agents that might have differential effects on specific developmental stages.

Materials and Methods

Biological Material. The coffee berry borers used in this study were obtained from a population reared on a meridic diet (Villacorta and Barrera 1993) in the laboratory at El Colegio de la Frontera Sur, Tapachula, Chiapas, Mexico. A wild population used to initiate the colony was obtained from coffee berries (*C. canephora*) infested by the coffee berry borer in coffee plantations in Soconusco, Chiapas. The berries were dissected to obtain coffee berry borers in their immature stages.

Duration of Larval Development and Associated Morphological Changes. Morphological changes of larvae reared on a meridic diet were observed directly. Immature stages were obtained by

[1] El Colegio de la Frontera Sur, Carretera Antiguo Aeropuerto Km. 2.5, Tapachula, Chiapas, México 30700.
[2] Corresponding author, e-mail: jgomez@ecosur.mx.
[3] Sustainable Perennial Crops Laboratory, United States Department of Agriculture, Agricultural Research Service, Bldg. 001, Beltsville, MD 20705-2350.

placing adults (five females and one male) in test tubes containing the meridic diet. Twenty days later, 300 eggs were removed from the tubes and placed in Petri dishes until the larvae hatched; 160 of these recently emerged larvae were separated and placed in a plastic box divided into cells in which small pieces of diet were offered as food. The conditions of the rearing chamber were maintained at an average temperature of 31°C with an average relative humidity of 73%. Using a stereoscope, morphological changes and development time were recorded daily until larvae reached the adult stage. Separation of the larval instars was done based on the presence of exuviae. The larval stages were described morphologically using scanning electron microscope images.

Scanning Electron Microscopy. Immature coffee berry borers were washed superficially with distilled, filtered sterile water and fixed for 24 h with a modified Karnovsky solution consisting of 2% formaldehyde and 2.5% glutaraldehyde in a 0.1 M phosphate buffer with a pH of 7.2. They were then washed in a 0.1 M phosphate buffer solution for 5 min. The larvae were dehydrated in solutions of methanol from 30 to 100% and then dried to CO_2 critical point. The samples were covered with a layer of gold–palladium in a Denton Vacuum Desk II unit (Denton Vacuum LLC, Moorestown, NJ) and were observed with a Topcon SM-510 scanning electron microscope (Topcon, Tokyo, Japan).

Size of Larval Head Capsule. The size of the larval head capsules was measured in insects obtained from coffee berries. Red coffee berries (*C. canephora*) infested with the insect were brought to the laboratory from the field and placed on a white absorbent paper. One week later, berries that had dark green powder around the hole made by the colonizing female were dissected to obtain larvae and prepupae. Heads of all of the larvae and prepupae were separated from the bodies and placed on a strip of adhesive tape. The width of the head capsules of 134 larvae was measured with a micrometric ruler attached to the eyepiece of the stereoscopic microscope.

Statistical Analysis. The head capsule measurements were used in the application of Dyar's rule (Taylor 1931). Our data were compared with Bergamin's data (1943) using simple frequency tables and frequency distribution graphs, as well as by estimating the probability density function of data and discriminant analysis for univariate data for nonparametric methods (Fraley et al. 2012).

Results and Discussion. The data obtained by direct observation indicate that *H. hampei* larvae reared on artificial diet molted only once: only one exuvium per larva was observed during the entire larval development up to their metamorphosis into pupae, confirming the presence of only two larval instars. Nevertheless, the morphological changes exhibited by larvae were dramatic (Fig. 1) and may have thus caused confusion in the past in determining the number of instars. When the larvae emerge from the egg, they are apodous and translucent, the head is dark brown, and the setae are long. In addition, larvae move very little and the body remains straight. As they grow, they become more voracious. During ecdysis, detachment of the old cuticle and head capsule occurs, and the

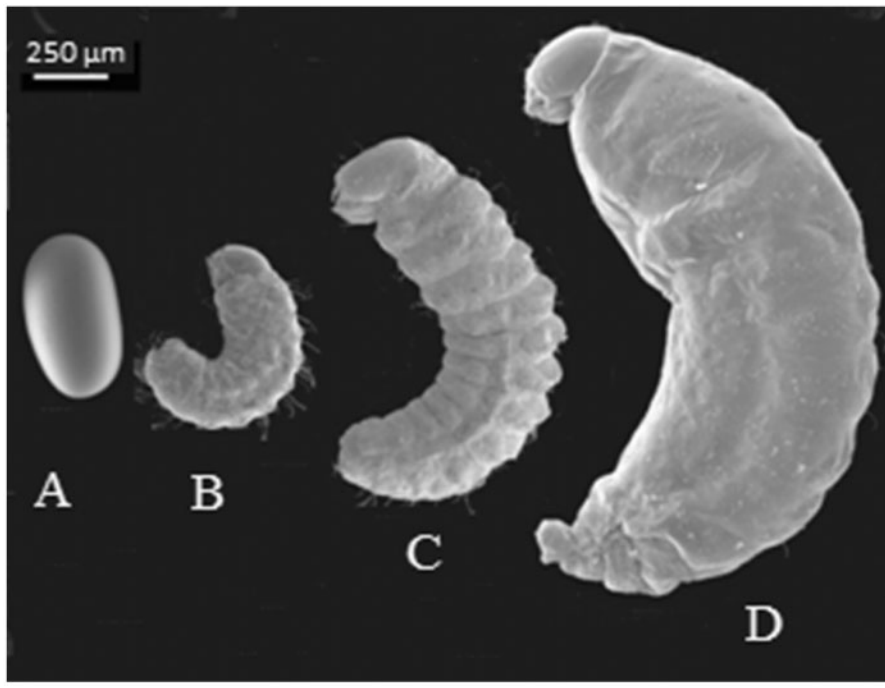

Fig. 1. Scanning electron micrographs of egg and larval stages of female *H. hampei*. A, egg; B, first-instar larvae; C, second-instar larvae; D, prepupa.

larva, including the cephalic mass, becomes white, whereas the mouthparts become brown. A few hours after molting, the larval head turns brown and the abdomen acquires an intense whiteness.

Three to four days after ecdysis, a morphological change occurs (with no molting). The larva ceases to feed and move; it becomes whiter and wider in the thorax region, making the cephalic mass look very small. A v-shaped depression in the ventral area is seen when observed laterally (Fig. 1D). This stage is known as the "prepupa" (Abraham et al. 1990), but our results show that it continues to be the second larval instar.

The biological cycle of *H. hampei*, from egg to adult, was 22 d. The average duration of each of the stages observed is presented in Table 1, with values similar to those reported by Oliveira Filho (1927), Ruiz et al. (1996), and Fernández and Cordero (2007). The small variations are attributed to the number of observations performed in the different studies (Fernández and Cordero 2007) and to the environmental conditions in the laboratories, mainly temperature (Ruiz et al. 1996, Costa and Villacorta 1989).

Dyar's rule is frequently used to separate the larval instars of coleopterans (Mizell and Nebeker 1979, Pershing and Linit 1988, Logan et al.1998, Pantoja et al.1999, Rodríguez-Quiroz et al. 2000, Bailez et al. 2003, Hammack et al. 2003, Fernández and Cordero 2007). Fernández and Cordero (2007) were the first to use this technique on *H. hampei* and determined that the insect has two larval instars: the first corresponding to the stage described morphologically as larva and the second to prepupa. However, when analyzing the frequency values of the measurements of *H. hampei* larval and prepupal head capsule of both the present study and that of Bergamin (1943), the prepupal head capsule falls within the range of the second larval instar (Table 2), indicating that the insect only has two larval instars because there is no growth of the head capsule after the only observed ecdysis, despite the increase in size and the morphological change of the prepupae.

Moreover, the graphs showing the frequency histograms of the head capsule measurements, in both the present study and that of Bergamin (1943) (Fig. 2), show a very clear separation between the values of head capsule widths for the first and second larval instar, and the inclusion of the prepupae within the second larval instar. According to Gaines and Campbell (1935), Dyar's rule can be used with different groups of insects only if the measurements of the different larval instars do not overlap; therefore, it can be confirmed that female *H. hampei* goes through two larval instars, and the prepupal stage is part of the second instar (Fig. 2).

Fernández and Cordero (2007) reported two peaks in the frequency distribution graph (0.33 and 0.52 mm), corresponding to the first and second instar, respectively. We also found two peaks, 0.20 and 0.30 mm, while Bergamin (1943) found peaks of 0.24 and 0.34 mm (Fig. 2), which are different from those reported by Fernández and Cordero (2007).

Estimation of the probability density function using data from measurements of the head capsule (Fig. 3) clearly defines and separates the two larval instars of *H. hampei* and includes the prepupa in the second instar, as there is no head growth after the larva passes to the second instar. The discriminant analysis shows the prepupa data grouped within the second instar (Table 3), with both our data (ratio of error = 15.67) and Bergamin's (1943; ratio of error = 8.96), meaning that they do not form a separate group. Taylor (1931) states that a larval instar cannot be characterized only by the average values of the head capsule width (Dyar's rule) and that other traits can also be used, such as body size, head color (if it is pale immediately following ecdysis), any abnormal appearance in head shape, amongst others. The conclusions reached in this study were based not only on Dyar's rule but were also supported by daily direct observations of a set of larvae.

It is noteworthy that after >60 years since Bergamin (1943) reported measurements for the head capsule of *H. hampei* larvae in Brazil, our data coincides with his results. Despite the differences in geography, climate, and time lapse between the two studies, no differences in number of instars of the borer larvae were found. This suggests that there is no intraspecific variability

Table 1. Duration of *H. hampei* developmental stages when reared on a meridic diet

Developmental stage	Replicates	Avg (days)	SD	95% CI
Egg	163	4.18	0.38	4.12 – 4.24
First larval instar	160	4.33	0.74	4.22 – 4.45
Second larval instar	130	5.21	0.84	5.06 – 5.35
Prepupa	110	2.46	1.73	2.14 – 2.78
Pupa	101	5.49	0.87	5.32 – 5.66

Table 2. Head capsule width (mm) for *H. hampei* larval stages obtained from coffee berries compared with results reported by Bergamin (1943)

Larval stage[a]	Present article			Bergamin (1943)		
	L1	L2	Pp	L1	L2	Pp
Range	0.20–0.22	0.28–0.32	0.28–0.30	0.220–0.252	0.320–0.340	0.320–0.340
Mode	0.2	0.3	0.3	0.24	0.33	0.33
Mean	0.21	0.3	0.3	0.24	0.33	0.33
Probable error of the mean	±0.0007	±0.0007	±0.0006	±0.0005	±0.0005	±0.0012
SD	0.008	0.008	0.004	0.006	0.007	0.006
Coefficient of variation	4.4	2.8	1.56	2.66	2.15	1.96

[a] L1, first larval instar; L2, second larval instar; Pp, prepupa.

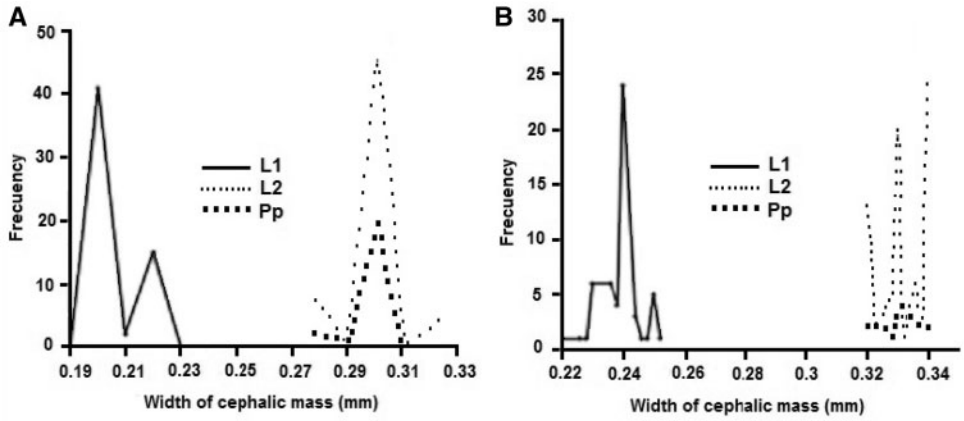

Fig. 2. Frequency distribution of the width of the head (cephalic mass) of larvae and prepupa of *H. hampei*. L1, first larval instar; L2, second larval instar; Pp, prepupa. (A) = present data; (B) = Bergamin's data (1943).

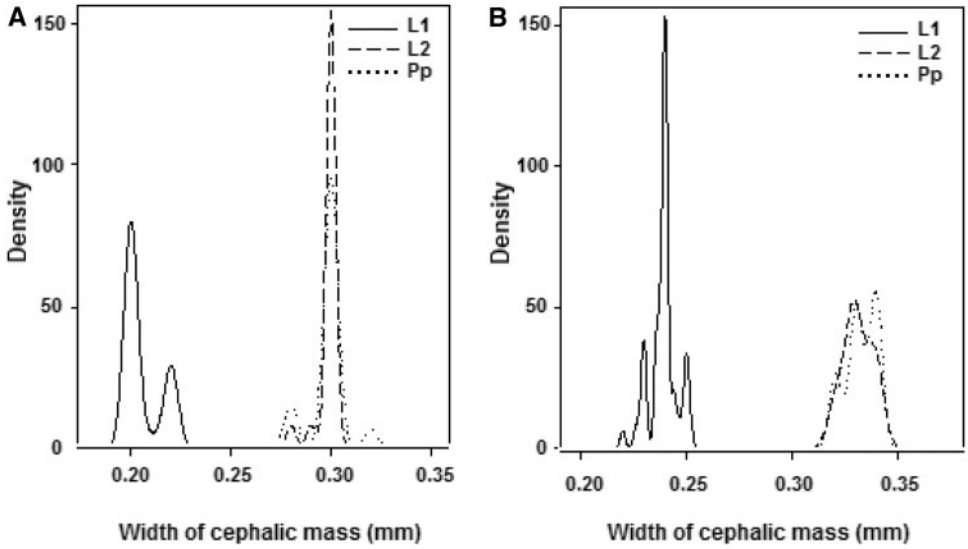

Fig. 3. Estimation of probability density function of width of head (cephalic mass) data for larvae of *H. hampei* obtained from coffee berries. L1, first larval instar; L2, second larval instar; Pp, prepupa. (A) = present data; (B) = Bergamin's data (1943).

Table 3. Mixture discriminant analysis applied to data from measurements of the head capsule of each larval stage of *H. hampei*

Data		Classification obtained[a]		
		L1	L2	Pp
Present article	L1 = 58	58	0	0
	L2 = 55	0	55	0
	Pp = 21	0	21	0
Bergamin (1943)	L1 = 54	54	0	0
	L2 = 78	0	78	0
	Pp = 13	0	13	0

[a] L1, first larval instar; L2, second larval instar; Pp, prepupa.

among *H. hampei* larvae in terms of number of instars, as has been found in other insects, including Curculionidae (Esperk et al. 2007).

Based on the results, which show that there is no morphometric overlap of the first and second larval instars, but there is an overlap of the prepupa with the second instar, we can conclude that the larvae of the *H. hampei* females only have two larval instars and that the prepupa is a phase of the second larval instar. This study is of relevance for research requiring knowledge of specific larval stages or for standardizing bioassays in the laboratory (Charles et al. 2000), principally in the search for biological alternatives in the control of the coffee berry borer.

Acknowledgments

We thank Guadalupe Nieto for the scanning electron microscope photos, and Enrique López and Giber González for technical assistance.

References Cited

Abraham, Y. J., D. Moore, and G. Godwin. 1990. Rearing and aspects of biology of *Cephalonomia stephanoderis* and *Prorops nasuta* (Hymenoptera: Bethylidae) parasitoids of the coffee berry borer, *Hypothenemus hampei* (Coleoptera: Scolytidae). Bull. Entomol. Res. 80: 121–128.

Bailez, O. E., A. M. Viana-Bailez, J. O. G. de Lima, and D. D. O. Moreira. 2003. Life-history of the guava weevil, *Conotrachelus psidii* Marshall (Coleoptera: Curculionidae), under laboratory conditions. Neotrop. Entomol. 32: 203–207.

Baker, P. S. 1999. La broca del café en Colombia. Informe final del proyecto MIP para el café DFID - Cenicafé - CABI Bioscience (CNTR 93/1536A). Chinchiná (Colombia), DFID - CENICAFÉ, p. 148.

Barrera, J. F., A. García, V. Domínguez, and C. Luna. 2007. La broca del café en América tropical: hallazgos y enfoques. Sociedad Mexicana de Entomología y El Colegio de la Frontera Sur. Tapachula, Chiapas, México.

Bergamin, J. 1943. Contribuição para o conhecimento da biologia da broca do café "*Hypothenemus hampei* (Ferrari, 1867)" (Col. Ipidae). Arq. Inst. Biol. São Paulo 14: 31–72.

Charles, J.-F., A. Delécluse, and C. Nielsen-LeRoux. 2000. Entomopathogenic Bacteria: from Laboratory to Field Application. Kluwer Academic Publishers, Dordrecht, The Netherlands, p. 524.

Corbett, G. H. 1933. Some preliminary observations on the coffee berry beetle borer, *Stephanoderes* (*Cryphalus*) *hampei* Ferr. Malayan Agric. J. 21: 8–22.

Costa, T. C., and A. Villacorta. 1989. Modelo acumulativo para *Hypothenemus hampei* (Ferrari, 1867) (Coleoptera: Scolytidae) com base em suas exigências térmicas. An. Soc. Entomol. Bras. 18: 91–99.

Daly, H. G. 1985. Insect morphometrics. Annu. Rev. Entomol. 30: 415–438.

Decazy, B. 1993. Descripción, biología, ecología y control de la broca del cafeto, *Hypothenemus hampei* (Ferr.), pp. 133–139. *In* 50 años de Cenicafé 1938–1988, Conferencias Conmemorativas, Chinchiná, Colombia.

Esperk, T., T. Tammaru, and S. Nylin. 2007. Intraspecific variability in number of larval instars in insects. J. Econ. Entomol. 100: 627–645.

Fernández, S., and J. Cordero. 2007. Biología de la broca del café *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae: Scolytidae) en condiciones de laboratorio. Bioagro (Venezuela) 19: 35–40.

Ferrari, J. A. G. 1867. Die Forst- und Baumzuchtschädlichen Borkenkäfer (Tomicides Lac.) aus der Familie der Holzverderber (Scolytides Lac.), mit besonderer Berücksichtigung vorzüglich der europäischen Formen, und der Sammlung des k. k. zoologischen Kabinetes in Wien. Carl Gerold's Sohn, Vienna.

Fraley, C., A. E. Raftery, T. Brendan Murphy, and L. Scrucca. 2012. Mclust Version 4 for R: Normal Mixture Modeling for Model-Based Clustering, Classification, and Densitly Estimation. Techical Report No. 597, Department of Statistics, University of Washington, Seattle, WA.

Gaines, J., and F. Campbell. 1935. Dyar's rule as related to the number of instars of the corn earworm; *Heliothis obsoleta* (Fab.) collected in the field. Ann. Entomol. Soc. Am. 18: 445–461.

Guharay, F., J. Monterrey, D. Monterroso, and C. Staver. 2000. Manejo integrado de plagas en el cultivo del café. Centro Agronómico Tropical de Investigación y Enseñanza (CATIE), Managua, Nicaragua.

Hammack, L., M. M. Ellsbury, R. L. Roehrdanz, and J. L. Pikul, Jr. 2003. Larval sampling and instar determination in field populations of northern and western corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol. 96: 1153–1159.

Hargreaves, H. 1926. Notes on the coffee berry borer (*Stephanoderes hampei*, Ferr.) in Uganda. Bull. Entomol. Res. 16: 347–354.

Le Pelley, R. H. 1968. Las Plagas del Café. Editorial Labor, S.A., Barcelona España.

Logan, J. A., B. J. Bentz, J. C. Vandygriff, and D. L. Turner. 1998. General program for determining instars distribution from head capsule widths: example analysis of mountain pine beetle (Coleoptera: Scolytidae) data. Environ. Entomol. 27: 555–563.

Mizell, R. F., and T. E. Nebeker. 1979. Number of instars of the southern pine beetle (Coleoptera: Scolytidae) and some comparisons of head capsule widths. Ann. Entomol. Soc. Am. 72: 313–316.

Morallo-Rejesus, B., and E. Baldos. 1979. The biology of coffee berry borer *Hypothenemus hampei* (Ferr.) (Scolytidae, Coleoptera) and its incidence in the southern Tagalog provinces. Philippine Entomol. 4: 303–316.

Oliveira Filho, M. L. de. 1927. Contribuição para o conhecimento da broca do café, *Stephanoderes hampei* (Ferrari 1867). Modo de comportar-se e ser combatida em S. Paulo-Brasil. Secretaria da Agricultura, Commercio e Obras Publicas. Comissão do Estudo e Debellação da Praga Caféeira 20: 1–95.

Pantoja, A., L. M. Ramírez, L. E. Escalona, H. Bastidas, and M. C. Duques. 1999. Numbers of instars of *Lissorhoptrus kuscheli* (Coleoptera: Curculionidae) from rice in Venezuela. Ann. Entomol. Soc. Am. 92: 236–238.

Pershing, J. C., and M. J. Linit. 1988. Variation in number of instar of *Monochamus carolinensis* (Coleoptera: Cerambycidae). J. Kans. Entomol. Soc. 61: 370–378.

Rodríguez-Quiroz, M., J. Valdez-Carrasco, J. Vera-Graziano, and A. Castillo-Morales. 2000. Identificación de ínstares larvales de *Zabrotes subfasciatus* (Boh.) (Coleoptera: Bruchidae) mediante las dimensiones de sus cápsulas cefálicas. Agrociencia 34: 83–90.

Ruiz, S. L., A. E. Bustillo-Pardey, F. J. Posada Flórez, and M. T. González. 1996. Ciclo de vida de *Hypothenemus hampei* en dos dietas merídicas. Cenicafé 47: 77–84.

Taylor, R. L. 1931. On "Dyar's Rule" and its application to sawfly larvae. Ann. Entomol. Soc. Am. 24: 451–466.

Vega, F. E., F. Infante, A. Castillo, and J. Jaramillo. 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.

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

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

Received 1 September 2014; accepted 22 January 2015.