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Development of an improved laboratory production technique for the coffee berry borer *Hypothenemus hampei*, using fresh coffee berries

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Abstract

The suitability of a mixture of plaster of Paris and charcoal as a means to regulate the moisture content of coffee berries and the relative humidity (moisture conditions) of the rearing environment and its impact on rearing the coffee berry borer (CBB), *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae), was evaluated under laboratory conditions using two types of coffee. Coffee berries were kept individually in vials with a 1-cm layer of the mixture, and the fresh weight of the berries was assessed, as well as the penetration of CBB into the berries, its survival, and its progeny production over a period of 55 days. Significantly higher survival and progeny production was achieved when using the mixture regardless of the coffee type. Compared to the vials without plaster of Paris/charcoal, a six- to sevenfold increase in survivorship of the F1 was recorded when using plaster of Paris/charcoal and in the latter treatment berries harboured on average more than 100 individuals, whereas only 1.7 in the vials without plaster of Paris.

Introduction

The coffee berry borer (CBB), *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae), is the most important coffee pest worldwide (LePelley, 1968; Jaramillo et al., 2006). Females bore galleries into the endosperm of the coffee berries, causing qualitative and quantitative losses through feeding of the larvae inside the berries (Damon, 2000). One of the main control strategies is application of broad-spectrum synthetic insecticides (e.g., Mejía & López, 2002), but growing environmental concerns and increasing problems with insecticide resistance in *H. hampei* (Brun et al., 1994; Góngora et al., 2001) have stimulated the search for environmentally friendly control strategies (Jaramillo et al., 2006). Moreover, sustainable coffee production and certification schemes stress the safety aspects of pest control, which indirectly leads to an increased demand for biological control solutions (Jaramillo et al., 2006). Prerequisites for successful inundative biological control are sound knowledge of the pest's biology, and, in the case of *H. hampei*, rearing of large numbers of healthy females at low costs for the production of natural enemies.

In spite of the economic importance of the pest, there are still major gaps in our understanding of its biology. For instance, conflicting data on important life-table parameters are reported in the literature (Bergamin, 1943; Ticheler, 1963; Decazy, 1990; Barrera, 1994; Montoya & Cardena, 1994; Ruiz, 1995; Fernandez & Cordero, 2007). Such discrepancies are most likely due to the difficulties of studying a concealed pest like CBB under controlled conditions and they reveal problems with the existing methodologies (Damon, 2000). Because of the difficulties to maintain CBB on fresh coffee berries in the laboratory, some of the previously cited studies have been conducted under field conditions. Yet varying environmental factors lead to variations in reported biological parameters of the insect (Ruiz, 1995; Fernández & Cordero, 2007).

Most of the previous efforts in mass rearing of CBB were aimed at the development of suitable production systems for natural enemies, especially parasitoids. To date several

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effective techniques exist, such as artificial diets for *H. hampei* (Villacorta, 1985; Brun et al., 1993; Portilla, 1999), a rearing method that uses parchment coffee (Benavides & Portilla, 1990), and a rearing method for the endoparasitoid of CBB females *Phymastichus coffea* LaSalle that uses fresh coffee (Infante et al., 1994). However, for basic studies of the biology of the beetle, all these techniques have considerable drawbacks. For instance, the existing artificial diets of CBB negatively affect fecundity and sex ratio of the beetles (Portilla & Streett, 2006). The method developed by Infante et al. (1994) does not permit the F1 of the colonizing female to complete its life cycle because of desiccation of the berries. Finally, parchment coffee is comparatively expensive and not the natural substrate of the beetle. Thus, to study the beetle's biology under controlled conditions a new and affordable methodology for its mass rearing is needed, that uses fresh coffee berries, the natural host of the pest, thereby mimicking field conditions in the laboratory. We report a technique that enables efficient production of CBB in the laboratory on fresh coffee berries.

Materials and methods

General procedure

The study was carried out in the laboratories of the International Centre of Insect Physiology and Ecology (ICIPE) in Nairobi, Kenya. For this study, coffee berries were collected from two locations in two coffee growing regions of Kenya, i.e., the Kisii district of Western Kenya (00°25′S, 34°28′E, 1 720 m a.s.l.) and the Kiambu district of Central Kenya (1°10′S, 36°49′E, 1 723 m a.s.l.). In the two plantations, coffee [*Coffea arabica* L. var. Ruiru 11(Rubiaceae)] was produced organically without use of any pesticides. The two coffee plantations were chosen based on the differences in crop management as well as differences in environmental conditions, mainly water deficiency/availability. The coffee from Kisii was produced by poor small-scale farmers without any fertilizer input. In addition, this coffee plantation suffered from water deficiency during the fruiting period, resulting in a 'low quality' coffee. On the other hand, the coffee from Kiambu is cultivated using high standards of organic production, was fertilized with compost and manure, and did not suffer from water stress, making it a 'high quality' coffee. Thus, the present study was aimed at testing whether the new rearing technique would be affected by coffee berry quality.

Coffee berries used in all experiments were collected in Kisii and Kiambu between October 2006 and January 2008. Berries older than 120 days of developmental time contain more than 20% of dry matter content and are therefore suitable for the development of the coffee berry borer (Alonzo, 1984; Ruiz, 1995). Coffee berries approximately 150 days old (n = 1 500) were randomly sampled in the field. Coffee berries were collected, brought into the laboratory and checked for CBB infestation. Only non-infested berries of uniform shape and weight were used for the experiments. In total 1 200 berries were used for the experiments.

Hypothenemus hampei females used in this study were obtained from a colony maintained at the ICIPE laboratories. The colony was established in July 2005 with CBB-infested coffee berries collected from different plantations in Kisii. The field-collected beetles were reared in plastic jars filled with 3 cm of plaster of Paris (Pattex; Henkel, Nairobi, Kenya) on fresh coffee berries of approximately 150 days of development collected in the Kisii area. Every month, new insects were brought to the colony. All experiments were conducted at 25 ± 1 °C, 70 ± 5% r.h., and L12:D12 photoperiod.

Artificial infestation of the coffee berries with *Hypothenemus hampei* females

Non-infested healthy coffee berries approximately 150 days old were selected for the experiments. Initially, berries were surface sterilized using the protocol developed by Pérez et al. (2005), where the berries were washed with detergent for 15 min, rinsed with tap water, then dipped in a 2% (wt/vol) sodium hypochlorite solution for 10 min, rinsed again with sterile distilled water, thereafter soaked in a 2% (wt/vol) potassium sorbate solution, and finally rinsed with sterile distilled water. Subsequently, the coffee berries were allowed to dry at room temperature (25 ± 1 °C). Thereafter, the berries were placed in a round plastic container (23 cm diameter × 6.8 cm depth) and exposed to large numbers of *H. hampei* females from the stock culture. After 2 h exposure, berries that were attacked by one female per berry were selected and transferred individually into the vials (see below).

Rearing procedure

The rearing containers used in this experiment were conical polystyrene vials, 5 cm in height, 3 cm in diameter at the top, and a square bottom of 2 × 2 cm. The containers had plastic lids with a 1.5-cm diameter opening, covered with insect gauze to prevent the escape of CBB females. Depending on the treatment, the vials were either filled or not with a 1-cm layer of a mixture of plaster of Paris and charcoal (9:1) as used by Premachandra et al. (2005). Infested or non-infested coffee berries were placed individually in vials with or without the plaster of Paris mixture, thus leading to four treatments. Sterile distilled water was added every 3 days to the vials that contained the plaster of Paris mixture to keep them moist and prevent

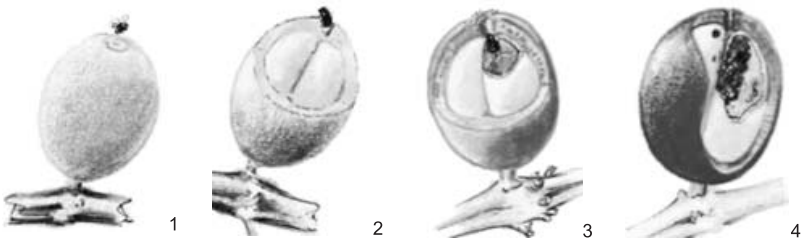

Figure 1 Positions of the coffee berry borer, *Hypothenemus hampei*, in coffee berries (from Bustillo et al., 1998, drawing by Gonzalo Hoyos, Cenicafé).

desiccation of the berries. The vials that did not contain the layer of plaster of Paris were not watered to prevent fungal or bacterial contamination arising from completely soaked coffee berries. In addition, the emergence of CBB females is triggered when infested coffee berries are soaked in water (Baker, 1999). All vials were completely randomized. Six hundred vials were used for each treatment, giving a total of 300 CBB females reared per treatment. The whole procedure was replicated three times for the two coffee berry sources (i.e., Kisii or Kiambu). The vials were kept at room temperature (25 ± 1 °C, 70 ± 5% r.h., and L12:D12) to mimic normal rearing conditions.

Evaluation of treatments

According to Bustillo et al. (1998), the positions of the *H. hampei* female in the coffee berry (Figure 1) are defined as: (1) the female is starting to colonize a new berry and the penetration of the exocarp begins; (2) CBB has commenced penetrating the berry but has not yet reached the endosperm; (3) the beetle has started to bore into the endosperm but has not yet oviposited; and (4) CBB has produced a gallery in the endosperm, and one or more of its immature stages are found inside the gallery. For each position 1, 2, 3, and 4, numbers of live and dead CBB females and CBB life stages (i.e., eggs, larvae, or adults) were recorded at 5-day increments, from 10–55 days after infestation. At each evaluation date, a sample of five berries per treatment and coffee source (i.e., Kisii or Kiambu) was taken. After recording the position of the colonizing female inside the berry, the coffee berry was dissected under a stereomicroscope (10×), and CBB eggs, larvae, and females were counted. For the non-infested berries, their wet weight was recorded at each evaluation date.

Statistical analysis

Percentages were calculated of colonizing females in each position (1, 2, 3, and 4) and of mortality/survival of colonizing females and life stages estimated across evaluation dates. The differences in mortality/survival between treatments and sources of berries (i.e., Kisii and Kiambu) were analysed using a χ^2 test. Differences in oviposition period, total number of eggs, total progeny, sex ratio, and egg–adult survival were analysed by ANOVA, using the general linear model (GLM) procedure of SAS (SAS, 1999). Bonferroni's test was used to compare mean differences between treatments.

The change in the coffee berries' wet weight over time was described by fitting the data to a modified equation of Sequeira & Mackauer (1992) using a non-linear least square regression: $H = (1/a_1) \times [1 + \exp(a_2 - a_3 \times t)]$, where, H is the weight of the coffee berry (g), t is days after infestation with CBB, and a_1, a_2, and a_3 are fitted coefficients. All fitted coefficients were estimated using the non-linear model (NLR) procedure (SAS, 1999). The Proc difference in the change of the coffee berries' wet weight between treatments over time was analysed by ANOVA using the area-under-curve method in the GLM procedure for repeated measures over evaluation dates (SAS, 1999). An F-test was used to test the significance of mean differences, and least square mean values were computed.

Results

Effect of plaster of Paris on colonization of coffee berries and mortality of colonizing females

The presence of plaster of Paris in the experimental units significantly affected the mortality/survival of the CBB females ($\chi^2 = 182.19$, d.f. = 1, $P<0.0001$; n = 472) (Figure 2). In the treatments with and without plaster 87 and 26% live females were found, respectively (Figure 3). No significant differences were found between coffee sources ($\chi^2 = 1.838$, d.f. = 1, $P = 0.18$; n = 472).

The percentage of colonizing CBB females that were found in positions 2, 3, or 4 (none were found in position 1) was significantly affected by coffee source ($\chi^2 = 28.09$, d.f. = 1, $P<0.0001$; n = 602) and plaster of Paris treatment ($\chi^2 = 76.73$, d.f. = 1, $P<0.0001$; n = 602) (Figure 4). In the plaster of Paris treatment the highest proportion of CBB females was found in position 4 (75%), followed by 3 (21%), whereas for the non-plaster treatment, the proportions were 48 and 40% for positions 3 and 4, respectively.

Effect of plaster of Paris on the reproductive potential of CBB inside coffee berries. Data on the oviposition period, total

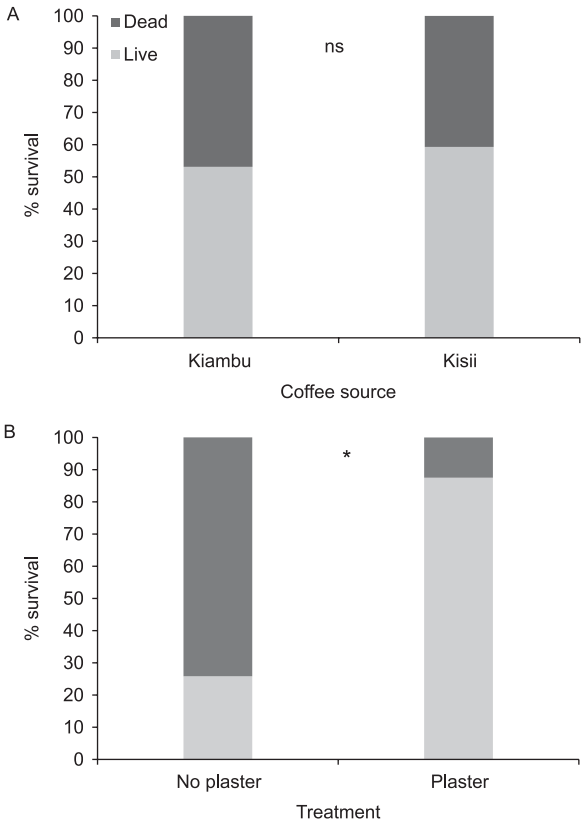

Figure 2 Percentage of live and dead coffee berry borer (*Hypothenemus hampei*) females attacking coffee berries in vials, grouped on basis of (A) source of the berries (Kisii or Kiambu Districts), and (B) presence of plaster of Paris; ns and * indicate non-significant and significant differences (χ^2: P<0.0001), respectively.

number of eggs and progeny per coffee berry, sex ratio, and egg–adult mortality are presented in Table 1. Dissections of the coffee berries revealed that in the vials without plaster of Paris most of the CBB died in the larval and pupal stages.

The plaster of Paris in the vials for both coffee sources had significant effects on oviposition period (F = 90.25 and 361, P = 0.0007 and < 0.0001, for Kiambu and Kisii, respectively), total number of eggs per berry (F = 64.35 and 11.47, P = 0.0013 and 0.0267), total progeny per berry (F = 24.12 and 84.9, P = 0.008 and 0.0008), and egg–adult survival (F = 330.61 and 103.99, P<0.0001 and 0.0005). No F1 females were found in the berries that were reared on the non-plaster treatment for both coffee sources (Table 1).

Effect of plaster of Paris on coffee berry weight and developmental period of CBB. Coffee berry wet weight decreased significantly ($F_{9,72}$ = 292.28, P<0.001) over evaluation time,

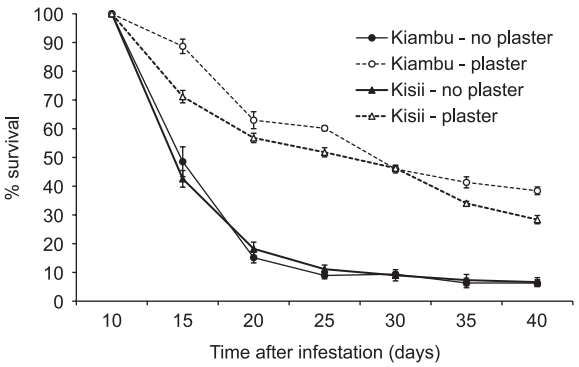

Figure 3 Survival of coffee berry borer (*Hypothenemus hampei*) females in rearing vials with or without plaster of Paris and with coffee berries from Kisii or Kiambu District, Kenya.

across all treatments (Figure 5), and the weight decrease varied between treatments ($F_{3,8}$ = 10.31, P = 0.004). Total wet weight reduction was significantly smaller (P<0.01) in Kiambu coffee berries reared on plaster of Paris than in the other three treatments (Figure 5).

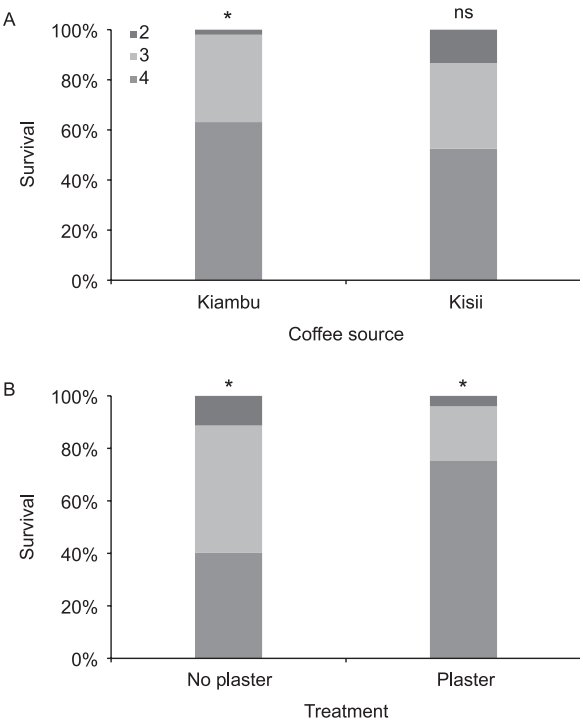

Figure 4 Percentage of coffee berry borer (*Hypothenemus hampei*) females found in one of three positions in coffee berries (2, 3, or 4; see Figure 1), grouped on basis of (A) the source of the berries (Kisii or Kiambu Districts), and (B) presence of plaster of Paris; ns and * indicate non-significant and significant differences (χ^2: P<0.0001), respectively.

Table 1 Development and reproduction parameters of coffee berry borer (*Hypothenemus hampei*) females in rearing vials with or without plaster of Paris and with coffee berries from Kisii or Kiambu District

Coffee source	Treatment	Oviposition period (days)	Total eggs per berry	Total progeny per berry	Sex ratio[1]	Egg–adult survival (%)
Kiambu	No plaster	15.0 ± 2.9	51.0 ± 26.2	1.7 ± 0.9	0	6.3 ± 1.2
	Plaster	46.7 ± 1.7	287.7 ± 13.5	107.3 ± 21.5	88.0 ± 3.3	38.4 ± 1.3
Kisii	No plaster	18.3 ± 1.7	44.7 ± 15.5	2.3 ± 0.9	0	6.6 ± 1.6
	Plaster	50.0 ± 0.1	144.7 ± 25.1	41.7 ± 4.2	88.2 ± 1.5	28.4 ± 1.4

All means with vs. without plaster (within a coffee source and within a column) are significantly different (Bonferroni test: $P<0.05$).
[1]Percentage of daughters in total progeny.

The non-linear model gave a good fit to the data sets for both coffee sources and the two treatments: $r^2 = 0.438$, 0.704, 0.283, and 0.804 ($F = 1107.58$, 1505.25, 773.92, and 1206.84), for Kiambu without and with plaster, and Kisii without and with plaster, respectively ($P<0.0001$ for all four combinations). The fitted parameters of the model are presented in Table 2.

Table 2 Fitted parameters of the model $H = (1/a_1) \times [1 + \exp(a_2 - a_3 \times t)]$ (Sequeira & Mackauer, 1992), estimated using the non-linear model procedure (SAS, 1999)

		Parameters		
Coffee source	Treatment	a_1	a_2	a_3
Kiambu	No plaster	0.9837	–14.3648	–6.8408
	Plaster	8.8943	2.4792	–0.0150
Kisii	No plaster	8.4093	3.0214	–0.0328
	Plaster	1.1397	–54.8397	–5.4475

Discussion

A coffee berry starts to desiccate the moment it is picked from the tree. The physiological development from the flower to the harvest of the ripe berry takes around 240 days (Salazar et al., 1993). In our study we used berries that were approximately 150 days old, with an average of 64% moisture content (Montoya & Cardena, 1994). Because CBB can only thrive in berries with a moisture content between 40 and 80% (Montoya & Cardena, 1994; Bustillo et al., 1998), moisture conditions were good for the development of the beetles' progeny. Once moisture content drops below the critical level, CBB mortality increases sharply (Benavides & Portilla, 1990). Field data from Colombia suggest that berries, harbouring the pest, that fall to the ground are an important source for the re-infestation of next season's coffee (Baker, 1999; Bernal et al., 1999; Bustillo et al., 1999). Bergamin (1944) and Salazar et al. (1993) noted that CBB continues to reproduce and develop in the fallen berries, and assumed that the wet soil surface slows down the desiccation process of the berries, thereby increasing the survivorship of the beetles inside.

The experimental set-up used in this study tried to mimic this process in the laboratory by utilizing a mixture of plaster of Paris and charcoal. The combination of these two materials allows better regulation of the relative humidity in the environment, thus slowing down the dehydration of berries, and at the same time preventing them from rotting. The buffering potential of this mixture has been successfully used in colonies of insects like thrips (Premachandra et al., 2005), springtails (Fox et al., 2007;

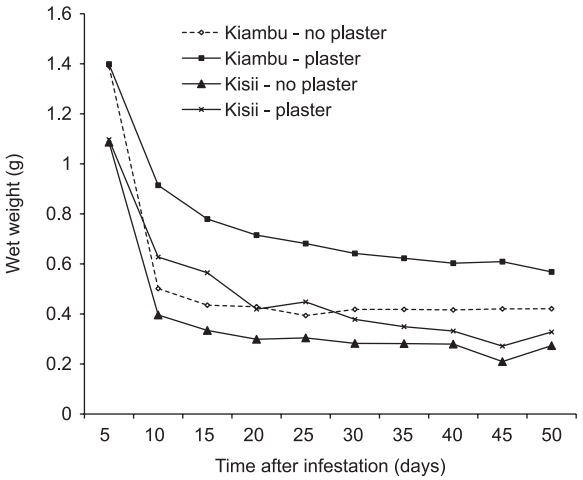

Figure 5 Wet weight (g) over time of coffee berry borer (*Hypothenemus hampei*) females in rearing vials with or without plaster of Paris and with coffee berries from Kisii or Kiambu District.

Park, 2007), and mites (Muma & Denmark, 1967). Relative humidity and moisture content of the berries are important factors for development and progeny production of CBB, hence their optimization is critical for any rearing system (Baker et al., 1992a,b, 1994). In our methodology, the microclimate inside the vials with plaster of Paris kept the berries fresh longer, as shown by the slower decrease of weight compared to the control. This provided sufficient time for the eggs to hatch and the larvae to develop into pupae. By the time the berries started to desiccate further, a considerable proportion of the F1 had already moulted into pupae or adults. In general older CBB broods can better withstand desiccation than younger ones (Baker et al., 1994). Compared to the control, a six- to sevenfold increase in survivorship of the F1 was recorded when using plaster of Paris, and in the latter treatment berries harboured on average more than 100 individuals, whereas only 1.7 in the control. Beetles in the plaster of Paris treatment were less affected by the different qualities of the two coffee berry types.

Coffee berries in the control dehydrated fast, forcing the colonizing females to stop penetrating into the berry in positions 2 or 3 (Figure 4B). Offspring of females in position 4 either died of desiccation or was carried out of the berry by the colonizing females (J Jaramillo, unpubl.). The latter behaviour was also observed by Baker et al. (1994), and they hypothesized this to be a form of brood hygiene. However, we believe that the colonizing female tries to move the brood to a more suitable (moist) environment to increase its chance of survival; hence, it could be an act of brood care, rather than brood hygiene. Yet, no F1 adults were observed in the control.

In this study, CBB progeny production was assessed over a period of 55 days. The survivorship of the F1 started to fall below 50% 40 days after infesting the berries in the plaster of Paris treatment (Figure 3); thus maximum survivorship of the F1 did not exceed 37% (Table 1). Most likely, berries started to dehydrate under our experimental conditions beyond the critical 40% moisture content (Bustillo et al., 1998) after 40 days. Using multi-well plates with 12 berries, i.e., one berry per well, 12 per plate, instead of an individual berry in a vial, progeny production can be extended until the F2-F3, probably because of the higher relative humidity in such an experimental set-up (J Jaramillo, unpubl.).

Although we did not quantify the microbial contamination of the berries over time, it was marginal and never affected the CBB brood, probably due to effective initial surface sterilization of the berries and because the plaster of Paris effectively buffered the relative humidity in the experimental units. This capacity may be particularly important for CBB production systems in coffee-growing regions of the Americas, where ambient relative humidity can reach up to 100% for several months. For example in Mexico, the use of fresh coffee berries for CBB production had to be abandoned and replaced by artificial diets (Villacorta, 1985; Villacorta & Barrera, 1993) mainly because of problems with microbial contaminations (F Infante, pers. comm.).

The methodology presented in this paper is cheap, easy to implement, and not labour intensive. It is particularly well suited for conducting experiments under controlled laboratory conditions or to establish small CBB colonies.

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