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Federación Nacional de Cafeteros de Colombia

Boletín Técnico Cenicafé 48

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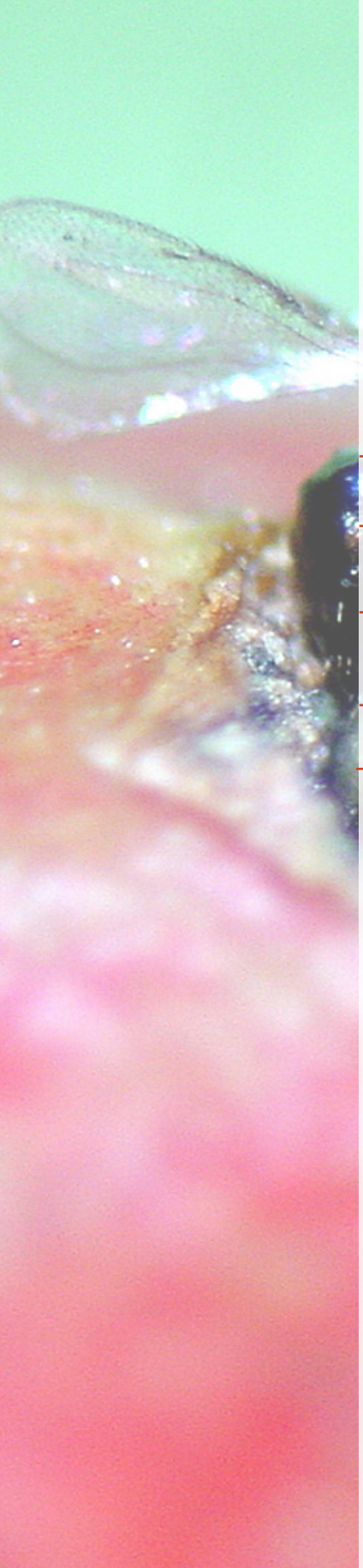

Tabla de **CONTENIDO**

Coffee Berry Borer (CBB) initial population

For the rearing of CBB parasitoids, the first step is to have enough berry borers, which are obtained following this protocol:

a. In the field, collect CBB infested coffee berries, with infestation rates above 20%. At this moment, we should take into consideration that 1 kg of infested coffee berries produce around 1,300 CBB female adults. This procedure should be performed frequently depending on the number of CBB adults required for parasitoid production.

b. Transfer the collected coffee to a rearing laboratory and place it in sieves 1.7 m long x 85 cm wide x 4 cm high (10 kg per sieve) (**Figure 1a**), leave it in the open air (**Figure 1b**); disinfect this material with separate sprays of fungicide and mite pesticide, one liter to sprinkle 60 kg of coffee. (**Figure 1c**). Let the material soak and stir the infested coffee (**Figure 1d**).

c. After disinfection, take the sieves containing the treated CBB infested coffee berries **(Figures 2a and 2b)**, to a room covered with a cloth to prevent the borers from escaping (**Figure 2c**). After 8 days, CBB adults are released if the infestation is lower than 20% (**Figure 2d)**.

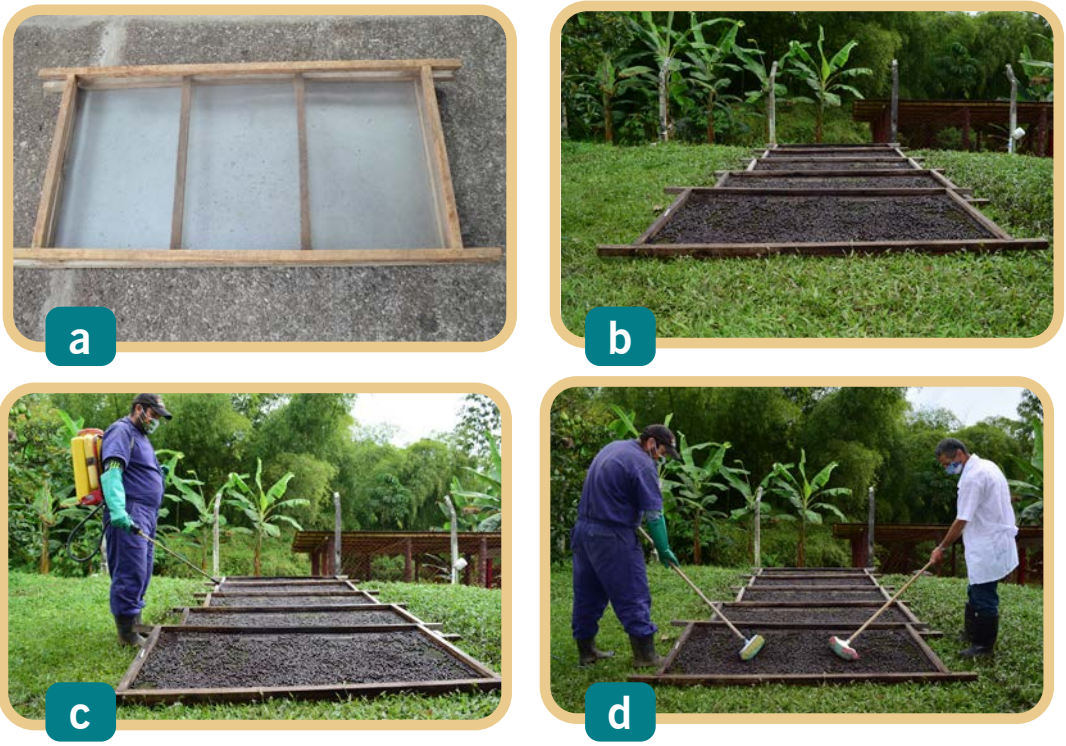

Figure 1. a. Sieves 1.7 m long x 85 cm wide x 4 cm high, **b.** CBB infested coffee berries on open air, **c.** Disinfection of CBB infested coffee berries, **d.** Soaking and stirring the infested coffee.

Figure 2. a. and **b.** Transporting sieves containing treated CBB infested coffee berries, **c.** Room covered with a cloth **d.** CBB adults release to increase infestation above 20%.

d. Cover the sieves with white muslin, then ventilate the room with the coffee containers for 15 days to dry the mucilage of the coffee fruits, during this time stir the coffee berries weekly. Mark the sieves with the place and date of collection of the infested coffee berries. Eliminate impurities using a ventilator (**Figure 3**).

e. Clean after 15 days, for this, use a broom and rub the infested material. Carry out a new disinfection with fungicide and acaricide as in section b, then, transfer the CBB infested coffee to metal trays 87.5 cm long x 37 cm wide x 5.0 cm high with 16 side holes covered with white muslin cloth (**Figure 4a**). Each tray should contain between 10 to 12 kg of this material; move the trays to a dark rearing room at a constant temperature

Figure 3. Ventilation CBB infested coffee berries.

of 25°C and 75% relative humidity **(Figure 4b)**. Mark the trays with the place and date of collection of the coffee berries. In this room, the material will remain for 60 to 150 days until CBB adults are collected.

f. Clean at 8:00 a.m. every CBB infested coffee metal tray after 15 days in order to remove sawdust and others, so to avoid contaminants. In each cleaning the sieve is changed and disinfected again with fungicide and acaricide.

g. At the moment of CBB female adult collection (60 to 150 days), use the trays containing the oldest date of CBB infested coffee, clean it using sifters, then wet the material for two hours (using a sprinkler system). Place it at a collecting room

Figure 4. **a.** CBB infested coffee in metal trays 87.5 cm long x 37 cm wide x 5.0 cm high **b.** Room at a constant temperature of 25 °C and 75% relative humidity.

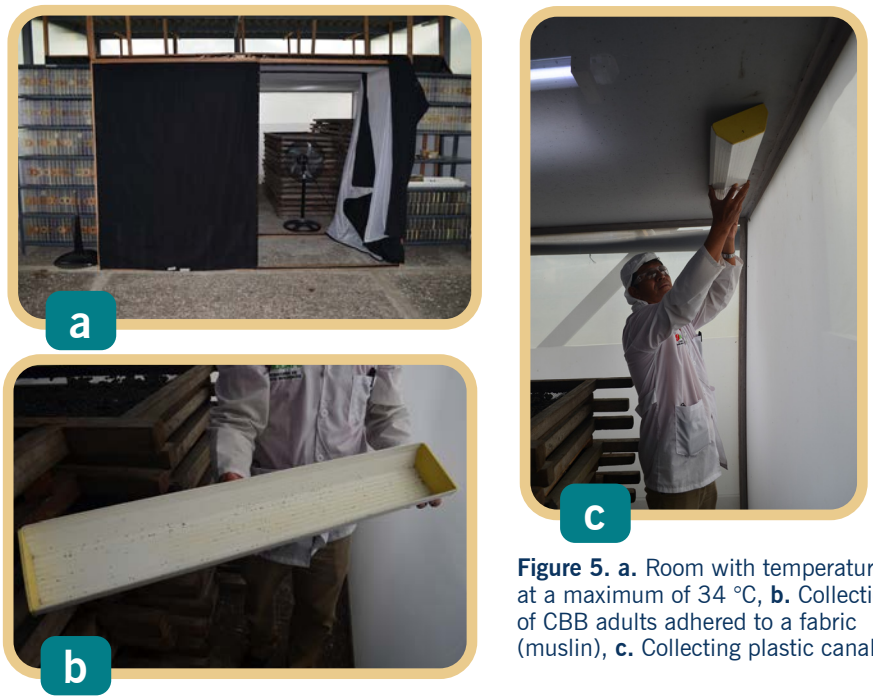

Figure 5. a. Room with temperatures at a maximum of 34 °C, **b.** Collection of CBB adults adhered to a fabric (muslin), **c.** Collecting plastic canal.

rising the temperatures at a maximum of 34°C and 75% relative humidity. **(Figure 5a).** Then, collect the CBB adults adhered to a fabric (muslin) **(Figure 5b)**, using a plastic canal, as they fly in search of light **(Figure 5c).** Every 2 days, take out the sieves, spray the coffee with water for 1 hour using the splinkers, disinfect again with fungicide and acaricide and take it back to the collection room.

h. The collected insects are passed through a sieve which allows classifying the borer from other insects.

i. Place the collected CBB adults in a rectangular acrylic box 21.5 cm long x 10 cm wide x 7.5 cm high, with ventilation holes covered with white muslin cloth and inside place confetti paper to avoid insect mutilation, plug the box **(Figure 6)**.

j. The adult CBB are ready to be used after three to 14 hours; then remove the confetti paper using an acrylic box of 32 cm long x 20 cm wide and 11 cm high (**Figure 7a)**, with mesh at the base that allows the passage of the adults, but not the confetti paper **(Figure 7b).** Cover the box containing the CBB adults.

Figure 6. CBB adults in a rectangular acrylic box 21.5 cm long x 10 cm wide x 7.5 cm high.

Figure 7. a. Acrylic box of 32 cm long x 20 cm wide and 11 cm high, **b.** Adult CBB separated from the confetti paper.

Preparing wet parchment coffee for rearing *Phymastichus coffea* on CBB adults

a. Obtain premium quality wet parchment coffee (around 45% humidity content).

b. Wash the wet coffee parchment; disinfect it by immersion for 12 to 15 hours with the fungicide thiabendazole in a concentration of 3 cm^3/liter **(Figure 8).**

c. Dry the coffee on sieves 60 cm wide x 91 cm long x 4 cm high, to guarantee around 45% humidity **(Figure 9)**. This process takes between 1.5 to 2 days.

d. Once the required moisture percentage is obtained, the coffee is selected, eliminating grains with defects **(Figure 10).**

e. Store the wet parchment in metal trays to preserve humidity at 45% and place it in a room at 23ºC and 75% Relative humidity; up to eight days.

Figure 8. Wash and desinfect the wet coffee parchment. Used one bucket with holes.

Figure 9. Deposit 5 kg of wet coffee parchment on sieves 60 cm wide x 91 cm long x 4 cm high.

Figure 10. Elimination of grains with defects.

Rearing *Phymastichus* coffea on CBB infested wet parchment coffee

Once the CBB adults are collected and the wet parchment coffee has 45% humidity, the parasitoid rearing can be pursued; for this:

Disinfect CBB adults by immersion in 0.5% sodium hypochlorite solution for 10 minutes stirring permanently **(Figure 11a)**; rinse three times with distilled water **(Figure 11b)** then take them out and place them on a muslin fabric to remove wet excess using a disposable towel **(Figure 11c).**

a. Place the CBB adults in a box with a towel on the base, cover the box, and let them dry for 10 minutes.

b. In a 17 cm long x 12 cm wide x 7,0 cm high acrylic box with a ventilation hole in the lid covered with white muslin cloth, place 200 wet parchment coffee arranged with the center cut facing up **(Figure 12)**.

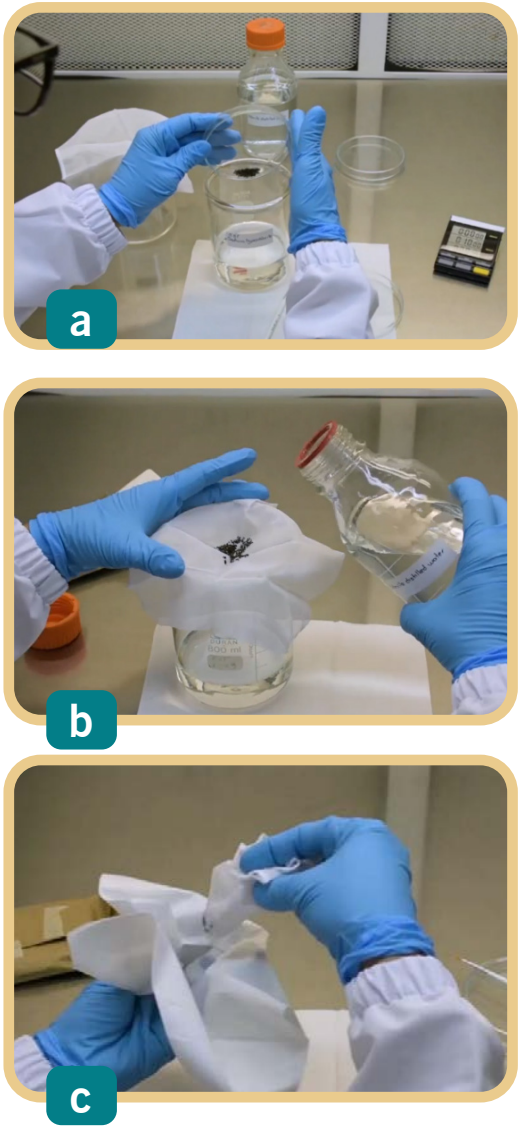

Figure 11. a. Disinfection of CBB in 0.5% sodium hypochlorite solution. **b.** Filtered of CBB with muslin cloth. **c.** Remove wet excess using a disposable towel.

Figure 12. 200 wet parchment coffee arranged with the center cut facing up in acrylic box.

c. Infest the wet parchment coffee with the sanitized CBB adults in a 1: 5 ratio, for a total of 1,000 CBB adults per box containing 200 wet parchment coffees (**Figures 13a and 13b**).

d. After 15 hours of infestation, the CBB adults are boring the wet coffee parchment and are exposing the abdomen. At this moment, parasitize releasing newly emerged *P. coffea* females in a 1:10 ratio, for a total of 100 wasps per box (**Figures 14a, 14b, 14c and 14d**).

e. Place the boxes with the parasitized CBB adults in a dark room for six days at a constant temperature of 25 °C and 75 % ± 3 relative humidity **(Figures 15a and 15b).**

f. After six days, perform the first cleaning to remove sawdust produced by CBB adults, as well as fungi contaminated wet parchment **(Figures 16a and 16b).**

Figure 13. a. 1,000 CBB adults, **b.** Box containing 200 wet parchment coffees and 1,000 CBB.

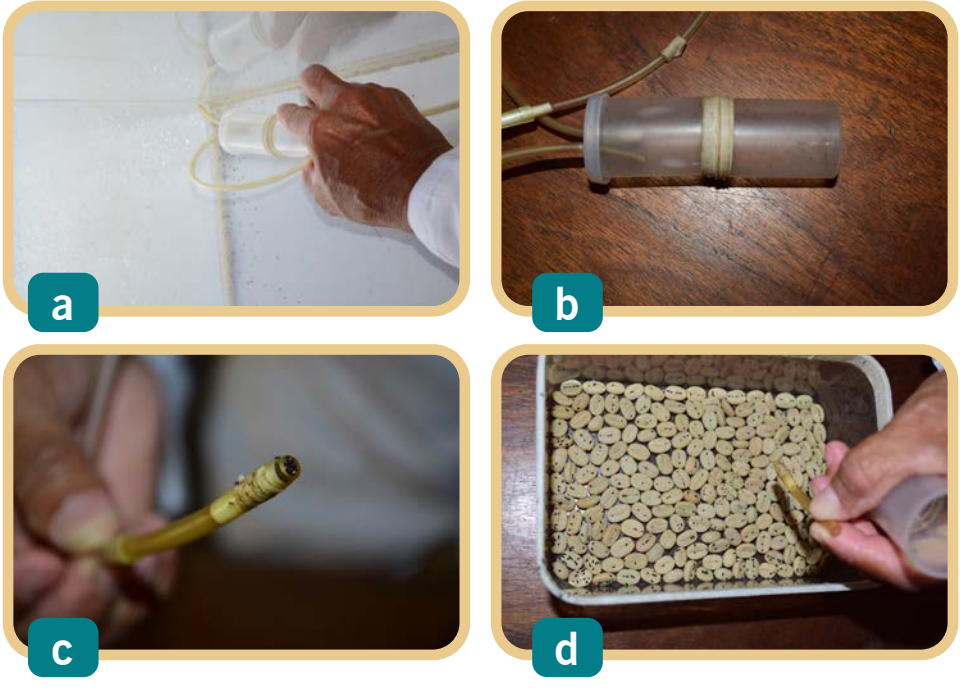

Figure 14. a, b, c, d. Parasitizing with newly emerged P. *coffea* females in a 1:10 ratio, for a total of 100 wasps per box.

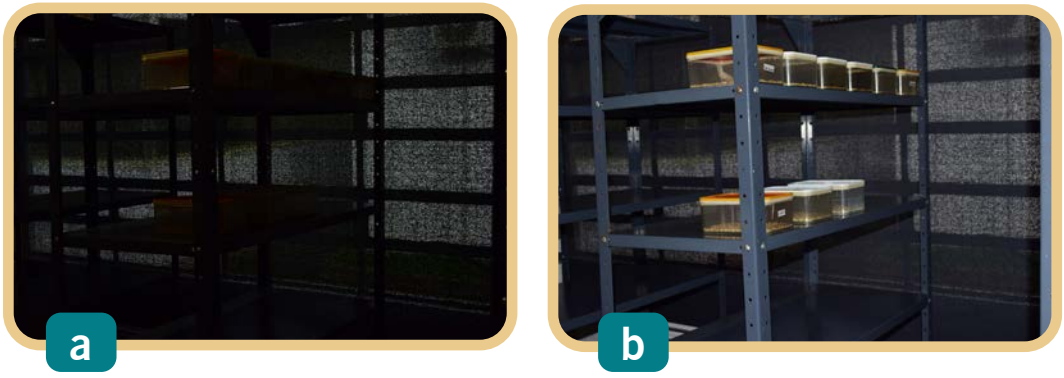

Figure 15. a. Dark room, **b.** Boxes with the parasitized CBB adults in a dark room.

Figure 16. a. and **b** Cleaning to remove sawdust produced by CBB and fungi infected adults.

g. After cleaning, transfer the sawdust-free parasitized CBB in wet parchment coffee into a new 32 cm long x 20 cm wide and 11 cm high acrylic box with two vents on the sides covered with white muslin. For each box put 1,000 parchments, leaving the not infested wet coffee parchments to preserve moisture **(Figure 17).**

h. Place the acrylic boxes in a dark room at 25 °C constant temperature and 75% constant relative humidity.

After 20 days, clean again and remove material contaminated by fungi (**Figure 18**).

i. After cleaning, place 5,000 parasitized CBB wet parchment coffee for each metal tray 87.5 cm long x 37 cm wide x 5 cm high, with 16 vents covered with white muslin cloth and leave them for ten more days (**Figure 19**).

j. After this time, the parasitoids are in the pupal stage, transfer them to sieves 76 cm long x 33 cm wide x 2 cm high **(Figure 20a)** and take them to the *P. coffea* collecting room at a temperature between 25 to 29 °C and a relative humidity between 60 to 65%. For collecting, you should have an acrylic display case 100 cm long x 50 cm wide x 70 cm high, covered with black cloth and a horizontal LED lamp. Finally, *P. coffea* adults are collected using a vacuum pump (**Figure 20b**).

k. Eliminate living CBB (not parasitized) placing parasitized CBB wet parchment coffee between 4 to 5 hours in a dark chamber at a temperature of 29°C and humidity higher than 90%.

Figure 17. 1,000 parasitized CBB in wet parchment coffee into a new 32 cm long x 20 cm wide and 11 cm high acrylic box with two vents on the sides covered with white muslin.

Figure 18. Acrylic boxes in a room at 25 °C constant temperature and 75% constant relative humidity.

Figure 19. 5,000 parasitized CBB wet parchment coffee in metal tray 87.5 cm long x 37 cm wide x 5 cm high.

To carry out releases in the field, pack the parasitized CBB wet parchment coffee in mesh bags with holes that allow the exit of the wasps (**Figure 21a**). These are covered with plastic to protect them from the rain. For each bag, 500 grains are packed; then transferred to the field in Styrofoam coolers (**Figures 21b and 21c).**

The remaining 30% of the grains are taken to the collecting room to recover ***P. coffea*** wasps that will be used to continue the rearing cycle.

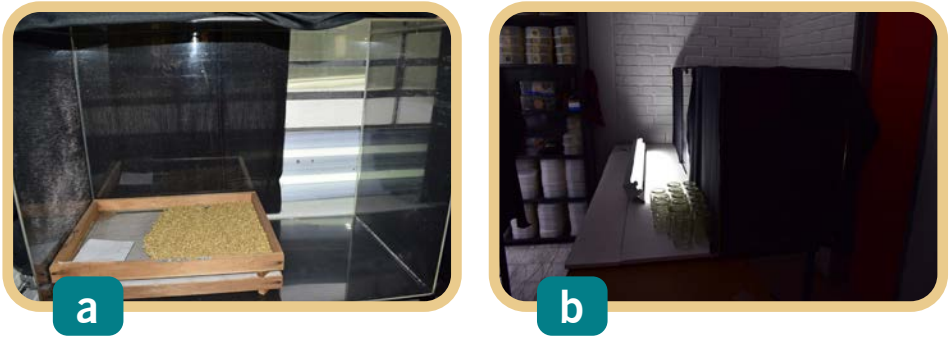

Figure 20. a. Sieves 76 cm long x 33 cm wide x 2 cm high with parasitized CBB wet parchment coffee. **b.** Acrylic display case 100 cm long x 50 cm wide x 70 cm high, covered with black cloth and a horizontal LED lamp.

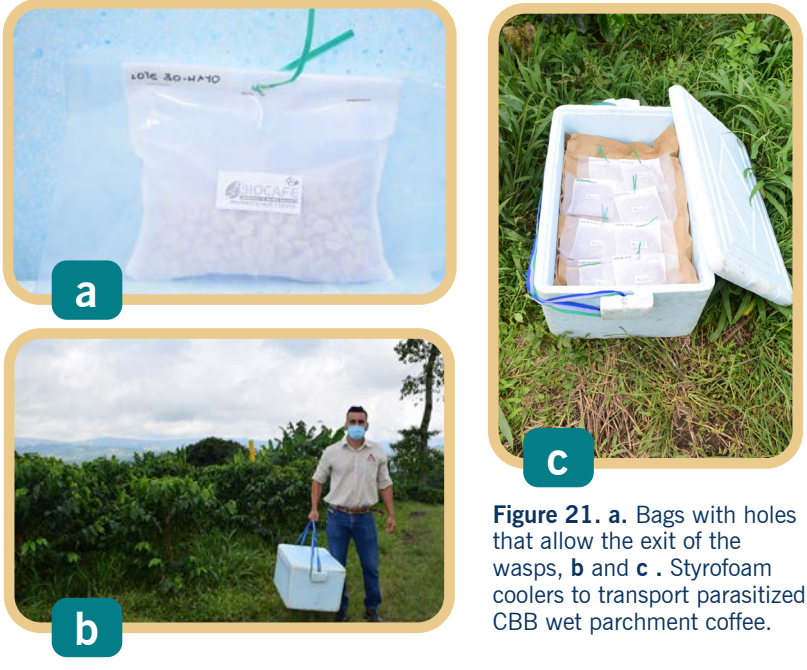

Figure 21. a. Bags with holes that allow the exit of the wasps, **b** and **c** . Styrofoam coolers to transport parasitized CBB wet parchment coffee.

Rearing CBB in artificial diets

Preparation of the artificial diet for the rearing of CBB

Tabla 1. Ingredients to prepare a liter of artificial diet for rearing *Hypothenemus hampei* **(Figure 22).**

Ingredients	Amount
Gelcarin (Carragenina) (CAS 9000–07-1)	13 g
Distelled water	820 ml
Green ground coffee	150 g
Saccharose	10 g
Casein (CAS 9000-71-9)	15 g
Brewer´s yeast (CAS 68876-77-7)	15 g
Benzoic acid (CAS 65-85-0)	1 g
Vanderzant vitamin solution	0.66 g
Wesson salts	0.8 g
Formaldehyde 37% (CAS 50-00-0)	2.66 ml
Methyl parahydroxybenzoate (Nipagin®) (CAS 99763)	1.33 g

Figure 24. Ingredients to prepare the artificial diet for CBB rearing.

a. Dissolve 13 g of gelcarin in 820 ml of water and autoclave for 30 minutes at 121°C **(Figure 23**); when the temperature is below 80°C, mix with 150 g of green ground coffee (granulometry of around 600 micras, achieved with the use of a sieve No. 30 0.0234 opening in inches), 15 g of brewer's yeast, 15 g of casein and 10 g of saccharose, using a mixer at low and constant speed for half spin 12 minutes.

b. When the temperature in the previous mix is below 48°C, add: 1 g Benzoic acid, 0.66 g Vaderzant vitamin solution, 0.8 g Wesson salts, 2.66 ml Formaldehyde 37% and 1.33 g Nipagin. Keep mixing at low speed for 3-4 minutes **(Figure 24)**.

c. Immediately serve 60 ml of the mix in a 90 mm Petri dish (for larger recipients maintain servings of around 0.9 ml/cm^2) **(Figure 25a)**; cover the boxes and let it cool for 12 hours at room temperature; after this time, dry the moisture condensed on the lid using a disposable sterile towel and close again. Then place it in an oven for 15 hours at a constant temperature of 45-47°C until a humidity of 50-65% is archived. Determine the humidity by difference in weight, until a 20% loss is obtained. **(Figure 25b)**.

Figure 23. Mixing the ingredients to prepare the artificial diet for rearing CBB.

Figure 24. Mixing Vaderzant vitamin solution, antifungal and antibacterial ingredients into the artificial diet for rearing CBB.

Figure 25. a. CBB artificial diet serving process (60 ml diet per Petri dish). **b.** CBB diet drying process at 45-47°C.

d. For the CBB rearing it is advisable to use CBB founders coming from artificial diets, which starts by depositing 40 CBB eggs on the diets (in 90 mm Petri dishes). This will provide a starting colony to guarantee a better infestation. The harvesting of CBB adults starts after 60 days when around 30 females per dish are obtained.

e. Then infest artificial diets with the 30 founder female adults of CBB **(Figure 26).**

f. Then place the infested artificial diet in a room at 25°C, and relative humidity of 75% and scotophase 24h. For each founding female it is possible to obtain an average production of 62±6.53 (average±SE) CBB stages every 60 days to start the colony.

Figure 26. Infested artificial diet with 30 female adults of CBB per petri dish.

Artificial substrate for *Phymastichus coffea* rearing on adult CBB individuals

Tabla 2. Ingredients to prepare a liter of artificial substrate for *Phymastichus coffea* rearing on adult CBB individuals.

Ingredients	Amount
Gelcarin (Carragenina CAS 9000–07-1)	13 g
Benzoic acid (CAS 65-85-0)	1 g
Green ground coffee	150 g
Distilled water	820 ml
Nipagin (CAS 99763)	1.32 g
Formaldehyde 37% (CAS 50-00-0)	2.66 ml
Saccharose	10 g

For the preparation of the artificial substrate for *P. coffea*, the process developed in the coffee berry borer diet is similar; the ingredients are in **Table 2, Figure 27**.

a. Dissolve 13 g of gelcarin in 820 ml of water and autoclave for 30 minutes at 121°C (**Figure 28b**); when the temperature is below 80°C, mix with 150 g of ground coffee and 10 g of saccharose, using a mixer at low and constant speed for 12 minutes **(Figure 28b).**

b. When the temperature in the previous mix is below 48°C, add: 1 g Benzoic acid, 2.66 ml Formaldehyde 37% and 1.32 g Nipagin. Keep mixing at low speed for 3-4 minutes**.**

c. Immediately serve 90 ml of the mix in an acrylic box 17 cm long x 12 cm wide x 7 cm high with a ventilation hole in the lid covered with white muslin cloth **(Figure 29)**. For larger recipients maintain a proportion of around 0.45ml/ cm^2**,** cover the box and let it cool for approximately 15 hours at room temperature.

d. Incubate the acrylic box with the artificial substrate in an oven for 15 hours at a constant temperature of 45-47°C, to reach a final humidity of 60-62% **(Figure 30**).

e. Disinfect CBB adults by immersion in 0.5% sodium hypochlorite solution for 10 minutes stirring permanently **(Figure 31f)**; then take them

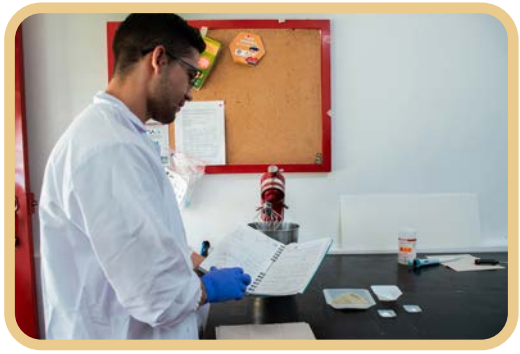

Figure 27. Ingredients to prepare a liter of artificial substrate for *Phymastichus coffea* rearing on adult CBB individuals.

Figure 28. a. Autoclaved water and gelcarin for preparation of artificial substrate to rear *Phymastichus coffea*, **b**. Mixture of green ground coffee and saccharose.

Figure 29. *Phymastichus coffea* artificial substrate serving process (90 ml per acrylic box).

out and place them on a muslin fabric **(Figure 31a)** to remove wet excess using a disposable towel **(Figure 31b).**

f. Infest the artificial substrate with the sanitized CBB adults for a total of 5,000 CBB adults per acrylic box **(Figure 34).**

Figure 30. *Phymastichus coffea* artificial substrate drying process at 45-47°C.

Figure 31. a. Disinfection of CBB in 0.5% sodium hypochlorite solution. **b.** Rinse three times with distilled water using a muslin cloth. **c.** Remove wet excess using a disposable towel.

Figure 32. Acrylic box containing artificial substrate and 5,000 CBB.

g. Place the acrylic boxes in a dark room at 25°C, 75% relative humidity and 24 hours scotophase.

h. After 24 hours of infestation, the CBB adults are penetrating the artificial substrate and are exposing the abdomen. At this moment, parasitize releasing newly emerged *P. coffea* females in a 1:10 ratio, for a total of 500 wasps per acrylic box **(Figure 33).**

i. Place the acrylics boxes with the parasitized CBB adults in in a BOD-type chamber at a constant temperature of 25 °C¸ relative humidity of 60 % and 24-hour scotophase.

j. After fifteen days, for each acrylic box, eliminate contaminated CBB **(Figure 34).**

Recover *Phymastichus coffea* in CBB mummies from artificial substrate using electromagnetic sieves **(Figures 35 a-d).**

k. To harvest adult *Phymastichus coffea* to continue the rearing process, after 32-35 days, take the boxes containing the CBB parasitized, at 8:00 am, inside a transparent acrylic chambers 90 cm long x 60 cm wide x 50 cm high, with a front LED lamp **(Figure 36).** Around 11:00 am, open the lid of the boxes to initiate the emergence of *P. coffea* adults.

Collect the newly emerged wasps with a vacuum pump (DVR II- model DVP 3A free air displacement 6 cfm-170l/m), connected to a 4 mm diameter plastic hose, which at the other end connects to a plastic tube where the harvested adults are deposited **(Figure 37).**

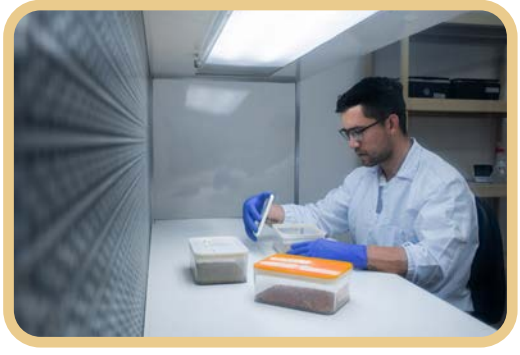

Figure 33. Parasitizing with newly emerged *P. coffea* females in a 1:10 ratio, for a total of 500 wasps per acrylic box.

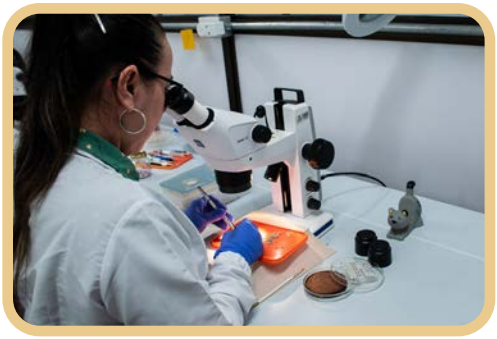

Figure 34.
Eliminating contaminated CBB.

Figure 35. a, b, and **c.** Detail of recovering *Phymastichus coffea* in CBB mummies from artificial substrate using electromagnetic sieves. **d.** CBB mummies separated from the artificial substrate.

Figure 36. Acrylic chambers 90 cm long x 60 cm wide x 50 cm high with a horizontal LED lamp.

Figure 37. Collecting adults *P. coffea* with a vacuum pump.


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