The Coffee Berry Borer: An Overview

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Beltsville, MD
Cofee berry borer – Hypothenemus hampei
(Coleoptera: Curculionidae: Scolytinae)

Photos by E. Erbe (left) and P. Grebb (right), USDA, ARS.
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.

Kurz revidirt
von

J. A. Graf Ferrari,

Beamter am k. k. zoolog. Kabinete in Wien, mehrerer in- und ausländischer
gelahrten Gesellschaften Mitglied.

Wien.
Druck und Verlag von Carl Gerold's Sohn.
1867.
The Tropics
Coffee berry borer first reported in:

Gabon 1901
Democratic Republic of the Congo (ex Zaire) 1903
Java (Indonesia) 1908-1909
Uganda 1908
Brazil 1913
Borneo and Sumatra (Indonesia) 1919
Côte d’Ivoire 1922
Kenya 1928
Malaysia 1929
New Caledonia 1948
Peru 1962
Tahiti 1963
Nicaragua 1969
El Salvador 1969
Guatemala 1971
Honduras 1977
Mexico 1978
Jamaica 1978
Fiji 1979
El Salvador 1981
Ecuador 1981
Colombia 1988
Nicaragua 1988
India 1990
Venezuela 1995
Dominican Republic 1995
Costa Rica 2001
Puerto Rico 2007
Hawaii 2010
Coffee berry borer
Basic biology:

- Berries are susceptible ca. 100-150 days post-flowering
- Female has been reported to oviposit up to 300 eggs
- From eggs to adults: 25-35 days
- Skewed sex ratio: 10 females for every male
- Longevity ♀ (days):
  - 35-112 Uganda
  - 87-102 Java
  - 120 Malaysia
  - 157 Brazil
- ♂ can’t fly and remain inside the berry
Damage

[Diagram showing the relationships between different stages of coffee bean damage, including an intact bean, an infected bean, and a damaged bean with visible damage to the internal layers.]
Infestation levels

• 60% Colombia and Mexico
• 75% Jamaica
• 50-90% Malaysia
• 80% Uganda and Côte d’Ivoire
• 90% Tanzania
Estimated losses

> $500 MILLION!
Artificial diet
Wolbachia infection

- FEMALE-BIASED SEX RATIOS

Vega et al., Annals of the Entomological Society of America 95:374-378
Can we manipulate *Wolbachia* to reduce the number of female progeny?
Caffeine
CBB-associated yeasts

*Pichia burtonii* and *Candida fermentati*
How does the CBB breaks down caffeine?
Analysis of microbial communities associated with the coffee berry borer

Eoin L. Brodie

Javier Ceja Navarro

C. A. Santee, N. J. Bouskill, U. Karaoz, M. Zemla, and M. Auer
Percentage distribution of bacterial phylogenetic groups associated with the CBB. Combined counts for all samples: family level (except “Firmicutes”)

Pyrosequencing of bacteria and fungi associated with the CBB.
Shown to degrade caffeine

Previously found in association with the coffee berry borer
Fungi associated with the CBB in Mexico

✓ 40 fungal species in 22 genera
Penicillium brocae, a new species associated with the CBB in Chiapas, Mexico
Pest management
Cultural control

Frequent harvest/sanitation:
• interrupts the life cycle of the insect
• reduces the # infested berries that fall on the ground
MANEJO INTEGRADO DE LA BROCA DEL CAFÉ

Hypothemus hampei (Ferrari) en Colombia

Alex E. Bustillo P.
Reinaldo Cárdenas M.
Diógenes A. Villalba G.
Pablo Benavides M.
Jaime Orozco H.
Francisco J. Posada F.

Cenicafé
CENTRO NACIONAL DE INVESTIGACIONES DE CAFÉ
"PEDIRO URIBE MEJÍA"
Cultural control
Cultural control
Natural enemies

• Parasitoids

• Predators

• Nematodes

• Fungal entomopathogens

Aaron Davis, Kew Gardens
Parasitoids

• *Phymastichus coffea* (Eulophidae)
• *Cephalonomia stephanoderis* (Bethylidae)
• *Prorops nasuta* (Bethylidae)
• *Heterospilus coffeicola* (Braconidae)
• *Cryptoxilos* (Braconidae) – Colombia
• *Cephalonomia hyalinipennis* – Mexico
Parasitoids

Phymastichus coffea

Cephalonomia stephanoderis
Predators - thrips

Karnyothrips flavipes

Top left photo by L. Mound, CSIRO; others by J. Jaramillo, ICIPE
Molecular diagnosis of a previously unreported predator–prey association in coffee: *Karnyothrips flavipes* Jones (Thysanoptera: Phlaeothripidae) predation on the coffee berry borer

Juliana Jaramillo • Eric G. Chapman • Fernando E. Vega • James D. Harwood

Abstract The coffee berry borer, *Hypothenemus hampei*, is the most important pest of coffee throughout the world, causing losses estimated at US $500 million/year. The thrips *Karnyothrips flavipes* was observed for the first time feeding on immature stages of *H. hampei* in April 2008 from samples collected in the Kisii area of Western Kenya. Since the trophic interactions between *H. hampei* and *K. flavipes* are carried out entirely within the coffee berry, and because thrips feed by liquid ingestion, we used molecular gut-content analysis to confirm the potential role of *K. flavipes* as a predator of *H. hampei* in an organic coffee production system. Species-specific COI primers designed for *H. hampei* were shown to have a high degree of specificity for *H. hampei* DNA and did not produce any PCR product from DNA templates of the other insects associated with the coffee agroecosystems. In total, 3,327 *K. flavipes* emerged from 17,792 *H. hampei*-infested berries collected from the field between April and September 2008. Throughout the season, 8.3% of *K. flavipes* tested positive for *H. hampei* DNA, although at times this figure approached 50%. Prey availability was significantly correlated with prey consumption, thus indicating the potential impact on *H. hampei* populations.
Contributed Paper

Ecological and Economic Services Provided by Birds on Jamaican Blue Mountain Coffee Farms

JHERMIE L. KELLERMANN,*† MATTHEW D. JOHNSON,* AMY M. STERCHO,* AND STEVEN C. HACKETT†
*Department of Wildlife, Humboldt State University, Arcata, CA 95521, U.S.A.
†Department of Economics, Humboldt State University, Arcata, CA 95521, U.S.A.

Abstract: Coffee farms can support significant biodiversity, yet intensification of farming practices is degrading agricultural habitats and compromising ecosystem services such as biological pest control. The coffee berry borer (Hypothenemus hampei) is the world's primary coffee pest. Researchers have demonstrated that birds reduce insect abundance on coffee farms but have not documented avian control of the berry borer or quantified avian benefits to crop yield or farm income. We conducted a bird-exclusion experiment on coffee farms in the Blue Mountains, Jamaica, to measure avian pest control of berry borers, identify potential predator species, assess predator abundance and borer reductions with vegetation complexity, and quantify resulting increases in coffee yield. Coffee plants excluded from foraging birds had significantly higher borer infestation, more borer broods, and greater berry damage than control plants. We identified 17 potential predator species (73% were Neotropical migrants), and 3 primary species composed 67% of migrant detections. Average relative bird abundance and diversity and relative resident predator abundance increased with greater shade-tree cover. Although migrant predators overall did not respond to vegetation complexity variables, the 3 primary species increased with proximity to noncoffee habitat patches. Lower infestation on control plants was correlated with higher total bird abundance, but not with predator abundance or vegetation complexity. Infestation of fruit was 1-14% lower on control plants, resulting in a greater quantity of salable fruits that had a market value of US$4.4-8.1/50lb in 2005/2006. Landscape heterogeneity in this region may allow mobile predators to provide pest control broadly, despite localized farming intensities. These results provide the first evidence that birds control coffee berry borers and thus increase coffee yield and farm income, a potentially important conservation incentive for producers.

Keywords: coffee berry borer, coffee farms, ecosystem services, Hypothenemus hampei, Jamaican coffee, pest control

Servicios Ecológicos y Económicos Proporcionados por Aves en Fincas Cafetaleras en Blue Mountains, Jamaica

Resumen: Las fincas cafetaleras pueden soportar biodiversidad significativa. Sin embargo, la intensificación de las prácticas agrícolas está degradando los hábitats naturales y comprometiendo los servicios del ecosistema tal como el control biológico de plagas. El barrenador del café (Hypothenemus hampei) es la principal plaga del café a nivel mundial. Los investigadores han demostrado que las aves reducen la abundancia de insectos en las fincas cafetaleras pero no han documentado el control de aves sobre el barrenador del café ni cuantificado los beneficios de las aves a la producción o al ingreso de la finca. Realizamos un experimento de exclusión de aves en fincas cafetaleras en las Blue Mountains, Jamaica, para medir el control de barrenadores del café, identificar especies potencialmente depredadoras, cuantificar la abundancia de depredadores y la reducción de barrenadores con la complejidad vegetal y cuantificar los incrementos en la producción de café. Las plantas de café excluidas del forrajeo de aves tuvieron significativamente mayor infestación y reproducción de barrenadores, mayor daño de frutos que las plantas control. Identificamos 17 especies potencialmente depredadoras (73% fueron especies migratorias neotropicales), y 3 especies primarias.

*email: jkellermann@gmail.com
Paper submitted April 27, 2007; revised manuscript accepted January 30, 2008.
Predators - ants

- Azteca
- Dorymyrmex
- Mycocepurus
- Pheidole
- Pseudomyrmex
- Solenopsis
- Tetramorium
Nematodes

*Heterorhabditis* and *Steinernema* – commercially available
Nematodes

Nematode life cycle courtesy of David Shapiro-Ilan, USDA-ARS
Nematodes

Coffee berry borer = 2 mm long

*Steinernema* infective juvenile = 0.5 mm long

adult = 2-3 mm long

![Infected and Healthy Nematodes](image)

*Heterorhabditis bacteriophora*

Photos courtesy of Alfredo Castillo, ECOSUR, Mexico
Nematodes

Metaparasitylenchus hypothenemisi

Poinar et al., J. Parasitol. 90:1106-1110
Nematodes

*Metaparasitylenchus hypothenemi*

*Panagrolaimus* sp.
Nematodes

Sampling in Kenya - 2006-2009

Mermithidae – new genus

Juliana Jaramillo

George O. Poinar, Jr.
Fungal entomopathogens

- *Beauveria bassiana*
- *Metarhizium anisopliae*
- *Isaria farinosa*
- *Isaria fumosorosea*
- *Lecanicillium lecanii*
- *Nomuraea rileyi*
- *Ophiocordyceps entomorrhiza*
Fungal entomopathogens

Beauveria bassiana

over 20 phylogenetic species
*B. bassiana* natural infestations in the field:

- Venezuela 30%
- India 60%
- Mexico <10%
- Brazil <1%
A new method to evaluate the biocontrol potential of single spore isolates of fungal entomopathogens
Life cycle (days) for *B. bassiana* (IBL 03047)

- **Conidia discharged:** 2.3 ± 0.1 (n=40)
- **Production of conidia:** 0.7 ± 0.1 (n=40)
- **Insect covered with mycelium:** 1.2 ± 0.1 (n=40)
- **Mycelium starts to grow:** 0.9 ± 0.0 (n=40)
- **Fungal Inoculation**
- **Time to kill:** 4.8 ± 0.2 (n=40)
- **Death:**

**Summary:**

- **9.9 ± 0.2 (n= 40)** ([X± S.E.])
Mycotrol® ES
EMULSIFIABLE SUSPENSION MYCOINSECTICIDE

For use in controlling Whitefly, Aphids, Thrips, Psyllids, Mealybugs, Leafhoppers, Weevils, Plant Bugs, Borer and Leaf-feeding Insects in Field, Agronomic, Vegetable and Orchard Crops; also in Forestry; Grasshoppers, Mormon Crickets, Locusts and Beetles in Rangeland, Improved Pastures and Agronomic Crops; Whitefly, Aphids, Thrips, Psyllids and Mealybugs in Ornamentals and Vegetables, Indoor/Outdoor Nursery, Greenhouse, Shadehouse, Commercial Landscape, Interiorscape and Turf.

Active Ingredient: Beauveria bassiana Strain GHA........11.3%**
Inert Ingredients: ...........................................88.7%
Total: ......................................................100.0%

*Contains petroleum distillates
**Based on the weight estimate of 4.78 x 10^-12 grams per spore.
Mycotrol ES contains 2 x 10^13 viable spores per quart.

KEEP OUT OF THE REACH OF CHILDREN

CAUTION

Store between 40°F and 85°F

See additional precautionary statements and first aid statements in attached booklet.

LAVERLAM INTERNATIONAL CORPORATION
117 S Parkmont; P.O. Box 4109-Butte, MT 59702; Ph: (406)782-2386; Fax: (406) 782-9912
EPA Registration Number 82074-1
EPA Establishment Number 65626-MT-02

Edition: 080923
Lot No.: ESO090605
Net Contents: 1 Gallon
Expiration Date: 6.30.2010
Traps/Attractants
Traps/Attractants

The BROCAP® trap

- Trap suspension hook
- Diffusion space
- Dispenser holder
- Dispenser
- Capture funnel
- Lid
- Capture recipient
- Overflow
- Capture liquid

[Image of the BROCAP® trap]

[Image of a red BROCAP® trap in a natural setting]
The BROCAP® trap

A novel solution for controlling the coffee berry borer

The coffee berry borer (CBB), Hypothenemus hampei, is a major coffee insect pest. As traditional control methods have their limitations, an economical environment-friendly solution is proposed.

Limitations of chemical control
Insecticide use has well-known drawbacks:
• the risk of toxic residues,
• insecticide with limited selective, depleting CBB natural enemies,
• CBB resistance to insecticides.

The BROCAP® trap is an effective integrated control instrument
With its design adapted to the insect's biology and its powerful anticoagulant, these traps effectively reduce CBB populations in coffee plantations.

Advantages of the BROCAP® trap
Using the BROCAP® trap means:
• Exchanging the traditional concept of CBB control for a reality: integrated solution adapted to quality coffee production.
• Improving producer incomes.

In quality terms
• Production of guaranteed pesticide-free coffee.
• Lower beans damaged by CBB, so less risk of contamination by mycoexhin producing fungi.
• Preserves the environment and biodiversity.
• Through selective trapping, 97% of the insects caught are coffee berry borers.

In quantity terms
• 10 to 16% increase in the weight of green coffee yields.

In quality terms

The BROCAP® trap

The trap, the funnel with red blades, a colour that attracts coffee berry borers.
• Centre, the dispenser containing the volatile attractant.
• Bottom, the capture recipient, transparent for visual inspection.

Trap efficiency
Trap efficiency has been confirmed by tests in several countries, notably in certain regions of El Salvador.

Up to 10,000 CBB caught per trap per day in a major migration period, in highly infested regions.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean rate of reduction of CBB at harvest (%)</th>
<th>Mean rate of reduction (%)</th>
<th>Mean rate of reduction (%)</th>
<th>Mean rate of reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hiping</td>
<td>1.09</td>
<td>4.45</td>
<td>9.06</td>
<td>16.3</td>
</tr>
<tr>
<td>Control</td>
<td>4.46</td>
<td>13.20</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Recommended use

When should traps be installed?
In the pre-harvest period, when CBB populations are preparing to leave residual fruits to seek new food sources. The traps are installed for 4 months each year; e.g., from the beginning of March to the end of June in El Salvador.

When should the dispenser be replaced?
After around 2 months use, two dispensers will therefore be required per season.

How many traps, installed how?
At least 18 traps on hectare (12 traps/manzana), 24 metres apart and 1.20 metres from the ground.

Should the traps be replaced each year?
No. The trap can be used for several years.

An economical solution

The trap is cheap to buy and amortized over several years, so it is cheaper to use than insecticide treatments.

Packaging
Traps: in boxes of 36 units.
Dispensers: in boxes of 36 units.

The first year, for 2 hectares or 3 manzanas, one box of 36 traps and two boxes of dispensers (72 dispensers in all) are required. Thereafter, dispensers can be obtained separately for replacement.

For further information

CIRAD
Tree Crops Department
Export service
Telephone: +33 4 67 61 75 65 / 66
Fax: +33 4 67 61 71 20
brocap@cirad.fr
**When should traps be installed?**

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*Up to 10,000 CBB caught per trap per day in a major migration period, in highly infested regions!*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Bored fruits at the start of infestation (%)</th>
<th>Bored fruits in the first harvest (%)</th>
<th>Reduction in CBB populations trapping/control (%)</th>
<th>Harvest improvement by green coffee weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trapping</td>
<td>1.01</td>
<td>4.60</td>
<td>81.06</td>
<td>16.3</td>
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<tr>
<td>Control</td>
<td>4.46</td>
<td>13.20</td>
<td>____</td>
<td>____</td>
</tr>
</tbody>
</table>
BROCAP trap in Mexico (Garcia et al., 2004)

• sampling for 16 weeks using 16 traps

• 679,107 insects (= 2,653 CBB per trap per week)

• Dufour (2002) has reported reductions of 12-85% in CBB infestation

• Villacorta et al. (2001) reported 50% reduction in infestation

• Barrera (2006) reported no significant differences
PASOS PARA LA ELABORACIÓN DE LA TRAMPA ARTE SALAL

TRAMPA ARTE SALAL OPCIÓN UNO: Se usa una botella plástica de bebida gaseosa (1), se corta la parte superior de la botella (2) luego se invierte la parte superior de la botella (3) y se introduce en la parte inferior que es la más larga de la botella que cortamos (4). Después de tener la estructura plástica armada (4), se coloca el alambre de tal forma que atraviese el ancho de la botella y se forma un gancho que servirá para colgar la trampa (5), luego se colocara el difusor o atrayente en el alambre de forma individual amarrándolo al alambre (6) o si lo prefiere, puede colocar o amarrar el difusor o atrayente utilizando el mismo alambre con el que se forma el gancho (7).

TRAMPA ARTE SALAL OPCIÓN DOS: Se usa una botella plástica de bebida gaseosa (1), se cortan cuatro partes iguales de forma vertical (2) y a cada una se les hacen cortes horizontales dejando la parte superior más larga con las cuales se formarán las aletas y eliminando la parte inferior (3). Después de tener armada la estructura plástica que forma la trampa, se elabora con alambre el soporte del difusor o atrayente (4) y (5) luego se coloca el alambre que formará el gancho para ser colocada la trampa en el cafetal y se le coloca el soporte del difusor o atrayente en la estructura plástica y de esta manera se tiene la trampa armada (6). Por último se puede pintar de color rojo la trampa si así lo desea.

COMO UTILIZAR AMBOS MODELOS DE TRAMPAS
- Llenar el fondo de las trampas con agua, destapar los difusores, cortar su punta y colocarlos en las trampas. Se recomienda agregar 3 o 4 gotas de jabón líquido sin olor al agua, para evitar el mal olor por la acumulación de brocas muertas.
- Se debe colgar cada trampa en una rama, a una altura de 1.2 m y a una distancia de 21 a 22 m. entre sí.
- Se recomienda revisar las trampas cada 8 a 10 días. Dependiendo de la cantidad de broca capturada y del nivel de agua, se limpian y llenan nuevamente de agua limpia. El cambio de difusores se realiza inmediatamente se termine el que está en uso.

Si necesita más información sobre el trampeo, puede solicitar la asesoría de un técnico en los 15 Centros de Atención al Caficultor con que cuenta PROCAFE.

Oficina Central y Región I: PBX: 286-3588, Fax: 286-0669 Región I: Tel. 487-8475 y Telefax 487-8474
Región III: Tel: 683-0669 y 683-0153

PROCAFE, El Salvador
Traps – artificial berries

Robert Behle (USDA-ARS)
<table>
<thead>
<tr>
<th>compound</th>
<th>( t_R )</th>
<th>green</th>
<th>half-ripe</th>
<th>ripe</th>
<th>overripe</th>
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<tr>
<td>1,3-butadiene, 2-methyl-</td>
<td>2.68</td>
<td>160</td>
<td>97</td>
<td>3017</td>
<td>1839</td>
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<td>3.04</td>
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<td>138</td>
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<td>acetaldehyde</td>
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<td>2201</td>
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<td>9731</td>
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<td>711</td>
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<td>89</td>
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<td>231</td>
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<tr>
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<td>8.39</td>
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<td>231</td>
<td>955</td>
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<td>5050</td>
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<td>955</td>
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<td>2-butanol, 3-methyl</td>
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<td>149</td>
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<td>86</td>
<td>1121</td>
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<td>9495</td>
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<td>toluene</td>
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<td>72</td>
<td>132</td>
<td>231</td>
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<td>2317</td>
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<td>593</td>
<td>256</td>
<td>138</td>
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<td>1-propanol, 2-methyl</td>
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<td>15.97</td>
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<tr>
<td>1-butanol</td>
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<td>593</td>
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<tr>
<td>( \beta )-ocimene</td>
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<td>73</td>
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<tr>
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<td>2-butene-1-ol, 2-methyl</td>
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<td>73</td>
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<tr>
<td>1-octen-3-ol</td>
<td>23.19</td>
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<td>332035</td>
<td>2422796</td>
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</tbody>
</table>
Table 1. Mean ± SEM per female for each insect stage 40 d after placing one, two, or five females in vials containing artificial diet.

<table>
<thead>
<tr>
<th>Developmental stage</th>
<th>1</th>
<th>2</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eggs</td>
<td>7.1 ± 1.72 (143)</td>
<td>6.1 ± 2.66* (243)</td>
<td>2.6 ± 3.81* (262)</td>
</tr>
<tr>
<td>Larvae</td>
<td>13.1 ± 3.01 (263)</td>
<td>4.1 ± 2.15* (164)</td>
<td>1.7 ± 2.76* (168)</td>
</tr>
<tr>
<td>Pupae</td>
<td>1.3 ± 0.38 (27)</td>
<td>0.9 ± 0.55 (36)</td>
<td>0.1 ± 0.28 (14)</td>
</tr>
<tr>
<td>Adults</td>
<td>3.6 ± 0.84 (73)</td>
<td>1.8 ± 0.65* (74)</td>
<td>1.3 ± 0.54* (127)</td>
</tr>
<tr>
<td>Total</td>
<td>25.1 ± 2.38 (506)</td>
<td>12.9 ± 2.21 (517)</td>
<td>5.7 ± 2.99 (571)</td>
</tr>
</tbody>
</table>

Table 2. Mean ± SEM per female for each insect stage 40 d after placing one female or two females in vials containing artificial diet.

<table>
<thead>
<tr>
<th>Developmental stage</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eggs</td>
<td>6.3 ± 1.49 (253)</td>
<td>3.8 ± 1.99* (454)</td>
</tr>
<tr>
<td>Larvae</td>
<td>4.9 ± 1.00 (190)</td>
<td>2.8 ± 1.37* (296)</td>
</tr>
<tr>
<td>Pupae</td>
<td>0.5 ± 0.42 (34)</td>
<td>1.0 ± 0.6 (91)</td>
</tr>
<tr>
<td>Adults</td>
<td>1.1 ± 0.62 (47)</td>
<td>1.1 ± 1.0 (138)</td>
</tr>
<tr>
<td>Total</td>
<td>13.1 ± 1.24 (530)</td>
<td>8.1 ± 1.59 (982)</td>
</tr>
</tbody>
</table>
Can we develop better CBB attractants?

Can we develop CBB repellents?
Climate change: coffee on the move?

Thermal Tolerance of the Coffee Berry Borer Hypothenemus hampei: Predictions of Climate Change Impact on a Tropical Insect Pest

Juliana Jaramillo¹,²*, Adenirin Chabi-Olaye², Charles Kamonjo², Alvaro Jaramillo³, Fernando E. Vega⁴, Hans-Michael Poehling¹, Christian Borgemeister²

¹ Institute of Plant Diseases and Plant Protection, University of Hannover, Hannover, Germany, ²International Center of Insect Physiology and Ecology (icipe), Nairobi, Kenya, ³Centro Nacional de Investigaciones de Café, Manizales, Colombia, ⁴Sustainable Perennial Crops Laboratory, United States Department of Agriculture, Agricultural Research Service, Beltsville, Maryland, United States of America
Field survey of Ethiopia's coffee-growing regions conducted in the late 1960s found no trace of CBB, but in 2003 researchers reported that the pest was widespread (J. Jaramillo, pers. comm.).

The average minimum temperature for CBB to reproduce is about 68°F; the mountainous regions of Ethiopia did not reach that temperature until 1984.

For every 1.8°F increase in temperature, Colombian Arabica growers will have to move their plants up about 160 masl to maintain quality and quantity.

Two possibilities:

- Move (altitude or latitude)
- Adapt
Using shade trees in Central America and East Africa:

• a reduction of 3.6–10.8°F when compared to coffee grown at full sun

Adaptation Strategy:

• Shade trees

Temperature reduction:

• ca. 7.2°F at low elevations
• ca. 3.6°F at mid- to high elevations
Beauveria bassiana as a fungal endophyte
Beauveria bassiana endophytic in:

- Corn
- *Pinus radiata*
- Banana
- Date palm
- Potato, cotton, cocklebur, jimsonweed
- Tomato
- *Theobroma cacao, T. gileri*
- Opium poppy
- Coffee berries
- Sorghum
**Beauveria** activity against plant pathogens:

- *Gaeumannomyces graminis* (take-all disease of wheat)
- *Pythium, Septoria*
- *Fusarium oxysporum, Armillaria mellea, Rosellinia necatrix*
- *Rhizoctonia* damping-off
Francisco Posada · Fernando E. Vega

Inoculation and colonization of coffee seedlings (*Coffea arabica* L.) with the fungal entomopathogen *Beauveria bassiana* (Ascomycota: Hypocreales)


Establishment of the fungal entomopathogen *Beauveria bassiana* (Ascomycota: Hypocreales) as an endophyte in cocoa seedlings (*Theobroma cacao*)

Francisco Posada
Fernando E. Vega
Inoculation of coffee plants with the fungal entomopathogen Beauveria bassiana (Ascomycota: Hypocreales)

Francisco POSADA\textsuperscript{a}, M. Catherine AIME\textsuperscript{b}, Stephen W. PETERSON\textsuperscript{c}, Stephen A. REHNER\textsuperscript{a}, Fernando E. VEGA\textsuperscript{a,*}
Beauveria bassiana inoculation methods

- Spraying: 7 x 10^6 spores ml⁻¹
- Injection: 5 x 10^5 spores ml⁻¹
- Irrigation: 7 x 10^6 spores ml⁻¹
- Seed soaking
**Beauveria bassiana recovery**

<table>
<thead>
<tr>
<th>MONTHS POST-INOCULATION</th>
<th>% RECOVERY</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>31</td>
</tr>
<tr>
<td>4</td>
<td>5.5</td>
</tr>
<tr>
<td>6</td>
<td>2.7</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
</tr>
</tbody>
</table>
Fungal endophytes in coffee seedlings 2 and 4 months post-inoculation with *Beauveria bassiana*

<table>
<thead>
<tr>
<th>Alternaria sp.</th>
<th>Hypocreales sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arthopyrenaceae</td>
<td>Macrophomina sp.</td>
</tr>
<tr>
<td>Aspergillus tamari</td>
<td>Paecilomyces sp.</td>
</tr>
<tr>
<td>Aspergillus westerdijkiae</td>
<td><em>Penicillium citrinum</em></td>
</tr>
<tr>
<td><em>Beauveria bassiana</em></td>
<td><em>Penicillium brevicompactum</em></td>
</tr>
<tr>
<td>Bionectriaceae</td>
<td><em>Penicillium cecidicola</em></td>
</tr>
<tr>
<td>Chaetomium sp.</td>
<td><em>Penicillium glabrum</em></td>
</tr>
<tr>
<td>Cladosporium cf. <em>sphaerospermum</em></td>
<td><em>Penicillium janthinellum</em></td>
</tr>
<tr>
<td>Clavicipitaceae</td>
<td><em>Penicillium sp. near daleae</em></td>
</tr>
<tr>
<td><em>Colletotrichum gloeosporoides</em> complex</td>
<td><em>Penicillium steckii</em></td>
</tr>
<tr>
<td>Cylindrocarpon sp.</td>
<td><em>Penicillium toxicarium</em></td>
</tr>
<tr>
<td>Exobasidiomycetidae</td>
<td>Phyllachoraceae</td>
</tr>
<tr>
<td>Exophiala sp.</td>
<td><em>Plectosphaerella</em> sp.</td>
</tr>
<tr>
<td><em>Fusarium</em> cf. <em>oxysporum</em> f. sp. vasinfectum</td>
<td><em>Pleosporales</em> sp.</td>
</tr>
<tr>
<td><em>Fusarium oxysporum</em> complex (1)</td>
<td><em>Pseudallescheria</em> cf. <em>boydii</em></td>
</tr>
<tr>
<td><em>Fusarium oxysporum</em> complex (2)</td>
<td><em>Rhizopycnis</em> sp.</td>
</tr>
<tr>
<td><em>Fusarium</em> sp. (1)</td>
<td><em>Trichoderma</em> sp.</td>
</tr>
<tr>
<td><em>Fusarium</em> sp. (2)</td>
<td><em>Trichoderma hamatum</em></td>
</tr>
<tr>
<td><em>Fusarium</em> sp. (Lateritium clade 1)</td>
<td><em>Trichoderma harzianum</em></td>
</tr>
</tbody>
</table>
Fungal endophytes in coffee plants: the ecosystem within
Sampling of *Coffea arabica*

- **Colombia**
  - Puerto Rico
  - Hawaii
  - Mexico
Tissue sterilization for endophyte isolation

- 0.5% NaOCl – 2 min
- 70% ethanol – 2 min
- Sterile dist. water
Ribosomal RNA coding gene (rDNA)
<table>
<thead>
<tr>
<th>Location</th>
<th>Endophytes recovered</th>
<th>ITS genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colombia</td>
<td>267</td>
<td>113</td>
</tr>
<tr>
<td>Hawaii</td>
<td>393</td>
<td>126</td>
</tr>
<tr>
<td>Mexico</td>
<td>109</td>
<td>32</td>
</tr>
<tr>
<td>Puerto Rico</td>
<td>74</td>
<td>40</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>843</strong></td>
<td><strong>257</strong></td>
</tr>
</tbody>
</table>
Most abundant and richest taxa:
- *Colletotrichum*
- *Fusarium*
- *Penicillium*
- *Xylariaceae*

Of the 257 genotypes:
- 220 found in only one of the regions sampled
- 25 shared by two regions
- 10 shared by three regions
- 2 shared by four regions

Endophytes also isolated from *Coffea canephora*, *C. congensis*, *C. liberica*, *C. macrocarpa*, *C. racemosa*, and *C. stenophylla*.
“The human intestinal microbiota ... contains at least 100 times as many genes as our own genome.”

“...We have 10 times more microbes than human cells in our bodies.”

G. Weinstock, Washington Univ. in St. Louis

1,000 species of bacteria identified in mouth

128 species of bacteria in lungs of healthy people
### Fungal endophytes in green coffee seeds

<table>
<thead>
<tr>
<th>Fungal id</th>
<th>Country</th>
</tr>
</thead>
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<td>Acremonium sp.</td>
<td>Puerto Rico</td>
</tr>
<tr>
<td>Aspergillus sumatrense</td>
<td>Puerto Rico</td>
</tr>
<tr>
<td>Aspergillus fumigatus</td>
<td>India</td>
</tr>
<tr>
<td>Aspergillus fumigatus</td>
<td>Vietnam</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>Vietnam</td>
</tr>
<tr>
<td>Aspergillus pseudodeflectus</td>
<td>Papua New Guinea</td>
</tr>
<tr>
<td>Aspergillus pseudodeflectus</td>
<td>Vietnam</td>
</tr>
<tr>
<td>Aspergillus tubingiensis</td>
<td>Colombia</td>
</tr>
<tr>
<td>Aspergillus tubingiensis</td>
<td>Kenya</td>
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<tr>
<td>Clavicipitaceae sp. 1</td>
<td>Puerto Rico</td>
</tr>
<tr>
<td>Clavicipitaceae sp. 2</td>
<td>Puerto Rico</td>
</tr>
<tr>
<td>Aspergillus (Eurotium) ruber</td>
<td>India</td>
</tr>
<tr>
<td>Fusarium solani complex</td>
<td>Vietnam</td>
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<tr>
<td>Gibberella sp.</td>
<td>Colombia</td>
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<tr>
<td>Penicillium sp., subgenus Biverticillium</td>
<td>India</td>
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<tr>
<td>Penicillium crustosum</td>
<td>Guatemala</td>
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<tr>
<td>Penicillium olsonii</td>
<td>Colombia</td>
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</table>
Penicillium coffeaе, a new endophytic species isolated from a coffee plant
Coffee bacterial endophytes
Coffee bacterial endophytes

<table>
<thead>
<tr>
<th>Bacillus cereus</th>
<th>Gordona sp.</th>
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<tr>
<td>Bacillus megaterium</td>
<td>Klebsiella planticola</td>
</tr>
<tr>
<td>Bacilus subtilis</td>
<td>Klebsiella pneumoniae</td>
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<tr>
<td>Bacillus megaterium</td>
<td>Klebsiella trevisanii</td>
</tr>
<tr>
<td>Burkholderia cepacia</td>
<td>Kocuria kristinae</td>
</tr>
<tr>
<td>Burkholderia gladioli</td>
<td>Methylobacterium radiotolerans</td>
</tr>
<tr>
<td>Burkholderia glathei</td>
<td>Micrococcus sp.</td>
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<tr>
<td>Burkholderia pyrrocina</td>
<td>Pantoea agglomerans</td>
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<tr>
<td>Cedecea davisae</td>
<td>Pseudomonas chloroaphis</td>
</tr>
<tr>
<td>Chromobacterium sp.</td>
<td>Pseudomonas putida</td>
</tr>
<tr>
<td>Clavibacter michiganense insidiosum</td>
<td>Rhodococcus equi</td>
</tr>
<tr>
<td>Curtobacterium flaccumfaciens</td>
<td>Salmonella typhimurium</td>
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<tr>
<td>Enterobacter asburiae</td>
<td>Serratia liquefaciens</td>
</tr>
<tr>
<td>Enterobacter cancerogenus</td>
<td>Stenotrophomonas maltophilia</td>
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<tr>
<td>Enterobacter gergoviae</td>
<td>Variovorax paradoxus</td>
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<tr>
<td>Escherichia vulneris</td>
<td>Xanthomonas sp.</td>
</tr>
</tbody>
</table>

Vega et al., Journal of Basic Microbiology 45:371-380
What roles could endophytes be playing?

Can we establish fungal insect pathogens as systemic endophytes?
Summary

- **Wolbachia**
- Caffeine
- Natural enemies associated with the CBB: are there viruses, bacteria, or protozoa that might have a negative effect on the CBB?
- Attractants
- **Endophytes**
  - Introducing fungal insect pathogens
  - Fungal endophyte biodiversity
  - Bacterial endophytes