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Mycobiota associated with the coffee berry borer (*Hypothenemus hampei*) in Mexico

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Field surveys were carried out in coffee plantations in Chiapas, Mexico, to collect and identify fungi associated with the cuticle, gut, faeces and galleries of the coffee berry borer, *Hypothenemus hampei*. Insects and coffee berries containing galleries were collected in three coffee farms at different altitudes: Rosario Izapa (425 m), La Alianza (700 m) and Monteperla (950 m). An additional sample consisting of coffee berry borers reared in the laboratory on meridic diets was also included. Results show that there is a great diversity of fungi associated with this insect. 212 cultures, including 40 species distributed in 22 genera, were isolated. The recovery of fungi from the galleries was markedly less than from the borer's body. Three of the isolated species were undescribed; two belonging to the *Penicillium* and one to *Hanseniaspora*. Most of the species were collected from the cuticle of the insect, and the presence of fungi was not correlated with altitude. *Fusarium*, *Penicillium*, *Candida* and *Aspergillus* were the dominant genera with percentage abundance of 26.4, 18.7, 13.4 and 12.5 %, respectively. The present study provides a detailed description of the mycobiota associated with *H. hampei* and represents a significant advance in the understanding of the relationship among this insect and the fungi associated with it.

INTRODUCTION

The coffee berry borer (CBB), *Hypothenemus hampei*, is considered the key pest of coffee worldwide (Le Pelley 1968, Murphy & Moore 1990, Baker 1999). This beetle, indigenous to Central Africa, was accidentally introduced into Mexico in the late 1970s through the state of Chiapas, near the border with Guatemala (Baker 1984). At present the CBB has spread to the most important coffee areas of this country, where the damage has increased in the past 12 years (Ramirez & Reyes 2000). CBB damage is caused by both adult and larval stages, as they feed and reproduce in the endosperm of the coffee berry, thus reducing the quality and yield of coffee (Le Pelley 1968). The insect spends most of its time inside the coffee beans and its control is especially difficult due to its cryptic life cycle (Murphy & Moore 1990). Given the magnitude of the problem, the biology and ecology of the CBB has been intensively studied in recent years (Baker, Barrera & Rivas 1992, Baker & Barrera 1993, Baker *et al*. 1994, Baker 1999) to develop alternatives to chemical and cultural control methods.

Most of the 6000 species of *Scolytidae* are involved in a mutualistic relationship with fungi, which they cultivate and utilize as a food source (Wood 1982, Booth, Cox & Madge 1990). However, other species are also associated with fungi that can be pathogenic to the host plant or to the beetle (Whitney 1982; Waters, Stark & Wood 1985). The scolytids can be divided in two main subdivisions that describes their habits: bark beetles and ambrosia beetles (Rudinsky 1962). As a general rule, ambrosia beetles, which include the genus *Hypothenemus*, live in mutualistic associations with fungi (Batra 1967, Nakashima 1971). Therefore, for many years researchers suspected there were fungi associated with the CBB (Villacorta & Barrera 1993). Earlier investigations reported CBB associations with *Hirsutella eleutheratorum* (Posada, Bustillo & Saldarriaga 1993), *Fusarium* sp. (Pérez, Posada & González 1996), and

Paecilomyces lilacinus (Posada, Martin & Pérez 1998). Besides, spores of other fungi such as, *Aspergillus ochraceus*, *Penicillium chrysogenum* and *Verticillium s. lat*. sp., have been also recovered from CBB females in coffee plantations in Africa (Vega, Mercadier & Dowd 1999). Despite these reports, the role of fungi in the biology of the beetle has not been completely clear.

Morales-Ramos *et al*. (2000) reported the first documented case of a mutualistic association between *H. hampei* and *Fusarium solani*. Although the crucial experiments to demonstrate this association were not conducted, this is the only report of a mutualism for *H. hampei*. Since there are many species of fungi that have been isolated from the CBB, it is possible that there is not a specific fungal symbiont associated with the pest. In view of this, the objective of this paper was to survey the mycobiota associated with the coffee berry borer and its galleries in coffee plantations of Chiapas, Mexico. This study is part of a broader project to clarify the possible mutualistic relationships between *H. hampei* and fungi.

MATERIALS AND METHODS

Study areas

Coffee berries (*Coffea arabica*) infested with the CBB, were randomly collected from May to July (rainy season) in three coffee plantations near Tapachula, Chiapas: Rosario Izapa (14° 57′ 54.1″ N, 92° 09′ 6.4″ W; 425 m), La Alianza (15° 02′ 27″ N, 92° 10′ 22″ W; 700 m) and Monteperla (15° 02′ 50″ N, 92° 05′ 19″ W; 950 m). There was only one sample from each location, which consisted of approximately 500 female borers per site, obtained through the dissection of the berries. In addition, another group of 500 fourth generation CBB females reared on a meridic diet at laboratories of ECOSUR, was included.

Fungal isolation

The insects from each location were superficially disinfected in a solution of 0.5% sodium hypochlorite for 5 min and rinsed in sterile distilled water. Afterwards, they were submerged in a solution of 0.05% ascorbic acid + 0.05% citric acid for 5 min. Fungal spores were removed from the cuticle by immersion of a single CBB in 0.85% saline solution. The gut (proventriculus to rectum) was extracted, from 50 CBB, by grasping the abdomen and pulling out the anus with sterile forceps. Immediately after, the gut was macerated in 20 µl of saline solution to avoid dehydration (Gilliam & Prest 1972). Faeces were obtained by placing a group of 15 CBB females for 24 h in a sterile Petri dish (5 cm diam) lined with a piece of filter paper moistened in saline solution. Each group yielded one replicate of faeces.

Samples from cuticle ($n = 50$), gut ($n = 50$) and faeces ($n = 25$) were taken and placed in Eppendorf vials containing 100 µl of saline solution. These samples were shaken in ultrasonic cleaners at a frequency of 42 kHz for 10 s (Cazemier *et al*. 1997). Ten µl aliquots from each sample were spread on Petri dishes containing potato–dextrose–agar (PDA) acidified with lactic acid (44%) to inhibit bacterial growth (pH = 4.0–4.5) (Pérez, Posada & González 1996).

Ten samples of gallery walls from each location were subject to the disinfection process mentioned above. Each sample consisted of five pieces of gallery 2 mm long, which were placed directly on agar in Petri dishes. These were incubated at room temperature (±30 °C) for 7 d.

Fungal colonies were isolated and purified in PDA vials, and a preliminary identification based on macro and microscopic characteristics carried out (Barnett & Hunter 1998). *Fusarium*, *Penicillium* and yeast cultures were identified at the Laboratory of Microbial Genomics and Bioprocessing Research Unit, National Center for Agricultural Utilization Research in Peoria, IL. The remaining species were identified at The University of Alberta, Microfungus Collection and Herbarium (UAMH). Representatives cultures were accessed into both collections (Table 1).

Analyses

To compare fungal dominance among samples and locations, the percentage of abundance was calculated using the following equation (Ho *et al*. 2001):

$$P_i = \frac{n_i}{\sum_{i=1}^{S} n_i} \times 100, \quad i = 1, 2, 3, \ldots, S$$

Where P_i is the percentage abundance of the i species, n_i is the number of samples with i species, and S is the number of species found in all samples and locations. Fungal diversity was measured calculating four indices: (1) Richness index (Dulymamode, Cannon & Peerally 2001); (2) Shannon–Wiener index (Ricklefs & Miller 2000); (3) Simpson index (Ricklefs & Miller 2000); and (4) Evenness index (Stiling 1999). Because some isolates were not identified to species, the taxonomic unit of comparison to measure diversity was the genus (Ricklefs & Miller 2000). As it was not possible to identify the genus of some ascomycete yeasts, they were included in a separate group 'other yeasts'.

To compare the Shannon–Wiener indices among locations for each kind of sample (substrate), a Hutchenson t test was performed using a level of significance of 0.05 in all comparisons (Hutchenson 1970). To avoid problems of inflated error rates because of the number of tests, the Bonferroni's correction (Scheiner 1993) was applied giving a critical value of $\alpha = 0.008$ for borers and $\alpha = 0.016$ for galleries. Correspondence analysis were performed to visualize the effect of the numbers of fungi isolated from each substrate and the collection site (Everitt 1992).

Table 1. Representatives of some fungal species obtained from the coffee berry borer and its galleries in Chiapas, and place where they were accessed as live cultures.

Register	Identification	Substrate	Location
NRRL[1] 31462	*Penicillium brocae*[2]	Cuticle	Rosario Izapa, Cacahoatán
NRRL 31463	*P. brocae*	Cuticle	Rosario Izapa, Cacahoatán
NRRL 31465	*P. brocae*	Cuticle	Rosario Izapa, Cacahoatán
NRRL 31469	*P. brocae*	Cuticle	Laboratory (diet)
NRRL 31471	*P. brocae*	Cuticle	Rosario Izapa, Cacahoatán
NRRL 31472	*P. brocae*	Faeces	Laboratory (diet)
NRRL 31473	*P. brocae*	Cuticle	Laboratory (diet)
NRRL 31479	*P. brocae*	Cuticle	Rosario Izapa, Cacahoatán
NRRL 31485	*P. brocae*	Cuticle	Rosario Izapa, Cacahoatán
NRRL 31461	*P. citrinum*	Gut	Rosario Izapa, Cacahoatán
NRRL 31468	*P. citrinum*	Gut	Laboratory (diet)
NRRL 31475	*P. citrinum*	Gut	Monteperla, Unión Juárez
NRRL 31478	*P. citrinum*	Cuticle	Monteperla, Unión Juárez
NRRL 31481	*P. citrinum*	Cuticle	Rosario Izapa, Cacahoatán
NRRL 31486	*P. citrinum*	Cuticle	Rosario Izapa, Cacahoatán
NRRL 31466	*P. crustosum*	Faeces	Laboratory (diet)
NRRL 31480	*P. crustosum*	Gut	Rosario Izapa, Cacahoatán
NRRL 31487	*P. crustosum*	Cuticle	Laboratory (diet)
NRRL 31464	*P. olsonii*	Gut	La Alianza, Cacahoatán
NRRL 31467	*P. olsonii*	Cuticle	La Alianza, Cacahoatán
NRRL 31470	*P. biverticillate*	Cuticle	Laboratory (diet)
NRRL 31476	*P. biverticillate*	Cuticle	Laboratory (diet)
NRRL 31483	*P. biverticillate*	Faeces	Rosario Izapa, Cacahoatán
NRRL 31482	*P.* near *cyaneum*	Faeces	Rosario Izapa, Cacahoatán
NRRL 31484	*P.* near *oxalicum*	Gut	La Alianza, Cacahoatán
NRRL 31474	*Penicillium* sp.[3]	Cuticle	La Alianza, Cacahoatán
NRRL 31477	*Penicillium* sp.[3]	Cuticle	La Alianza, Cacahoatán
NRRL 31488	*Penicillium* sp.[3]	Cuticle	La Alianza, Cacahoatán
NRRL 31489	*Fusarium solani*	Cuticle	La Alianza, Cacahoatán
NRRL 31490	*F. solani*	Galleries	La Alianza, Cacahoatán
NRRL 31491	*F. solani*	Cuticle	Rosario Izapa, Cacahoatán
NRRL 31492	*F. solani*	Cuticle	Monteperla, Unión Juárez
NRRL 31493	*F. solani*	Galleries	Monteperla, Unión Juárez
NRRL Y-27431	*Candida diddensiae*	Cuticle	La Alianza, Cacahoatán
NRRL Y-27432	*C. diddensiae*	Gut	La Alianza, Cacahoatán
NRRL Y-27433	*C. fermentati*	Gut	Monteperla, Unión Juárez
NRRL Y-27434	*C. fermentati*	Cuticle	Rosario Izapa, Cacahoatán
NRRL Y-27435	*C. fermentati*	Galleries	Rosario Izapa, Cacahoatán
NRRL Y-27436	*Hanseniaspora* sp.[4]	Galleries	Monteperla, Unión Juárez
UAMH[5] 10117	*Pichia burtonii*	Cuticle	Rosario Izapa, Cacahoatán
UAMH 10118	*P. burtonii*	Faeces	Rosario Izapa, Cacahoatán
UAMH 10119	*Sporotrichum pruinosum*	Gut	La Alianza, Cacahoatán
UAMH 10120	*Aspergillus sclerotiorum*	Cuticle	Laboratory (diet)
UAMH 10121	*Metarhizium anisopliae*	Cuticle	Laboratory (diet)
UAMH 10122	*Fusarium merismoides*	Cuticle	La Alianza, Cacahoatán
UAMH 10123	*Tetracoccosporium praxianum*	Gut	Laboratory (diet)
UAMH 10124	*Ochroconis constricta*	Faeces	Laboratory (diet)
UAMH 10125	*Penicillium cfr variabile*	Cuticle	Rosario Izapa, Cacahoatán
UAMH 10126	*Acremonium terricola*	Cuticle	La Alianza, Cacahoatán
UAMH 10127	*Tritirachium oryzae*	Gut	Laboratory (diet)
UAMH 10128	*Curvularia pallescens*	Cuticle	La Alianza, Cacahoatán
UAMH 10129	*Coryne* sp.	Gut	Monteperla, Unión Juárez
UAMH 10134	*Coryne* sp.	Gut	Monteperla, Unión Juárez
UAMH 10133	*Didymosphaeria arachidicola*	Cuticle	Rosario Izapa, Cacahoatán
UAMH 10135	*Torula herbarum*	Cuticle	Monteperla, Unión Juárez

[1] Northern Regional Research Laboratory, USDA-ARS (Peoria).
[2] A new species described in Peterson *et al*. (2003).
[3] A new species of *Penicillium*, description pending.
[4] A new species of *Hanseniaspora*, description pending.
[5] University of Alberta Microfungus Collection and Herbarium.

Table 2. Percent of abundance of fungi isolated from the coffee berry borer in Chiapas.

	La Alianza			Rosario Izapa			Monteperla			Laboratory (diet)			
Species	CU	G	FE	CU	G	FE	CU	G	FE	CU	G	FE	Total
Acremonium terricola	3.5												0.44
Aspergillus niger										21.1			3.29
A. ochraceus				3.2						38.0		17.4	7.03
A. sclerotiorum										1.4			0.22
A. sydowii	3.5												0.44
A. versicolor										1.4			0.22
Aspergillus sp.	8.6	2.6											1.31
Candida diddensiae	22.4	43.6											6.59
C. fermentati				6.4				49.1					6.81
Chrysonilia sitophila	1.7												0.22
Cladosporium sp.	8.6		4.0					1.8		2.8	16.7	17.4	2.85
Coryne sp.								7.3					0.87
Curvularia pallescens	1.7												0.22
Didymosphaeria arachidicola				1.6									0.22
Fusarium merismoides	1.7												0.22
F. solani	32.8	15.4	64.0	1.6			84.4	34.6	100				24.61
Fusarium sp.	3.5			4.8				1.8		1.4			1.53
Metarhizium anisopliae										1.4			0.22
Ochroconis constricta												17.4	0.65
Paecilomyces variotii					3.9	2.6							0.44
Penicillum biverticillate						2.6				9.9	16.7		1.97
P. brocae				19.1						7.0		5.9	3.95
P. cfr *variabile*				1.6									0.22
P. citrinum				33.3	26.9		12.5	3.6			16.7		7.69
P. nr *crustosum*					3.9					14.1		5.9	2.63
P. nr *cyaneum*						5.1							0.44
P. nr *oxalicum*		2.6											0.22
P. olsonii	1.7	2.6											0.44
Penicillum sp. nov.	5.2												0.65
Penicillum sp.				3.2									0.44
Phoma sp.				1.6									0.22
Pichia burtonii				6.4	3.9	35.9							4.17
Sistotrema brinkmannii		2.6											0.22
Sporotrichum pruinosum		2.6											0.22
Tetracoccosporium praxianum											16.7		0.22
Torula herbarum							3.1						0.22
Trichoderma sp.	1.7												0.22
Tritirachium oryzae											16.7		0.22
Other yeasts	3.5	28.2	32.0	17.5	61.5	53.9		1.8		1.4	16.7	35.3	17.14

CU, cuticle; G, gut; FE, faeces.

RESULTS

Abundance of fungi in the insect

187 fungal isolates, representing 21 genera and 39 species, were found to be associated with the insect body. From this, 110, 48 and 29 cultures, were isolated from the cuticle, gut and faeces, respectively. *Fusarium*, *Penicillium*, *Candida* and *Aspergillus* were the dominant genera, with percentage abundance of 26.4, 18.7, 13.4 and 12.5%, in this order. The abundance of the remaining genera was $<5\%$ (Table 2). The distribution of the isolates according to the location was as follows: Rosario Izapa 73, La Alianza 56, Laboratory 30, and Monteperla 28.

Fusarium was present in all locations and was associated with all three types of substrata (cuticle, gut, and faeces). There were three species in this genus, the *F. solani* species complex being predominant. *Penicillium* was the genus with the most number of species (11) and was mainly associated with Rosario Izapa, where two new species of *Penicillium* were collected, one of them already described as *P. brocae* (Peterson *et al*. 2003).

Table 3. Percent of abundance of fungi isolated from galleries made by the coffee berry borer in coffee berries.

Species	La Alianza	Rosario Izapa	Monteperla	Total
Candida fermentati		36.4		10.3
Coryne sp.	7.7			2.6
Fusarium solani	76.9	18.2	13.3	35.9
Fusarium sp.		36.4	46.7	28.2
Hanseniaspora sp. nov.			26.7	10.3
Other yeasts	15.4	9.1	13.3	12.8

Yeasts were isolated from all substrata in all locations. However, only three species could be identified:

Table 4. Diversity and richness of genera of fungi associated with the coffee berry borer in Chiapas.

	Location	Genera	Samples	Frequency	Richness index	Shannon index	Simpson index	Evenness index
Cuticle	La Alianza	10	50	57	0.20	1.81	4.57	0.67
	Rosario Izapa	8	50	56	0.16	1.44	2.93	0.53
	Monteperla	3	50	32	0.06	0.51	1.37	0.19
	Laboratory (diet)	6	50	62	0.12	0.97	2.12	0.36
Gut	La Alianza	7	50	39	0.14	1.44	3.36	0.56
	Rosario Izapa	4	50	26	0.08	0.91	2.10	0.36
	Monteperla	6	50	55	0.12	1.17	2.63	0.46
	Laboratory (diet)	5	50	6	0.10	1.56	4.5	0.61
Faeces	La Alianza	3	25	25	0.12	0.78	1.95	0.37
	Rosario Izapa	4	25	38	0.16	1.00	2.38	0.48
	Monteperla	1	25	24	0.04	0.00	1.00	0.00
	Laboratory (diet)	5	25	17	0.20	1.54	4.31	0.74
Galleries	La Alianza	3	10	13	0.30	0.69	1.61	0.43
	Rosario Izapa	3	10	11	0.30	0.92	2.28	0.57
	Monteperla	3	10	14	0.30	0.96	2.33	0.59

Table 5. Hutchenson *t* test for diversity of fungi associated with the coffee berry borer in Chiapas.

Cuticle

Location	Rosario Izapa	Monteperla	Laboratory (diet)
La Alianza	$t=2.01$; $P=0.046$; df=111	$t=6.71$; $P=3.74\times10^{-9}$; df=72*	$t=4.88$; $P=3.25\times10^{-6}$; df=118*
Rosario Izapa		$t=4.52$; $P=2.13\times10^{-5}$; df=78*	$t=2.52$; $P=0.01$; df=113
Monteperla			$t=2.42$; $P=0.017$; df=71

Gut

Location	Rosario Izapa	Monteperla	Laboratory (diet)
La Alianza	$t=1.84$; $P=0.07$; df=60	$t=1.44$; $P=0.15$; df=83	$t=-0.39$; $P=0.70$; df=10
Rosario Izapa		$t=-1.34$; $P=0.18$; df=57	$t=-2.08$; $P=0.06$; df=10
Monteperla			$t=1.30$; $P=0.22$; df=8

Faeces

Location	Rosario Izapa	Monteperla	Laboratory (diet)
La Alianza	$t=-1.32$; $P=0.18$; df=92	$t=5.28$; $P=1.26\times10^{-5}$; df=28*	$t=-4.28$; $P=0.0001$; df=40*
Rosario Izapa		$t=8.64$; $P=5.84\times10^{-11}$; df=43*	$t=-3.18$; $P=0.002$; df=43*
Monteperla			$t=11.9$; $P=2.86\times10^{-10}$; df=19*

Galleries

Location	Rosario Izapa	Monteperla
La Alianza	$t=-0.55$; $P=0.58$; df=97	$t=-0.96$; $P=0.34$; df=23
Rosario Izapa		$t=-0.16$; $P=0.86$; df=23

* Significant statistical differences.

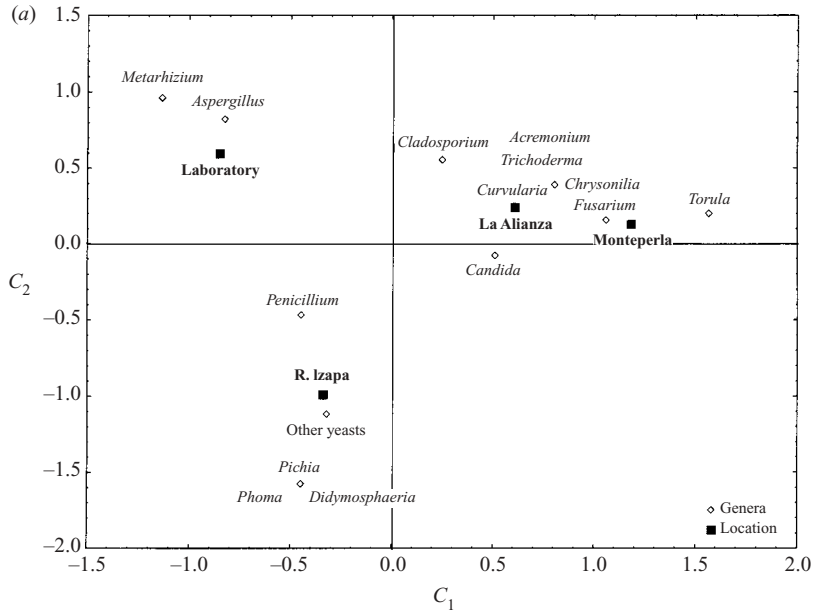
Candida diddensiae, *C. fermentati*, and *Pichia burtonii*. The first species was collected from samples of La Alianza only, and *P. burtonii* from Rosario Izapa. None of these species were present in insects from the laboratory, although Vega *et al.* (unpubl.) have isolated *P. burtonii* and *P. guilliermondii* from laboratory and field (Colombia) collected insects. In contrast, *Aspergillus* was the most abundant genus isolated in samples from the laboratory, with six species identified, *A. ochraceus* and *A. niger* being predominant.

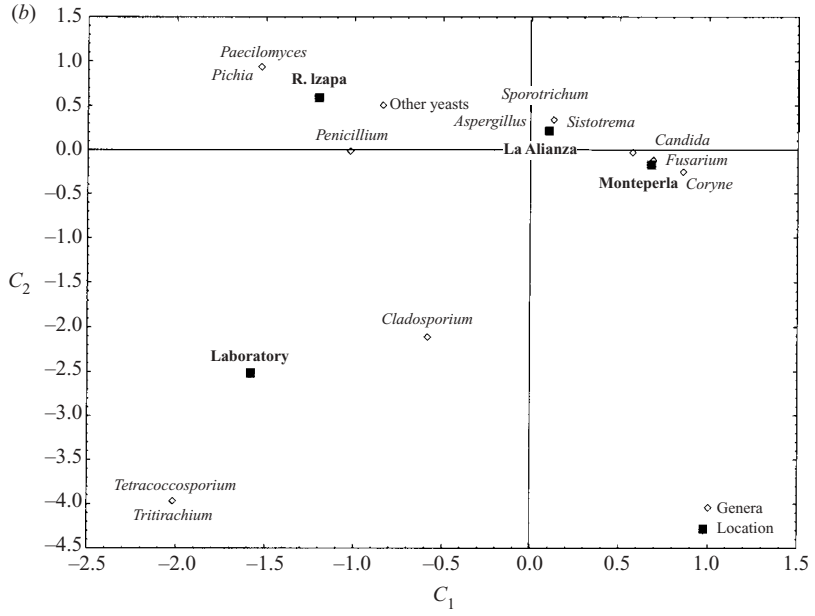
Abundance of fungi in the galleries

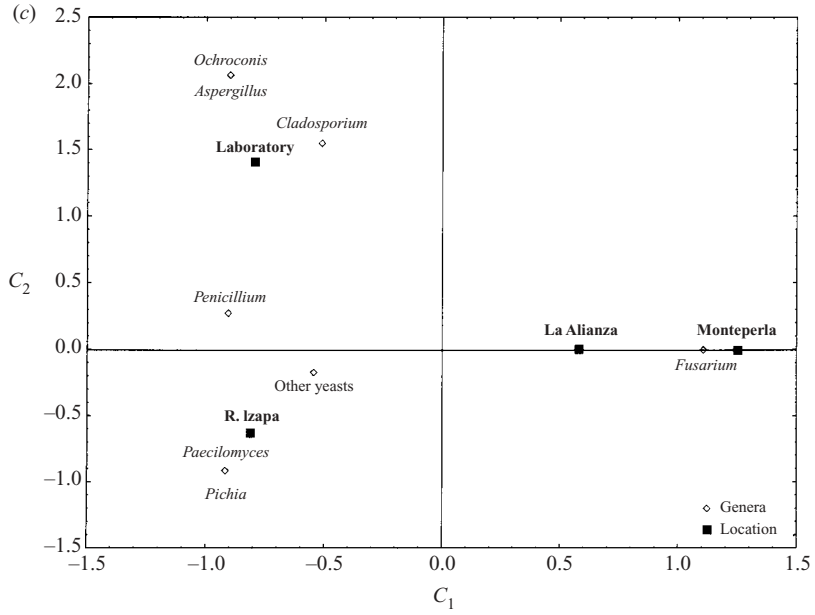
The recovery of fungi from galleries of coffee berries was markedly less than those obtained from the insects (Table 3). Only 25 isolates represented by four genera were obtained: *Fusarium* (64.1%), *Candida* (10.3%), *Hanseniaspora* (10.3%) and *Coryne* (2.6%). *Fusarium* was again the dominant genus and the only one isolated from all locations. The description of a new *Hanseniaspora* species close to *H. guilliermondii* is pending.

Indices of richness and diversity

The richness and diversity of fungi for each location and substrate is presented in Table 4. The highest richness of genera from the cuticle and the gut was in samples from La Alianza; and for faeces, in laboratory reared insects. In the galleries, the richness index was the same for the three locations.


(a)
Metarhizium
Aspergillus
Laboratory
Cladosporium
Acremonium
Trichoderma
Curvularia
Chrysonilia
Fusarium
La Alianza
Torula
Monteperla
Candida
Penicillium
R. Izapa
Other yeasts
Pichia
Phoma
Didymosphaeria
Genera
Location
C2
C1


(b)
Paecilomyces
Pichia
R. Izapa
Other yeasts
Sporotrichum
Aspergillus
Sistotrema
Penicillium
La Alianza
Candida
Fusarium
Monteperla
Coryne
Cladosporium
Laboratory
Tetracoccosporium
Tritirachium
Genera
Location
C2
C1


(c)
Ochroconis
Aspergillus
Cladosporium
Laboratory
Penicillium
La Alianza
Monteperla
Fusarium
Other yeasts
R. Izapa
Paecilomyces
Pichia
Genera
Location
C2
C1

Cuticle diversity was significantly higher in samples from La Alianza than Monteperla ($t=6.71$; df$=72$; $P<0.0001$) and Laboratory ($t=4.88$; df$=118$; $P<0.0001$). There were no significant differences between La Alianza and Rosario Izapa ($t=2.01$; df$=111$; $P=0.046$) (Table 5). The diversity of fungi isolated from the gut and galleries (Table 5) were not statistically different for any location. There were significant differences in fungal diversity in faeces from laboratory with respect to La Alianza ($t=-4.28$; df$=40$; $P=0.0001$), Rosario Izapa ($t=-3.18$; df$=43$; $P=0.002$) and Monteperla ($t=11.9$; df$=19$; $P<0.0001$) (Table 5).

Results from correspondence analysis of samples of cuticle, confirmed that *Fusarium*, althougth present in all locations, is better correlated to La Alianza and Monteperla; *Penicillium* and *Pichia* are more associated with Rosario Izapa, and *Aspergillus* with laboratory samples (Fig. 1a). In gut samples, *Fusarium* and *Coryne* were most associated with Monteperla, *Cladosporium* with Laboratory, and *Penicillium* and *Pichia* with Rosario Izapa (Fig. 1b). Finally, in faeces, *Fusarium* was connected to Monteperla and La Alianza, while *Pichia* and *Penicillium* were related to Rosario Izapa. *Aspergillus* and *Cladosporium* were correlated with Laboratory treatment (Fig. 1c).

DISCUSSION

This study demonstrates a great diversity of fungi associated with the CBB inside the berries coffee. Forty species were identified from the CBB body and its galleries. Amongst these, there were at least three new species; one has already been described (Peterson *et al.* 2003), and the others will be described shortly. Carrión, Bonet & Romero (2001) isolated only six species of fungi from CBB galleries. Therefore, this work represent the first substantive study of the mycobiota associated with the CBB, and is a significant contribution to understanding the interactions between this insect and the organisms cohabiting in the coffee fruits.

Because richness analysis was made taking into account the genus as a taxonomic unit, the indices were low for all locations. With respect to diversity indices, surprisingly, the diversity for faeces showed statistical differences in favour of insects from the Laboratory (diets). This can be explained if we considered that the Shannon–Wiener index gives more weight to the rare taxa than the abundance of individuals (Magurran 1988). Even though the gut samples had higher diversity indices for insects from Laboratory, the statistical analysis did not detect any significant differences.

Most of the fungi found associated with CBB are widely distributed in nature, and some of them have been isolated from other insects. For instance, different species of the genera *Aspergillus*, *Candida*, *Cladosporium*, *Fusarium*, *Pichia*, and *Penicillium* have been collected from bees (*Apoidea*) and their nests (Gilliam & Prest 1972, 1977, Batra, Batra & Bohart 1973). *Aspergillus*, *Candida*, *Cladosporium*, *Fusarium*, *Paecilomyces*, *Penicillium*, *Pichia*, and *Trichoderma*, are also found in association with the bark beetle *Dendroctonus* sp. (Whitney 1982). *Acremonium*, *Aspergillus*, *Cladosporium*, *Mucor*, *Fusarium*, and *Penicillium*, are associated with the termite *Reticulitermes flavipes* (Zoberi & Grace 1990). *Aspergillus*, *Fusarium*, *Mucor* and *Penicillium*, with the moth *Spodoptera litoralis* (Ismail & Abdel-Sater 1993). *Hanseniaspora*, *Pichia* and *Candida* have been found in the cuticle of *Drosophila* spp. (Morais *et al.* 1995). *Aspergillus*, *Mucor* and *Fusarium*, in the galleries of *Xyleborus fornicatus* (Kumar *et al.* 1998). Finally, *Aspergillus*, *Paecilomyces*, *Cladosporium*, *Fusarium*, *Acremonium*, *Tritirachium* and *Penicillium*, have been isolated from the gut of the true bug *Triatoma* sp. (Moraes *et al.* 2000).

We stress that in this study sampling was carried out only during the rainy season, and therefore the mycobiota associated with the CBB and its galleries could vary with respect to rainfall throughout the year. In the area where the study was conducted, the rainy season usually takes place from April to November, with September and October being the wettest months (Richter & Schmiedecken 1987). Thus, it is possible that fungal diversity increases during these months, and is reduced when rainfall decreases. However, this hypothesis has not been tested. On the other hand, there was not a correlation between altitude and fungal diversity.

The number of fungi isolated from the insects' body was markedly higher than those from the galleries. This could be due to the two methodologies we used to isolate the fungus from galleries *versus* the body; also the number of replicates was different. We decided to employ a different methodology to process the galleries, to avoid culturing microorganisms living outside of the gallery, as they do not necessarily have a relationship with the CBB.

It is important to mention that some genera of fungi were associated with a specific type of substrate. For instance, *Acremonium*, *Chrysonilia*, *Curvularia*, *Didymosphaeria*, *Phoma*, and *Trichoderma* were only obtained from the cuticle of the CBB, while *Sistotrema*, *Sporotrichum*, *Tetracoccosporium*, and *Tritirachium*, were isolated exclusively from the gut. *Ochroconis* was only detected in faeces, whereas *Hanseniaspora* was only present in galleries. Although the objectives of this work did not include the isolation of organisms other than fungi, that Gram-negative bacteria were abundant in all samples, except those from the laboratory.

Fig. 1. Correspondence analysis between locations and genera of fungi collected form different substrata of the coffee berry borer in Chiapas: (a) Cuticle. (b) Gut. (c) Faeces.

It has been suggested that some fungal species associated with CBB are in a mutualistic relationship with the insect. A case in point is *F. solani*, which has been mentioned as a symbiont of CBB (Rojas, Morales & Harrington 1999; Morales-Ramos *et al*. 2000). In the study, *Fusarium*, and particularly *F. solani*, was one of the most predominant fungi and was isolated from all the places studied. Although is possible that a mutualistic relationship between this fungus and the insect exists, conclusive evidence demonstrating this relationship is lacking. Due to the great diversity of fungi associated with the CBB, it is possible that a facultative mutualism involving a microbial community exists (Norris 1979, Kaufman *et al*. 2000), rather than this mutualism being based on a single microorganism. Additional studies are required to clarify the role played by the mycobiota associated with CBB and its galleries in the biology of this insect.

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