Does the Coffee Berry Borer (Coleoptera: Scolytidae) Have Mutualistic Fungi?

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ABSTRACT Laboratory bioassays were performed to determine if a mutualistic association exists between three species of fungi and the coffee berry borer, *Hypothenemus hampei* (Ferrari). The fungi *Fusarium solani* (Martius), *Penicillium citrinum* Thom and *Candida fermentati* (Saito) were evaluated on the reproduction and survivorship of *H. hampei*. The fungi were evaluated at three concentrations: 5×10^2; 5×10^4 and 5×10^6 spores/ml using coffee berries and meridic diets as substrate. The fungi did not affect the normal development of the insect and did not increase mortality at any concentration evaluated. When *H. hampei* was reared in berries, no significant differences in the total progeny production were detected between treatments involving fungi and the control. When reared on meridic diets, there was no clear trend to suggest a beneficial effect of a given species of fungus on *H. hampei*; at 40 d postinoculation, the total progeny production of the insect was higher than the control with *C. fermentati*; at 60 d it was higher with *F. solani*, and at 80 d there were no differences between treatments involving fungi and the control. Our results indicate that there were no clear positive effects of any fungi on the coffee berry borer either in berries or diets. Therefore, we conclude that *H. hampei* is not allied with fungi in mutualist relationships as was previously believed.

KEY WORDS *Hypothenemus*, coffee berry borer, mutualism, fungi

THE MAJORITY OF THE 6,000 described species of Scolytidae (Coleoptera) are involved in mutualistic relationships with microorganisms, particularly fungi (Wood 1982, Booth et al. 1990). Because mutualism is defined as "an interaction between two species that is beneficial to both" (Boucher et al. 1982, Ricklefs and Miller 2000), the fungi benefit by being transported to new hosts by the insect; in turn, the scolytids cultivate and use the fungi as a food source (Whitney 1982). The associated fungi seem to be carried in specialized cuticular structures termed mycangia, which are found on different parts of the insect (Batra 1963).

The coffee berry borer, *Hypothenemus hampei* (Ferrari), is a scolytid of the tribe Cryphalini (Wood 1982) in which reports of fungal associations or mycetophagous habits (feeding on fungi) are unusual (Beaver 1986). Nevertheless, a possible mutualistic relationship between *H. hampei* and fungi has been suspected for many years (Le Pelley 1968, Villacorta 1989, Villacorta and Barrera 1993). Such a hypothesis seems reasonable given that *H. hampei* is monophagous and insects feeding on a narrow food range often harbor populations of mutualistic microorganisms that supplement the restricted diet with critical nutrients (Ben-Beard et al. 2002).

More than 50 species of fungi have been recorded from *H. hampei* and its galleries (Posada et al. 1993, 1998; Pérez et al. 1996, 2003; Rojas et al. 1999; Vega et al. 1999; Morales-Ramos et al. 2000; Díaz et al. 2003; Peterson et al. 2003; Carrión and Bonet 2004). Of these, *Fusarium solani* (Martius) has been repeatedly isolated from *H. hampei* (Pérez et al. 1996, 2003; Vega et al. 1999; Rojas et al. 1999; Díaz et al. 2003; Carrión and Bonet 2004), although the role that it plays in the biology of the insect is in dispute. Rojas et al. (1999) and Morales-Ramos et al. (2000) suggested a mutualistic relationship between *H. hampei* and *F. solani*, whereas Pérez et al. (1996) and Díaz et al. (2003) reported *F. solani* and *Fusarium* sp., respectively, as *H. hampei* pathogens. Understanding the nature of the relationship between the coffee berry borer and associated microorganisms could provide important new clues applicable to management strategies for this pest.

In an attempt to clarify this situation, we performed a series of laboratory experiments. We hypothesized that if *H. hampei* lives in association with a mutualistic fungus, the insect should suffer a decrease in progeny production in the absence of the fungus and that the absence of this organism should be generally detrimental for the insect's development. The aim of the current study was to elucidate, by means of bioassays, whether *Fusarium solani* (Martius), *Penicillium citrinum* Thom, or *Candida fermentati* (Saito) are beneficial to the development and reproduction of *H. hampei*. These fungi were selected because they were

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found with the greatest prevalence in a field study carried out in Mexico (Pérez et al. 2003), and we assumed that they were most likely to be potential *H. hampei* mutualists. We had special interest in *F. solani* because this is the only species that has been reported as a mutualist of *H. hampei* (Morales-Ramos et al. 2000).

Materials and Methods

Life History of *H. hampei*. This insect feeds on coffee berries and does not damage other vegetative parts of the plant. The female bores into the fruit and lays eggs inside rearing chambers. Normally, they are laid at the rate of two or three eggs per day for several weeks, producing ≈30–70 progeny in a single berry (Le Pelley 1968). After hatching, the larvae immediately start to feed on berry tissues. The larval period ranges from 9 to 20 d, depending on temperature (Bergamin 1943). At the end of the larval stage, there is a nonfeeding prepupal stage lasting ≈2 d. The insect pupates in the galleries excavated by the larvae. Under field conditions, the generation time for this species is 45 d (Baker et al. 1992). Fertilization of the females is by siblings mating inside the coffee berry, so that *H. hampei* lives outside of the berry only for the short period when the female is searching for a new coffee berry to infest (Murphy and Moore 1990).

Insects. The experiments were performed with adults of *H. hampei* reared in the laboratory for three generations on a meridic diet (Villacorta and Barrera 1993). Before experiments, the insects were confined in a plastic container with small pieces of diet for 2 d to facilitate mating. Afterwards, they were superficially disinfected with distilled water, 2% formaldehyde, 2% sodium hypochlorite, and distilled water for 15 s in each solution. Only adult females were selected for bioassays.

Coffee Berries. Noninfested green coffee, *Coffea arabica* L., berries were collected in a coffee plantation near Cacahoatán, Chiapas, Mexico. The berries were placed in wooden trays lined with paper towel for 2 d before use. To remove the microbial population from the epidermis, berries were subject to the following superficial sterilization process: 1) washed in detergent solution for 15 min, 2) rinsed with tap water, 3) dipped in 2% sodium hypochlorite for 10 min, 4) rinsed with sterile distilled water, 5) rinsed with 2% potassium sorbate for 10 min, and 6) the berries were rinsed again with sterile distilled water and dried on sterile towel paper.

Fungal Isolation. *Fusarium solani*, *P. citrinum*, and *C. fermentati* were isolated from the cuticle and gut of *H. hampei* adult females collected in three coffee plantations near Tapachula, Chiapas, Mexico. *F. solani* in La Alianza (N 15° 02′ 27″, W 92° 10′ 22″, 700 m above sea level [masl]); *P. citrinum* in Rosario Izapa (N 14° 57′ 54.1″, W 92° 09′ 6.4″, 425 masl); and *C. fermentati* in Monteperla (N 15° 02′ 50″, W 92° 05′ 19″, 950 masl). From each location, we collected ≈500 females after the dissection of infested coffee berries collected randomly.

To obtain fungal cultures, the living *H. hampei* females were superficially disinfected in a solution of 0.5% sodium hypochlorite for 5 min and rinsed in sterile distilled water. Insects were then 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 female in 0.85% saline solution. The gut (proventriculus to rectum) was extracted by grasping the abdomen and pulling out the anus with sterile forceps. Immediately afterwards, the gut was macerated in 20 μl of sterile saline solution to avoid dehydration (Gilliam and Prest 1972).

All samples were individually placed in 1.5-ml plastic Eppendorf vials containing 100 μl of saline solution. These samples were agitated in an ultrasonic bath at a frequency of 42 KHz for 10 s (Cazemier et al. 1997). Ten-microliter 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). Isolates were incubated at room temperature (25–30°C) for 7 d. A preliminary identification of *F. solani*, *P. citrinum*, and *C. fermentati* based on macro- and microscopic characteristics was conducted (Barnett and Hunter 1998), and a final identification was made by specialists at the U.S. Department of Agriculture, Agricultural Research Service, Microbial Genomics and Bioprocessing Research Unit in Peoria, IL.

Before running the bioassays, the three isolates were cultivated on PDA for 8 d. A suspension of spores in a sterile solution of 0.1% Tween 80 was prepared for each species. The concentration of spores was determined by counting in a Neubauer hemacytometer and adjusted to concentrations of 5×10^2, 5×10^4, and 5×10^6 spores/ml.

Bioassays Using Coffee Berries. Spores were inoculated by immersion into the spore suspension for 1 min for *H. hampei* (insect treatment) and 2 min for berries (berry treatment). After inoculation, 10 berries (labeled 1–10) were place in a 1-liter plastic container and ten *H. hampei* adult females were placed inside. There were seven replicates. All treatments were kept in a dark room at 29 ± 2°C and 75 ± 10% RH. Five berries (even numbers) were dissected 20 d after the experiment was set up to verify the survivorship of the founder females and to count the number of progeny produced. The same procedure was performed at 40 d postinoculation with the remaining five berries (odd numbers).

Bioassays using berries were performed in the laboratory under a one-way completely randomized design with 19 levels and seven replicates of five berries each. The experiment involved three species of fungi (*F. solani*, *P. citrinum*, and *C. fermentati*), each applied at one of three spore concentrations (5×10^2, 5×10^4, and 5×10^6 spores/ml), one of two inoculation protocols (spores applied to berries or insects), and the control.

Bioassays Using Meridic Diets. The meridic diet was prepared according to Villacorta and Barrera (1993). Volumes of 5 ml of diet were poured into 7.5 × 1.5-cm vials and, after its solidification, 10 μl of each fungal

spore suspension was inoculated onto the diet surface. This procedure was followed for each of the three concentrations of each species of fungi. Control vials were inoculated with 10 μl of sterile distilled water. A single previously disinfested *H. hampei* female was placed in each vial. Bioassays were performed in the laboratory in a one-way completely randomized design with 10 levels and 10 replicates consisting of three vials each. There were three species of fungi (*F. solani*, *P. citrinum*, and *C. fermentati*), three spore concentrations (5×10^2, 5×10^4, and 5×10^6 spores/ml), and one control. All treatments were maintained under the previously mentioned conditions of temperature and relative humidity. At 40 d postinoculation, three vials were randomly selected from each replicate, and the diet was dissected to determine the numbers of adults and immature stages of *H. hampei* per vial. These evaluations were repeated at 60 and 80 d postinfestation with the remaining vials from each replicate.

Data Analysis. Data were examined for normality and homogeneity of variances. Because the data for progeny production of *H. hampei* did not conform to the requirements of a normal distribution, a rank transformation was used (Potvin and Roff 1993), but the reported means and standard errors in tables and graphs show the untransformed values. After transformation, a one-way analysis of variance (ANOVA) was conducted, and comparisons between treatment means were performed by least significant difference test. The survivorship of adults and immature stages of *H. hampei* were subjected to ANOVA by using the weighted means of treatments. In all tests, values of $P \leq 0.05$ were considered significant.

Results

Effect of Fungi on *H. hampei* Reared on Coffee Berries. *F. solani*, *P. citrinum*, and *C. fermentati* did not show any beneficial effect on the reproduction of *H. hampei* at any concentration or either inoculation method. Although most of the treatments produced more progeny than the control at day 20 (Fig. 1A), there were no statistical differences among treatments ($F = 1.08$; df = 18, 114; $P = 0.371$). Similarly, the production of progeny at day 40 (Fig. 1B), showed no significant differences for any treatment ($F = 1.56$; df = 18, 114; $P = 0.081$).

The mortality of adults and immature stages of *H. hampei* at 20 d postinoculation was very low (<1.2% in all treatments) and was not subjected to statistical analysis. At 40 d postinoculation, *H. hampei* mortality increased to 5.8% in the control, compared with 4.1–12.9% in treatments involving fungi, but no significant differences between treatments were detected ($F = 0.47$; df = 18, 114; $P = 0.963$).

Effect of Fungi on *H. hampei* Reared on Diets. At 40 d postinoculation, the highest progeny production was observed in the treatment involving 5×10^2 spores/ml of *C. fermentati* (Fig. 2A), which was significantly greater than the control and treatments involving *F. solani* (5×10^2 and 5×10^4 spores/ml), *P. citrinum* (5×10^2 and 5×10^4 spores/ml), and *C. fermentati* (5×10^6 spores/ml) ($F = 2.77$; df = 9, 90; $P = 0.006$). In contrast, at 60 d postinoculation, the highest progeny production was observed in the 5×10^4 spores/ml of *F. solani* treatment (Fig. 2B), which was significantly greater than the control, *P. citrinum* (5×10^4 and 5×10^6 spores/ml), and *C. fermentati* at the three concentrations ($F = 2.79$; df = 9, 90; $P = 0.006$). The same trend was observed in the third evaluation (day 80) (Fig. 2C), in which *F. solani* (5×10^4 and 5×10^6 spores/ml) treatments resulted in the highest averages, but there were no significant differences across treatments ($F = 1.89$; df = 9, 90; $P = 0.062$).

The mortality registered for adults and immature stages of *H. hampei* in diets was close to zero (maximum 0.15%) in all treatments and dates of evaluations.

Development of Immature Stages of *H. hampei*. To determine whether the fungi affect the normal development of immature stages of *H. hampei*, we compared the average number of each biological stage obtained in dissections of berries and diets. At 20 d postinoculation, the biological stages of *H. hampei* in berries did not differ significantly for eggs ($F = 1.65$; df = 18, 114; $P = 0.058$), first instars ($F = 1.07$; df = 18, 114; $P = 0.384$), second instars ($F = 1.54$; df = 18, 114; $P = 0.087$), or adults ($F = 0.55$; df = 18, 114; $P = 0.923$). Significant differences were only detected between treatments for the pupal stage ($F = 2.64$; df = 18, 114; $P < 0.001$), for *F. solani* 5×10^4 inoculated in berries, the treatment with the highest average of pupae (Table 1). At 40 d postinoculation, there were no significant differences for eggs ($F = 1.66$; df = 18, 114; $P = 0.055$), first instars ($F = 1.17$; df = 18, 114; $P = 0.295$), second instars ($F = 1.09$; df = 18, 114; $P = 0.364$), pupae ($F = 0.82$; df = 18, 114; $P = 0.668$), or adults ($F = 1.68$; df = 18, 114; $P = 0.052$) (Table 2).

In bioassays using meridic diets, we could not observe that a particular species of fungi were beneficial or detrimental for the development of immature stages of *H. hampei*. From 15 ANOVAs performed to evaluate the effect of fungi on the progeny production (five biological stages × three dates), 10 ANOVAs were not significant (Table 3). Conversely, in the analyses where significant differences were detected, there was an inconsistency with respect to the treatments that yielded significantly more progeny. For instance, at 40 d postinoculation the highest number of eggs was observed in the treatment 5×10^2 spores/ml of *C. fermentati*, which was significantly greater than the control ($F = 2.63$; df = 9, 90; $P = 0.009$). For the first instars, despite the fact that the treatments *F. solani* 5×10^6 and *P. citrinum* 5×10^6 spores/ml produced significantly more larvae than the control ($F = 2.25$; df = 9, 90; $P = 0.025$), most treatments involving fungi were not significantly different from one another. A different trend was observed in the second instars, where *F. solani* 5×10^6 spores/ml, *P. citrinum* 5×10^6 spores/ml, and the three concentrations of *C. fermentati* yielded significantly more individuals than the control ($F = 2.23$; df = 9, 90; $P = 0.026$). At 60 d postinoculation, the control had significantly less eggs than treatments involving *F. solani*

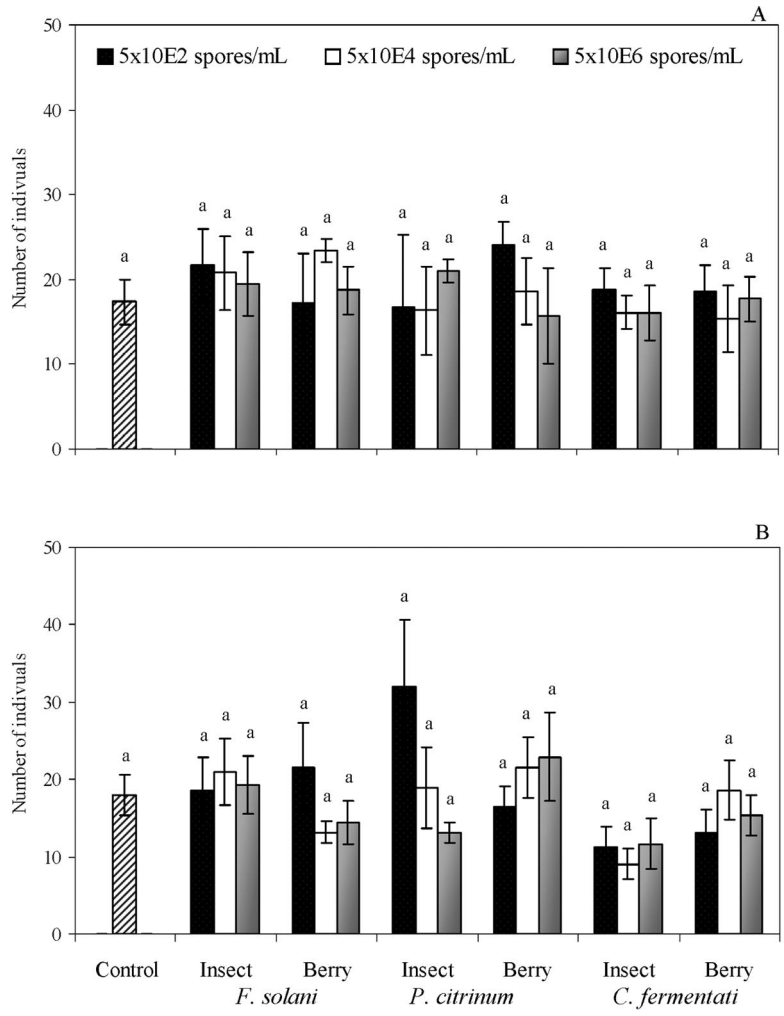

Fig. 1. Total number of progeny produced by *H. hampei* in coffee berries 20 (A) and 40 d (B) after being inoculated with on of three species of fungi. Each bar represents the mean value (±SE) of seven replicates (untransformed data).

(5×10^2 and 5×10^4 spores/ml) and *P. citrinum* 5×10^2 spores/ml ($F = 2.82$; df = 9, 90; $P = 0.005$), whereas at 80 d postinoculation the presence of second instars was significantly higher in *F. solani* (5×10^4 and 5×10^6 spores/ml) and *C. fermentati* (5×10^6 spores/ml) in comparison with the control ($F = 2.49$; df = 9, 90; $P = 0.013$).

Discussion

Our data strongly suggest that a mutualistic association between *H. hampei* and *F. solani*, *P. citrinum*, or *C. fermentati* does not exist. The results of this study show that none of the three species of fungi had a significant effect on *H. hampei*, in terms of increasing reproduction or survival. Because the number of adults and immature stages in most treatments of berries (at 20 and 40 d) and diets (at 40, 60, and 80 d) were not significantly different from the control, we deduce that these fungi did not affect the development of *H. hampei* when inoculated in borers, coffee berries or diets.

There is an apparent contradiction in the total progeny produced in treatments with berries, which was somewhat higher at day 20 than at day 40. However, these data only include live biological stages. If we considered that mortality at the first evaluation was near zero and increased to 12.9% in some treatments in the second evaluation, this would explain the differences in the total progeny produced at both dates. At 20 d postinoculation, berry tissues were still soft and with a suitable humidity for *H. hampei* reproduction. The humidity of coffee berries slowly declines over time (Gómez 1994), and at 40 d postinoculation most berries were hard and unsuitable for insect feeding. This could be the reason for the higher rates of mortality at the second evaluation.

When microorganisms are associated with insects in a mutualistic relationship, they usually provide sterols, compounds that are essential for normal insect

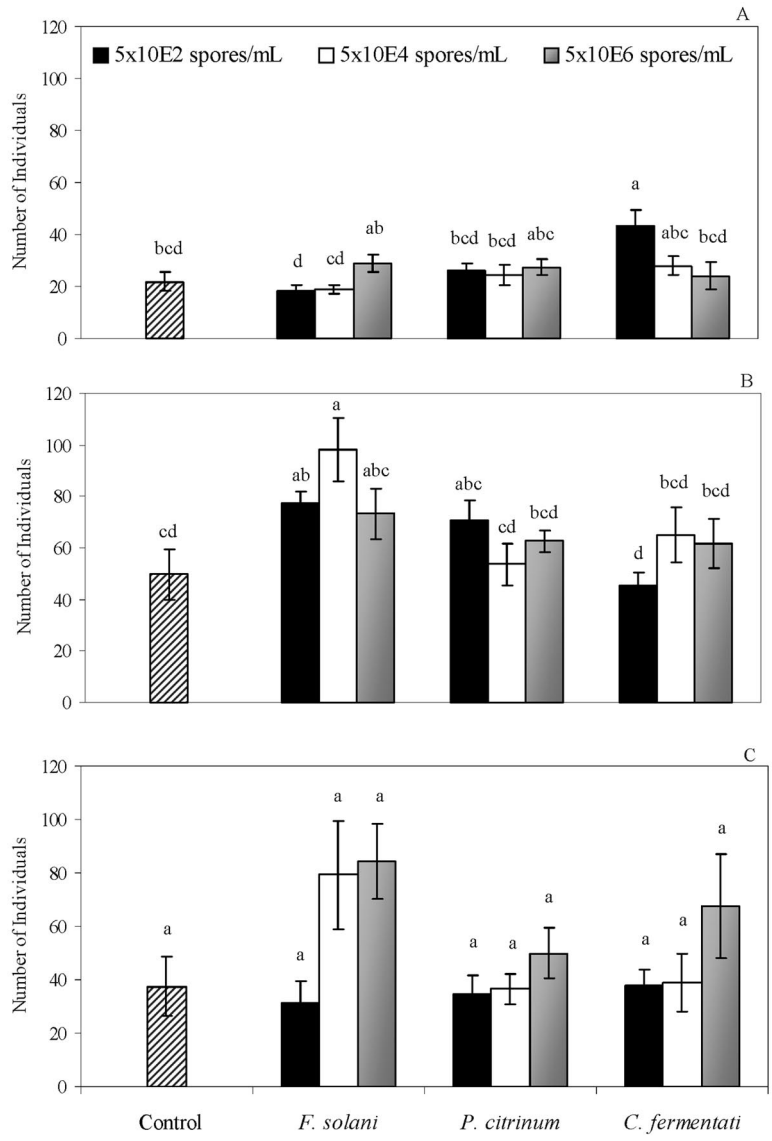

Fig. 2. Total number of progeny produced by *H. hampei* in meridic diets 40 (A), 60 (B), and 80 d (C) after being inoculated with one of three species of fungi. Each bar represents the mean value (±SE) of 10 replicates (untransformed data).

growth, development, and reproduction (Svoboda et al. 1978). Sometimes, the effect of sterols is not immediate and can only be reflected at the beginning of the second generation (Klepzig et al. 2001). Thus, we evaluated the progeny production in diets at three different times. Development from egg to adult of *H. hampei* reared in diets or on coffee berries, takes at least 23–24 d (Gómez 1994, Ruiz et al. 1996) and by the last evaluation (80 d), we should have had at least two generations of insects. In bioassays using coffee berries, we did not evaluate on the same dates as diets, because the humidity in berries declines drastically after 1 mo in culture (Gómez 1994). Thus, we decided to evaluate the fecundity of *H. hampei* at 20 d, where a great quantity of immature stages of the first generation was expected. We performed the second evaluation in berries at 40 d postinoculation, i.e., at the beginning of the second generation (Gómez 1994) to avoid the detrimental effects of dehydration of berries on the rearing of *H. hampei.*

Morales-Ramos et al. (2000) reported the first documented case of the existence of a mutualistic relationship between *H. hampei* and *F. solani.* Their study was based mainly on indirect evidence, such as 1) increased progeny in coffee berries where *F. solani* was growing compared with berries where the fungus was inhibited; 2) the presence of *F. solani* spores on the insect's body; and 3) an increase in progeny production when different concentrations of ergosterol were added to the diets. The crucial laboratory bioassays

Table 1. Number of adults and immature stages produced by *H. hampei* after either berries or insects were individually inoculated with three fungi

Treatment			Egg	Instar I	Instar II	Pupa	Adult
Control			7.5 ± 1.9a	4.9 ± 1.1a	2.7 ± 0.8a	0.9 ± 0.3abcd	1.3 ± 0.2a
F. solani	Insect	5×10^2	10.9 ± 2.4a	4.8 ± 0.6a	3.0 ± 0.7a	1.6 ± 0.6abc	1.3 ± 0.2a
		5×10^4	8.2 ± 1.0a	7.3 ± 1.6a	2.9 ± 0.6a	1.1 ± 0.4abcd	1.4 ± 0.2a
		5×10^6	9.3 ± 1.4a	3.4 ± 0.8a	3.6 ± 0.8a	1.8 ± 0.3a	1.3 ± 0.1a
	Berry	5×10^2	6.5 ± 1.3a	5.1 ± 1.4a	2.9 ± 0.8a	1.7 ± 0.3a	0.9 ± 0.1a
		5×10^4	8.5 ± 0.7a	6.7 ± 1.8a	4.4 ± 1.3a	2.6 ± 1.1a	1.3 ± 0.2a
		5×10^6	5.7 ± 0.8a	5.9 ± 1.6a	3.9 ± 1.1a	1.9 ± 0.7ab	1.3 ± 0.2a
P. citrinum	Insect	5×10^2	5.2 ± 0.7a	5.1 ± 1.3a	3.3 ± 0.5a	1.8 ± 0.3a	1.2 ± 0.1a
		5×10^4	6.0 ± 1.1a	4.6 ± 0.8a	3.4 ± 0.6a	0.9 ± 0.3abcd	1.4 ± 0.3a
		5×10^6	10.0 ± 1.1a	4.4 ± 0.9a	3.2 ± 0.6a	1.8 ± 0.4a	1.5 ± 0.1a
	Berry	5×10^2	12.0 ± 2.5a	4.2 ± 0.6a	4.5 ± 0.5a	2.1 ± 0.6a	1.4 ± 0.3a
		5×10^4	9.5 ± 1.6a	4.1 ± 1.1a	2.3 ± 0.9a	1.4 ± 0.4abc	1.1 ± 0.3a
		5×10^6	7.0 ± 1.5a	3.3 ± 0.8a	2.6 ± 0.4a	1.7 ± 0.5ab	1.1 ± 0.1a
C. fermentati	Insect	5×10^2	8.9 ± 2.3a	5.6 ± 1.7a	2.2 ± 0.8a	0.7 ± 0.2bcd	1.1 ± 0.1a
		5×10^4	7.1 ± 2.0a	5.9 ± 0.9a	1.6 ± 0.9a	0.3 ± 0.2d	1.3 ± 0.3a
		5×10^6	6.9 ± 2.1a	5.5 ± 1.0a	1.9 ± 0.4a	0.5 ± 0.4d	1.3 ± 0.2a
	Berry	5×10^2	11.4 ± 2.8a	3.8 ± 1.0a	1.8 ± 0.4a	0.5 ± 0.2cd	1.1 ± 0.2a
		5×10^4	8.2 ± 2.4a	3.2 ± 0.9a	1.2 ± 0.5a	1.0 ± 0.4abcd	1.6 ± 0.4a
		5×10^6	10.5 ± 1.1a	3.1 ± 0.8a	2.3 ± 0.4a	0.6 ± 0.2cd	1.2 ± 0.2a

Data represent mean ± SE of seven replicates of five coffee berries each, after 20 d in culture (untransformed data). Mean *H. hampei* productivity in treatments within columns followed by the same letter is not significant different (ANOVA; $P > 0.05$).

(i.e., experiments inoculating *F. solani*) to demonstrate this association were never carried out.

We believe that indirect evidence does not demonstrate a possible mutualism between *F. solani* and *H. hampei.* This fungus is ubiquitous in nature and has been isolated from many insects in nonmutualistic relationships (Batra et al. 1973, Barson 1976, Zoberi and Grace 1990, Kumar et al. 1998, Moraes et al. 2000). Morales-Ramos et al. (2000) did not mention direct evidence, such as adults or larvae feeding on mycelia or fungal spores of *F. solani*, that would support this mutualism. That they found spores of this fungus on the cuticle of *H. hampei* should be considered as fortuitous, because field surveys have revealed that *F. solani* is naturally present in a fraction of *H. hampei* adults, with a maximum prevalence of 25% (Pérez et al. 2003), 58% (Carrión and Bonet 2004), and 90% (Rojas et al. 1999). Given that mutualistic relationships are beneficial to both species (Ricklefs and Miller 2000), one would expect to find *F. solani* regularly in almost all individuals of the *H. hampei* population. In addition, *F. solani* has been reported as a pathogen of *H. hampei* (Pérez et al. 1996) and recently as a coffee parasite that can be found on coffee berries, even in the absence of *H. hampei* (Carrión and Bonet 2004). Furthermore, the coffee berry borer does not have specialized morphological structures in its body (i.e., mycangia) to shelter fungi, as observed in other scolytids (Rudinsky 1962, Whitney 1982, Wood 1982).

Table 2. Number of adults and immature stages produced by *H. hampei* after either berries or insects were individually inoculated with three fungi

Treatment			Egg	Instar I	Instar II	Pupa	Adult
Control			4.7 ± 0.6a	5.3 ± 1.7a	1.2 ± 0.3a	0.9 ± 0.2a	5.1 ± 0.6a
F. solani	Insect	5×10^2	4.5 ± 0.9a	5.4 ± 2.9a	1.8 ± 0.2a	0.6 ± 0.2a	5.6 ± 1.0a
		5×10^4	5.2 ± 0.9a	7.3 ± 2.2a	1.4 ± 0.4a	0.8 ± 0.3a	5.3 ± 0.9a
		5×10^6	4.4 ± 0.7a	5.8 ± 2.4a	1.4 ± 0.4a	0.9 ± 0.2a	5.8 ± 0.8a
	Berry	5×10^2	5.1 ± 1.4a	7.8 ± 2.9a	0.9 ± 0.2a	0.5 ± 0.2a	6.5 ± 1.4a
		5×10^4	3.2 ± 0.7a	3.3 ± 1.2a	1.3 ± 0.4a	0.4 ± 0.1a	3.9 ± 0.4a
		5×10^6	4.2 ± 1.4a	2.9 ± 1.2a	0.8 ± 0.1a	1.0 ± 0.2a	4.8 ± 1.0a
P. citrinum	Insect	5×10^2	9.6 ± 2.5a	11.0 ± 4.0a	2.2 ± 0.7a	0.5 ± 0.3a	7.8 ± 1.5a
		5×10^4	5.9 ± 1.7a	4.8 ± 2.1a	1.4 ± 0.5a	0.4 ± 0.1a	5.3 ± 1.5a
		5×10^6	3.3 ± 0.6a	2.3 ± 0.4a	0.7 ± 0.3a	0.7 ± 0.2a	4.7 ± 0.9a
	Berry	5×10^2	5.7 ± 1.0a	3.8 ± 1.0a	1.1 ± 0.2a	0.8 ± 0.3a	4.4 ± 0.9a
		5×10^4	5.7 ± 2.2a	8.3 ± 3.1a	1.1 ± 0.3a	0.8 ± 0.2a	5.0 ± 0.9a
		5×10^6	5.2 ± 0.8a	7.7 ± 3.2a	1.5 ± 0.5a	0.5 ± 0.2a	7.0 ± 1.7a
C. fermentati	Insect	5×10^2	3.5 ± 0.7a	2.8 ± 1.1a	0.8 ± 0.3a	0.3 ± 0.1a	3.3 ± 0.8a
		5×10^4	1.8 ± 0.7a	1.8 ± 0.6a	1.0 ± 0.2a	0.8 ± 0.4a	3.0 ± 0.7a
		5×10^6	2.8 ± 0.9a	2.8 ± 1.4a	1.6 ± 0.5a	0.8 ± 0.2a	2.7 ± 0.8a
	Berry	5×10^2	3.5 ± 1.0a	4.3 ± 1.9a	0.7 ± 0.2a	0.5 ± 0.2a	3.6 ± 0.8a
		5×10^4	6.3 ± 1.6a	4.9 ± 1.4a	1.0 ± 0.2a	0.7 ± 0.2a	5.0 ± 1.1a
		5×10^6	6.4 ± 2.0a	2.8 ± 1.3a	0.7 ± 0.2a	0.6 ± 0.2a	4.1 ± 0.6a

Data represent mean ± SE of seven replicates of five coffee berries each, after 40 d in culture (untransformed data). Mean *H. hampei* productivity in treatments within columns followed by the same letter is not significant different (ANOVA; $P > 0.05$).

Table 3. Number of adults and immature stages produced by *H. hampei* in diets inoculated with three fungi

Day	Treatment		Egg	Instar I	Instar II	Pupa	Adult
40	Control		19.6 ± 3.4bcd	0.1 ± 0.0bcd	0.1 ± 0.1d	0.1 ± 0.1a	1.8 ± 0.3a
	F. solani	5×10^2	13.8 ± 1.7d	0.1 ± 0.1abcd	0.5 ± 0.2abcd	1.0 ± 0.4a	2.6 ± 0.4a
		5×10^4	15.3 ± 1.7cd	0.0 ± 0.0bd	0.3 ± 0.1cd	0.4 ± 0.2a	2.8 ± 0.5a
		5×10^6	21.2 ± 2.4bc	0.7 ± 0.3a	1.6 ± 0.4a	1.4 ± 0.5a	4.0 ± 0.9a
	P. citrinum	5×10^2	22.9 ± 2.3ab	0.0 ± 0.0d	0.5 ± 0.2bcd	0.4 ± 0.1a	2.1 ± 0.4a
		5×10^4	19.7 ± 3.3bcd	0.5 ± 0.3abcd	0.6 ± 0.4cd	0.3 ± 0.1a	2.9 ± 0.7a
		5×10^6	20.5 ± 2.2bcd	0.5 ± 0.2a	1.7 ± 0.7ab	0.9 ± 0.3a	3.5 ± 1.1a
	C. fermentati	5×10^2	36.1 ± 5.2a	0.5 ± 0.2ac	0.7 ± 0.3abc	0.7 ± 0.2a	5.1 ± 0.9a
		5×10^4	22.2 ± 3.4bc	0.5 ± 0.3abc	1.3 ± 0.5abc	0.9 ± 0.5a	2.8 ± 0.4a
		5×10^6	19.0 ± 4.2bcd	0.4 ± 0.2abc	0.8 ± 0.3abc	0.5 ± 0.2a	3.2 ± 0.8a
60	Control		41.5 ± 8.2cd	0.3 ± 0.2a	0.7 ± 0.3a	0.4 ± 0.2a	6.5 ± 2.0a
	F. solani	5×10^2	62.0 ± 3.5ab	1.8 ± 0.4a	2.4 ± 0.6a	1.4 ± 0.5a	9.2 ± 0.9a
		5×10^4	81.3 ± 9.7a	1.7 ± 0.6a	4.4 ± 1.1a	0.9 ± 0.4a	8.9 ± 1.3a
		5×10^6	57.3 ± 7.6abc	2.4 ± 0.9a	1.4 ± 0.7a	1.8 ± 0.7a	9.7 ± 1.4a
	P. citrinum	5×10^2	57.0 ± 5.5ab	1.3 ± 0.4a	1.1 ± 0.4a	1.2 ± 0.3a	9.1 ± 2.2a
		5×10^4	45.0 ± 6.1bcd	0.7 ± 0.4a	1.2 ± 0.5a	0.4 ± 0.2a	5.5 ± 1.3a
		5×10^6	50.6 ± 4.0bcd	0.9 ± 0.4a	0.9 ± 0.4a	0.9 ± 0.3a	8.9 ± 1.0a
	C. fermentati	5×10^2	37.7 ± 4.0d	0.6 ± 0.3a	0.7 ± 0.3a	0.1 ± 0.1a	5.9 ± 1.1a
		5×10^4	51.0 ± 7.7bcd	0.8 ± 0.4a	1.8 ± 0.8a	0.5 ± 0.3a	10.2 ± 2.6a
		5×10^6	50.0 ± 6.8bcd	1.6 ± 0.6a	1.7 ± 0.6a	0.6 ± 0.2a	7.0 ± 1.8a
80	Control		31.4 ± 9.5a	0.3 ± 0.2a	0.3 ± 0.2c	0.3 ± 0.2a	4.7 ± 1.6a
	F. solani	5×10^2	27.2 ± 7.4a	0.3 ± 0.2a	0.3 ± 0.2bc	0.4 ± 0.4a	2.7 ± 0.7a
		5×10^4	58.1 ± 13.5a	2.9 ± 1.5a	3.7 ± 1.6ab	2.8 ± 1.2a	10.4 ± 3.6a
		5×10^6	68.9 ± 10.7a	1.7 ± 0.7a	3.4 ± 1.2a	2.4 ± 0.8a	6.6 ± 1.5a
	C. fermentati	5×10^2	29.5 ± 5.9a	0.1 ± 0.1a	0.5 ± 0.3bc	0.6 ± 0.3a	3.2 ± 0.6a
		5×10^4	32.6 ± 4.4a	0.2 ± 0.2a	0.3 ± 0.3c	0.1 ± 0.1a	3.0 ± 0.8a
		5×10^6	40.7 ± 7.2a	1.1 ± 0.5a	1.0 ± 0.6abc	1.0 ± 0.5a	5.3 ± 1.4a
	C. fermentati	5×10^2	32.1 ± 4.7a	0.7 ± 0.3a	0.9 ± 0.4abc	0.6 ± 0.3a	2.9 ± 0.5a
		5×10^4	31.7 ± 8.2a	0.7 ± 0.4a	0.7 ± 0.6bc	0.9 ± 0.5a	4.6 ± 1.6a
		5×10^6	51.7 ± 13.8a	2.2 ± 1.0a	2.3 ± 1.0ab	2.1 ± 1.0a	8.3 ± 2.8a

Data represent mean ± SE of 10 replicates (vials) after 40, 60, and 80 d in culture (untransformed data). Mean *H. hampei* productivity in treatments within columns and days followed by the same letter are not significant different (ANOVA; $P > 0.05$).

To our knowledge, this is the first detailed study attempting to evaluate experimentally the supposed mutualism between *H. hampei* and some associated fungi. According to our results, there is no evidence to designate any of the evaluated fungi as mutualists of *H. hampei*. Given that the three species of fungi evaluated in this study have been found in nature more frequently associated with *H. hampei* than others (Pérez et al. 2003, Carrión and Bonet 2004), it is unlikely that other species of fungus could act as mutualists of *H. hampei*.

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