Laboratory Evaluation of the Impact of Entomopathogenic Fungi on *Prorops nasuta* (Hymenoptera: Bethylidae), a Parasitoid of the Coffee Berry Borer

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ABSTRACT The entomopathogenic fungi *Metarhizium anisopliae* (Metschnikoff) Sorokin and *Beauveria bassiana* (Balsamo) Vuillemin and the bethylid parasitoid *Prorops nasuta* Waterston are natural enemies of the coffee berry borer, *Hypothenemus hampei* (Ferrari), and are considered valuable biocontrol agents in the coffee-growing regions of Central and South America. Laboratory evaluations were made on the impact of three isolates of each fungus on adult *P. nasuta.* These isolates were selected because of their high virulence toward *H. hampei* in previous studies. *B. bassiana* isolate Bb25 and *M. anisopliae* isolate Ma4 caused the lowest infection levels in *P. nasuta* with LC_{50} values of 8.31×10^6 and 4.08×10^6 spores per milliliter, respectively, by direct inoculation. Spore suspensions of each of these isolates were applied to coffee berry borer infested coffee berries. *P. nasuta* adult females were allowed to search and parasitize hosts within the treated berries. Despite the high virulence of these fungi to *P. nasuta*, neither pathogen significantly affected the predatory or parasitic capacity of *P. nasuta*, indicating that these isolates may be compatible with the action of the parasitoid under field conditions provided that pathogen applications and parasitoid liberations are timed not to coincide.

KEY WORDS *Beauveria bassiana*, *Metarhizium anisopliae*, *Prorops nasuta*, bioassay, virulence, nontarget organism

THE COFFEE BERRY borer, *Hypothenemus hampei* (Ferrari), is a small scolytid beetle of African origin that has become the principal pest of coffee worldwide (Le Pelley 1968, Singh and Ramani 1995). More than 350,000 ha of coffee are attacked by this pest in Mexico, Central America, and the Caribbean (Barrera et al. 1990a). Control of *H. hampei* by repeated applications of endosulfan has resulted in high levels of resistance to organochlorine insecticides in New Caledonia and possibly elsewhere (Brun et al. 1989, 1994).

Among the known natural enemies of *H. hampei*, the bethylid parasitoids *Prorops nasuta* Waterston and *Cephalonomia stephanoderis* Betrem are considered the most promising agents for use in biological pest control (Waterhouse and Norris 1989, Abraham et al. 1990). However, to achieve control of economic thresholds, additional measures such as the use of fungal bioinsecticides may be required (Moore and Prior 1988, Barrera et al. 1990b, Murphy and Moore 1990).

The most common fungal entomopathogens have a wide host range in nature and may infect insect natural enemies of the target pest (Hajek and St. Leger 1994). In particular, *Metarhizium anisopliae* (Metschnikoff) Sorokin and *Beauveria bassiana* (Balsamo) Vuillemin have been isolated from diverse species of parasitoids (Brooks 1993, Rosenheim et al. 1995), although aspects of the ecology of such interactions and their impact on levels of pest control remain poorly understood. It has been suggested that fungal infections of natural enemies are not particularly common and that many natural enemy species appear resistant to mycoses (Goettel et al. 1990).

Studies in Colombia and Mexico have indicated that a diversity of isolates of entomopathogenic fungi may be suitable for use alongside *C. stephanoderis* (Reyes et al. 1994; de la Rosa et al. 1997a, 1997b), whereas the mortality risk toward *P. nasuta* remains unknown. The aims of the current study were to assess the susceptibility of *P. nasuta* to two fungal pathogens in the laboratory, and to evaluate survival and host-attack abilities of *P. nasuta* in an environment with high densities of fungal spores, such as would be experienced after application of a fungal bioinsecticide. Such information represents an important first step in evaluation of the feasibility of using these distinct agents in an integrated program of coffee berry borer control.

Materials and Methods

Selection of Fungal Isolates and Insect Material. Three isolates of *B. bassiana*, designated as Bb4, Bb25, and Bb26 (Table 1) were originally isolated from infected *H. hampei* cadavers collected in Brazil or Mexico and have been shown to have high infectivity to this pest. Three isolates of *M. anisopliae* (Ma3, Ma4,

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Table 1. Origin of *B. bassiana* (Bb) and *M. anisopliae* (Ma) isolates used in the bioassays against *P. nasuta*

Name	Geographical origin	Host of origin
Bb4	Brazil	*H. hampei* (Coleoptera: Scolytidae)
Bb25	Mexico (Chiapas)	*H. hampei* (Coleoptera: Scolytidae)
Bb26	Mexico (Chiapas)	*H. hampei* (Coleoptera: Scolytidae)
Ma3	United States	Commercial product
Ma4	Mexico (Colima)	*Diatraea saccharalis* (F.) (Lepidoptera: Pyralidae)
Ma5	Mexico (Colima)	*Spodoptera frugiperda* J. E. Smith (Lepidoptera: Noctuidae)

and Ma5 originally isolated from a homopteran and two species of Lepidoptera) were also highly infective to adult coffee berry borers (de la Rosa et al. 1997a, 1997b).

Fungi were propagated in glass test tubes containing Sabouraud dextrose agar (0.65%), yeast extract (0.4%), and 0.05 g/liter tetracycline. Each inoculated tube was incubated for 14–15 d at 28–30°C to allow complete sporulation. Fungal conidia were harvested using a sterile bacterial loop and were checked for viability following the method of Jiménez (1992). Spore germination was not <90%.

A suspension of 1.0 g of spores in 100 ml of sterile distilled water was made using two drops of commercial wetter-sticker (AgralPlus, Zeneca, Mountain View, CA). This was considered a 1% suspension and represented 4.3×10^8 spores per milliliter for Bb4 and Bb26, 3.5×10^8 spores per milliliter for Bb25, 6.8×10^7 spores per milliliter for Ma3; 4.0×10^7 spores per milliliter for Ma4, and 3.3×10^7 spores per milliliter for Ma5. Serial dilutions of 0.1, 0.01, and 0.001% were made from these suspensions for testing against *P. nasuta.*

Adult *P. nasuta* of homogeneous age were obtained from a culture held in ECOSUR, Tapachula, Mexico. The sex ratio of cultured parasitoids was 0.20–0.25 male. Adult female *H. hampei* were taken from a culture maintained on semisynthetic diet (Villacorta and Barrera 1993).

Bioassays of Virulence Toward *P. nasuta.* Three bioassays were conducted with each isolate. In each bioassay, 40 insects were inoculated with each concentration of fungal spores described above. Control insects were treated with water containing wetter-sticker.

Groups of 10 parasitoids were placed in 13-ml glass tubes containing 500 μl of spore suspension. Insects were subject to immersion and gentle agitation for 30 s before being poured into a petri dish containing filter paper to absorb the excess liquid. Parasitoids were then placed individually in sterile glass tube sealed with muslin gauze to permit ventilation. Parasitoids were daily offered one or two drops of sugared water placed on the gauze for 10 d after exposure to fungal spores. Each replicate therefore comprised a group of 10 parasitoids treated with one of four concentrations of fungal spores between 1 and 0.001%, plus a group of 10 control insects treated with wetter-sticker solution alone.

Tubes containing experimental insects were held in environmental chambers at 27 ± 2°C, 90 ± 5% RH, and a photoperiod of 12:12 (L:D) h. Parasitoids were checked daily for mortality. Dead insects were placed in petri dishes containing a disk of damp sterile filter paper to allow fungal sporulation. Parasitoid mortality and the number of cadavers showing visible symptoms of sporulation were recorded and subject to Probit analysis after correction for control mortality (Abbott 1925). Mortality in the controls never exceeded 15% over the 10 d of the experiment.

These assays indicated that Bb25 and Ma4 had lower infectivity to *P. nasuta* than the other isolates tested. Consequently, bioassays of these two isolates were repeated using 10 different concentration steps between 1.0 and 0.001%. For each concentration, 50 parasitoids were used and the assay was performed three times. In this test, parasitoid mortality, time to death, and percentage sporulation of cadavers was recorded. The mean yield of spores from infected male and female parasitoids was estimated by shaking eight fully sporulated parasitoid cadavers in 500 μl sterile distilled water for 30 s, followed by quantification of the resulting spore suspension by counting using a Neubauer chamber. The experiment was performed three times.

Pest–Parasitoid–Pathogen Interaction. Mortality patterns of *H. hampei* were studied in the presence or absence of both parasitoids and pathogens. A surface disinfection of green-yellow coffee berries was performed by immersion in 3% sodium hypochorite solution for 30 min followed by three washes in distilled water each for 5 min. Berries were allowed to dry and were placed individually in 18-ml glass tubes together with a single female coffee berry borer. Tubes were capped with gauze and left to allow the pest to reproduce.

After 17 d, when *H. hampei* tunneling and oviposition had occurred (confirmed by dissection of a subsample of infested berries), a single *P. nasuta* female was introduced into each tube. In treatments involving fungi, infested berries were surface-contaminated by *B. bassiana* Bb25 or *M. anisopliae* Ma4 at 3 d or 10 d before the introduction of the *P. nasuta* female. This was achieved by 30-s immersion in 3.5×10^8 spores per milliliter of Bb25 suspension or 4×10^7 spores per milliliter of Ma4 suspension; which are concentrations predicted to cause >70% mortality by direct inoculation of the parasitoid. During the 3- or 10-d interval between treatment with spore suspension and introduction of the parasitoid, infested berries were kept individually in glass tubes as described above.

There were two control treatments, in the first, the parasitoid was introduced to an uncontaminated infested coffee berry. For the second, neither parasitoid nor pathogen was introduced to the *H. hampei* infested berries. Each treatment involved 20 infested berries and the experiment was performed five times.

Parasitoids remained confined with infested berries for 24 d under the conditions of temperature and humidity previously mentioned, after which all berries were dissected to determine the fate of pest and parasitoid. Predatory behavior by *P. nasuta* is a common characteristic and was detected by searching for

Table 2. Virulence of *B. bassiana* and *M. anisopliae* to adult *P. nasuta*

Isolate	LC_{50}[a] (*spores/ml*)	95% CL ($\times 10^6$)	Regression equation	LT_{50}[b]	χ^2
Bb4	6.63×10^6 a	0.353–11.8	Y = 6.68 + 0.93X	3.2	0.107
Bb25	8.31×10^6 a	3.78–17.8	Y = 6.10 + 0.66X	3.4	0.406
Bb26	4.14×10^6 a	1.55–9.16	Y = 6.26 + 0.62X	4.0	0.969
Ma3	6.61×10^5 b	0.184–1.71	Y = 6.01 + 0.50X	3.9	0.179
Ma4	4.08×10^6 a	1.98–9.47	Y = 5.60 + 0.62X	3.4	0.61
Ma5	2.60×10^5 b	0.0924–0.571	Y = 6.30 + 0.63X	3.3	0.398

Chi-square goodness-of-fit value indicates homogeneity of test population in Probit analysis of concentration-mortality response (df = 2).
[a] Values followed by the same letter are not significantly different as determined by examination of 95% CL of calculated LC_{50} values.
[b] At highest inoculum concentration tested.

evidence of decapitated adult *H. hampei* in each berry. Data were subject to a Kruskal–Wallis nonparametric analysis of variance (ANOVA), or an ANOVA analysis of arcsine transformed percentage data followed by least significant difference (LSD) mean separation (Sokal and Rohlf 1981, SPSS 1995).

Results

Bioassays of Virulence Toward *P. nasuta*. Examination of the 95% CL corresponding to the calculated LC_{50} values indicated that there were no significant differences among the three isolates of *B. bassiana* in terms of their infectivity to *P. nasuta* (LC_{50} values 4.14×10^6 to 8.31×10^6 spores per milliliter) (Table 2). LT_{50} values, calculated following the method given by Finney (1971), were also very similar among these isolates (3.2–4.0 d). Comparison of the *M. anisopliae* isolates indicated that Ma4 had a significantly lower infectivity toward *P. nasuta* than Ma3 or Ma5 (Table 2). Ma4 also had a significantly slower action that was most apparent at low inoculum concentrations (e.g., at the 0.1% inoculum concentration) Ma3 and Ma5 had LT_{50} values of 5.4 and 6.2 d, respectively, whereas the corresponding value for Ma4 was 8.3 d. This contrasts to the 3.2- to 4-d LT_{50} values calculated for the highest inoculum concentration (Table 2).

The isolates Bb25 and Ma4 were selected for further testing because they showed the least infectivity (highest LC_{50} values) toward *P. nasuta* and therefore these isolates may be more compatible with the use of this parasitoid in integrated programs of biocontrol. Additional bioassays of Bb25 and Ma4, involving 10 concentration steps, indicated that both LC_{50} and LT_{50} values for these assays for both species of fungus were statistically similar to those given in Table two (data not shown). The *B. bassiana* isolate Bb25 generally showed a moderate incidence of sporulation on cadavers of *P. nasuta* (Table 3). This difference was evident at the higher inoculum concentrations. The isolates of *M. anisopliae* were nearly identical in their degree of sporulation at all inocula concentrations. For both species of fungus there was a clear positive relationship between the incidence of sporulation and the concentration of inoculum to which the parasitoid had been exposed. The yield of *B. bassiana* Bb25 spores from each infected parasitoid was affected by inoculum concentration and sex of the parasitoid. Spore production was higher at high inoculum concentrations compared with lower concentrations and was invariably more prolific on female parasitoids compared with males (Fig. 1A).

Spore production by *M. anisopliae* Ma4 was lower than for *B. bassiana*, although the positive relationship between spore yields and inoculum concentration was similar to that observed in *B. bassiana*. Spore production was also more abundant on female compared with male *P. nasuta* cadavers (Fig. 1B).

Pest–Parasitoid–Pathogen Interaction. Dissection of infested coffee berries revealed evidence of the marked predatory behavior of *P. nasuta* (Table 4). In the absence of both parasitoid and pathogens, the mean number of living adult *H. hampei* (206.0) was more than an order of magnitude greater than in any treatment involving *P. nasuta* (Kruskal–Wallis H = 14.329; df = 5, 30; P = 0.013). The same pattern was observed for the number of healthy immature *H. hampei* (298.8) in the presence of the parasitoid compared with the no-parasitoid control (Kruskal–Wallis H = 14.107; df = 5, 30; P = 0.015).

It was clear that this difference was the result of the predation by the parasitoid rather than attack by one of the fungal pathogens because the incidence of fungal mycoses did not exceed 1.8 *H. hampei* cadavers per berry in any treatment involving fungi. The incidence of unexplained mortality in *H. hampei* was also low in all treatments (4.0–9.0 *H. hampei* per berry). Of these cadavers, between 44.4 and 69.2% subsequently showed signs of sporulation when incubated in moist

Table 3. Percent sporulation observed on cadavers of *P. nasuta* treated with different concentrations of *B. bassiana* or *M. anisopliae* spore suspension

Isolate	Inocula concn, %			
	1.0	0.1	0.01	0.001
Bb4	100.0a	91.5a	71.9a	13.3a
Bb25	68.0b	57.9b	13.7b	10.4a
Bb26	99.1a	84.7ab	37.7b	13.9a
Ma3	96.0a	95.2a	64.3a	32.5a
Ma4	95.6a	88.2a	45.0a	33.3a
Ma5	96.4a	94.2a	67.7a	36.1a

Values followed by different letters are significantly different at $P < 0.05$ (LSD, 1995 for Windows version 7.0, Sokal and Rholf 1981). For analysis, all percentages were arcsine transformed but are shown above as original mean percent sporulation. Comparisons were only made among isolates of the same species, not between fungal species.

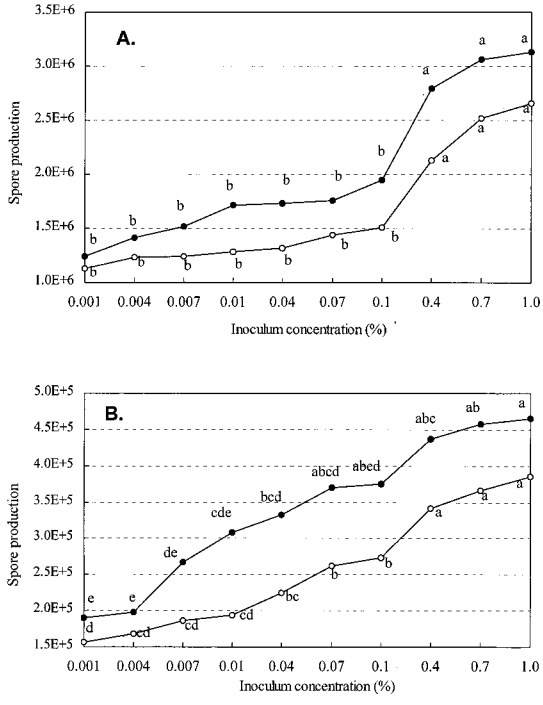

Fig. 1. Spore production of (**A**) *B. bassiana* Bb25 or (**B**) *M. anisopliae* Ma4 on cadavers of *P. nasuta* in relation to concentration of inoculum to which the parasitoids were exposed. Solid black circles represent females, open circles represent males. Data points with different letters are significantly different for comparisons of the same sex ($P < 0.05$). Spore production values are means of three replicate groups of eight individuals.

petri dishes, although no significant differences were detected with respect to species of fungus or time of inoculation ($F = 0.509$; df = 3, 16; $P > 0.05$). There were no significant differences among any of the treatments involving fungi in terms of the number of living adult or healthy immature *H. hampei*, the number of dead *H. hampei* (infected or not), or the incidence of predatory activity by *P. nasuta* (Table 4).

In the evaluation of parasitoid mortality, it was not possible to differentiate the founding female from her progeny and so the data in Table 4 include the *P. nasuta* foundress. Despite the high concentration of spores applied to the surface of the coffee berry, the incidence of fungal infection observed in *P. nasuta* was invariably very low (zero to 0.8 infected parasitoids per berry). For the treatments involving *B. bassiana*, no difference was seen when spores were applied 3 or 10 d before the introduction of the parasitoid (0.4 infected parasitoids per berry). In contrast, higher incidence of infection was seen in *P. nasuta* when *M. anisopliae* was applied 3 d (0.8 infected *P. nasuta* per berry) as opposed to 10 d (zero infection) before the introduction of the parasitoid (Table 4).

The number of living adult *P. nasuta* per berry did not differ among the fungal treatments although it was significantly less than the treatment involving *P. nasuta* in the absence of fungi. This difference was not observed in the number of healthy *P. nasuta* pupae found in each dissected berry that was statistically similar among all treatments involving the parasitoid (Table 4).

When dead *P. nasuta* adults (that showed no signs of sporulation) were incubated in moist petri dishes, 26.4–41.0% showed subsequent evidence of sporulation, although there were no significant differences with respect to species of fungus or time of inoculation (3 or 10 d before parasitoid) ($F = 0.128$; df = 3, 16; $P > 0.05$).

Discussion

Isolates of *B. bassiana* and *M. anisopliae* that showed high virulence toward the coffee berry borer were evaluated for their infectivity toward the parasitoid *P. nasuta.* Different concentrations of each isolate were used in the bioassays but as the range of concentrations of spores consistently spanned the LC_{10} to LC_{90} range, it was possible to determine accurately the LC_{50} value for each isolate. Differences among the isolates were observed in terms of their infectivity, speed of kill, and frequency of sporulation. There were also marked differences in spore production related to pathogen species, inoculum concentration and sex of the parasitoid. When spores were applied to the surface of berries infested with coffee berry borer, little pathogen-induced mortality was observed in either *H.*

Table 4. Mean number of adult and immature *P. nasuta* and *H. hampei* observed in dissected infested coffee berries following treatment with fungal spore suspensions or the introduction of an individual female *P. nasuta*

Treatments applied to *H. hampei* infested berries	*P. nasuta*				*H. hampei*				
	Living adults	Dead adults with mycosis	Dead adults, no mycosis	Healthy pupae	Living adults	Dead adults killed by *P. nasuta*	Dead adults with mycosis	Dead adults, no mycosis	Healthy immature stages
Bb25, 3d prior to *P. nasuta*	62.6b	0.4a	7.8ab	31.2a	5.0b	19.4a	1.2a	4.0a	14.0b
Bb25, 10d prior to *P. nasuta*	60.4b	0.4a	8.6a	33.0a	4.8b	17.0a	1.0a	5.4a	6.2b
Ma4, 3d prior to *P. nasuta*	51.0b	0.8a	7.8ab	32.0a	5.8b	18.6a	1.8a	5.2a	4.2b
Ma4, 10d prior to *P. nasuta*	57.0b	0.0b	10.6a	29.2a	11.8b	16.8a	0.4a	4.6a	16.6b
P. nasuta, no fungi	83.4a	0.0b	4.8b	37.4a	2.2b	20.4a	0.0a	4.2a	1.7b
No *P. nasuta*, no fungi	—	—	—	—	206.0a	—	0.0a	9.0a	298.8a

Values are means for each of five replicates of the experiment (1 replicate = 20 infested coffee berries). Values followed by different letters within each column are significantly different ($P < 0.05$, LSD, 1995 for Windows version 7.0, Sokal and Rholf 1981).

hampei or *P. nasuta*, regardless of pathogen species or the time interval between spore application and the introduction of the parasitoid. The predatory and parasitic behavior of *P. nasuta*, however, caused substantial mortality the coffee berry borer cohorts within each infested berry. This predatory ability, well recognized in *P. nasuta*, has also been documented in another *H. hampei* parasitoid, *C. stephanoderis* (Abraham et al. 1990, Murphy and Rangi 1991).

Previous studies have indicated that the *B. bassiana* isolates selected for this study induced >90% mortality by direct inoculation of *H. hampei* adult females at the 1% spore concentration (de la Rosa et al. 1997a), whereas the *M. anisopliae* isolates caused between 57% (Ma4) and 89% (Ma3, Ma5) *H. hampei* mortality under similar conditions (de la Rosa-Reyes et al. 1995). This difference may be related in part to the origin of the various isolates; all *B. bassiana* material was isolated from local populations of *H. hampei*, whereas the *M. anisopliae* material originated from Lepidoptera and Homoptera from northern Mexico and the United States.

The LC_{50} values of Bb25 and Ma4 toward the coffee berry borer were 4.1×10^6 and 4.2×10^6 spores per milliliter, respectively (de la Rosa et al. 1997a). These figures represent half the LC_{50} value of Bb25 to *P. nasuta* and a nearly identical value for the LC_{50} of Ma4 to *P. nasuta.* It is therefore evident that these isolates are highly virulent to both the coffee berry borer and the parasitoid *P. nasuta.*

Previous work on *B. bassiana* and *M. anisopliae* infection of the bethylid *C. stephanoderis* included all of the isolates used in the current study (de la Rosa et al. 1997b). LC_{50} values toward *C. stephanoderis* were 4.45×10^7 spores per milliliter for Bb25 and 1.00×10^8 spores per milliliter for Ma4, which were between one and two orders of magnitude greater than the LC_{50} values of these isolates when tested on *P. nasuta.* It appears that *C. stephanoderis* is substantially more resistant to fungal infection compared with *P. nasuta.*

Studies on the frequency of sporulation and the abundance of spores produced on cadavers of *P. nasuta* give insights into the potential of these hosts in the transmission of fungal infections. Sex-related differences in the spore production probably are a consequence of the different body size of male and female *P. nasuta.* With a mean body length (±SE) of 1.63 ± 0.05 mm ($n = 11$), males are markedly smaller than the females (body length 2.01 ± 0.04 mm, $n = 22$) and therefore represent a smaller food resource for the sporulating fungus.

Rosenheim's et al. (1995) review of intraguild interactions among pests, insect natural enemies, and pathogens indicated that most evaluations of such interactions have been by way of laboratory studies in which pathogen and insect natural enemy are placed in close association. Intraguild interactions are most commonly observed in fungi, protozoans, and nematodes because of the broad host range of these pathogens. Field evaluations of the impact of applications of entomopathogenic bioinsecticides to insect natural enemy populations are very limited in number. One of few examples is an evaluation of the impact of aerial applications of *Beauveria brongniartii* (Saccas) Petch spores to forest habitat for control of *Melolontha melolontha* L. This study reported that only 1.1% of nontarget insect and spiders were infected by the pathogen of >10,000 individuals sampled. This pathogen appeared to pose little risk to nontarget arthropods (Baltensweiler and Cerutti 1986).

In the current study it was clear that surface contamination had little effect on the incidence of fungal infection in the coffee berry borer. This pest has cryptic habits that make it difficult to control: the tunnels within the coffee berry appear to act as an effective refuge from pathogens and only actively searching enemies such as parasitoids are capable of attacking the pest once it has formed its galleries. Thus, it would be appropriate to apply the fungus in the field during the phase when the berries are beginning to be attacked by the borer. Subsequently, when the pest begins reproducing, liberation of parasitoids should be carried out. This would ensure that the main exposure to inoculum for the parasitoid would be by contact with contaminated surfaces, as tested in the current study.

As for any insecticide that acts by contact, the use of a fungal bioinsecticide will only be of value when the berries are beginning to be attacked by the pest. Periodic applications of spores during this period will have a greater chance of infecting *H. hampei* adult females while they search for suitable host berries and begin the tunneling process. The results of the current study indicate that it would be wise to avoid mass liberations of *P. nasuta* parasitoids during the period when fungal spore applications are being made. However, when application of the bioinsecticide has ceased, liberation of *P. nasuta* can occur with little risk of negative impact of the pathogen toward the parasitoid. The validity of this idea can only be confirmed by field testing.

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References Cited

Abbott, W. S. 1925. A method for computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265–267.

Abraham, Y. D., D. Moore, and G. Godwin. 1990. Rearing and aspects of biology of *Cephalonomia stephanoderis* and *Prorops nasuta* (Hymenoptera: Bethylidae) parasitoids of the coffee berry borer, *Hypothenemus hampei* (Coleoptera: Scolytidae). Bull. Entomol. Res. 80: 121–128.

Baltensweiler, W. and F. Cerutti. 1986. Bericht uber die Nebenwirkungen einer Bekampfung des Maikafers (*Melolontha melolontha* L.) mit dem Pilz *Beauveria brongniartii* (Sacc.) Petch auf die Arthropodenfauna des Waldrandes. Mitt. Schweiz. Entomol. Ges. 59: 267–274.

Barrera, J. F., P. S. Baker, J. E. Valenzuela, and A. Schwarz. 1990a. Introducción de dos especies de parasitoides africanos a México para el control biológico de la broca del café *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae). Folia Entomol. Mex. 79: 245–47.

Barrera, J. F., D. Moore, Y. J. Abraham, S. T. Murphy, and C. Prior. 1990b. Biological control of the coffee berry borer *Hypothenemus hampei,* in Mexico and possibilities for further action. Proc. Brighton Crop Protec. Conf. Pests Dis. 1990: 91–396.

Brooks, W. M. 1993. Host-parasitoid-pathogen interactions, pp. 231–272. *In* N. E Beckage, S. N. Thompson and B. A. Federici [eds.], Parasites and pathogens of insects, vol. 2. Pathogens. Academic, San Diego, CA.

Brun, L. O., C. Marcillaud, V. Gaudichon, and D. M. Suckling. 1989. Endosulfan resistance in *Hypothenemus hampei* (Coleoptera: Scolytidae) in New Caledonia. J. Econ. Entomol. 82: 1311–1316.

Brun, L. O., C. Marcillaud, V. Gaudichon, and D. M. Suckling. 1994. Cross resistance between insecticides in coffee berry borer, *Hypothenemus hampei* (Coleoptera: Scolytidae) from New Caledonia. Bull. Entomol. Res. 84: 195–178.

de la Rosa, W., R. Alatorre, J. Trujillo and J. F. Barrera. 1997a. Virulence of *Beauveria bassiana* (Deuteromycetes) strains against the coffee berry borer (Coleoptera: Scolytidae). J. Econ. Entomol. 90: 1534–1538.

de la Rosa, W., J. L. Godínez A., R. Alatorre and J. Trujillo. 1997b. Susceptibilidad del parasitoide *Cephalonomia stephanoderis* a diferentes cepas de *Beauveria bassiana* y *Metarhizium anisopliae.* Southwest. Entomol. 22: 233–242.

de la Rosa-Reyes, W., J. L. Godinez-Aguilar and R. Alatorre-Rosas. 1995. Biological activity of five strains on *Metarhizium anisopliae* upon the coffee berry borer *Hypothenemus hampei* (Col.: Scolytidae). Entomophaga 40: 403–412.

Finney, D. J. 1971. Probit analysis, 3rd ed. Cambridge University Press, Cambridge.

Goettel, M. S., T. J. Poprawski, J. D. Vandenberg, Z. Li, and D. W. Roberts. 1990. Safety to nontarget invertebrates of fungal biocontrol agents, pp. 209–231. *In* M. Laird, L. A. Lacey, and E. W. Davidson [eds.], Safety of microbial insecticides. CRC, Boca Raton, FL.

Hajek, A. E., and R. J. St. Leger. 1994. Interactions between fungal pathogens and insect hosts. Annu. Rev. Entomol. 39: 293–322.

Jiménez, G. J. 1992. Patogenicidad de diferentes aislamientos de *Beauveria bassiana* sobre la broca del café. Cenicafe 43: 84–98.

Le Pelley, R. H. 1968. Pests of coffee. Longmans, London.

Moore, D., and C. Prior. 1988. Present status of biological control of the coffee berry borer *Hypothenemus hampei.* Proc. Brighton Crop Protec. Conf. Pests Dis. 1988: 1119–1124.

Murphy, S. T., and D. Moore. 1990. Biological control of the coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae): previous programmes and possibilities for the future. Biocontrol News Inf. 11: 107–117.

Murphy, S. T., and D. K. Rangi. 1991. The use of the African wasp, *Prorops nasuta* for the control of the coffee berry borer, *Hypothenemus hampei* in Mexico and Ecuador: the introduction programme. Insect Sci. Applic. 12: 27–34.

Reyes, A. I., A. P. Bustillo, and B. C. Chaves. 1994. Efecto de los hongos *Beauveria bassiana* y *Metarhizium anisopliae* sobre el parasitoide de la broca del café *Cephalonomia stephanoderis.* Proceedings, XXI Congress of the Entomological Society of Colombia. Entomological Society of Colombia, Medellín, Colombia.

Rosenheim, J. A., H. K. Kaya, L. E. Ehler, J. J. Marois, and B. A. Jaffee. 1995. Intraguild predation among biological-control agents: theory and evidence. Biol. Control 5: 303–335.

Singh, S. P., and S. Ramani. 1995. Coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae). Technical Bulletin 12. Directorate of Biological Control, Bangiore, India.

Sokal, R. R., and F. J. Rohlf. 1981. Biometry, 2nd ed. Freeman, New York.

SPSS. 1995. User's manual, version 7.0, for Windows. SPSS, Chicago, IL.

Villacorta, A., and J. F. Barrera. 1993. Nova dieta meridica para cricão de *Hypothenemus hampei* (Ferrari, 1867) An. Soc. Entomol. Bras. 22: 147–152.

Waterhouse, D. F., and K. R. Norris. 1989. Biological control Pacific Prospects—Supplement 1. Australian Centre for International Agricultural Research, Canberra.

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