

Response of the Wasp *Cephalonomia tarsalis* (Hymenoptera: Bethyloidea) to *Beauveria bassiana* (Hyphomycetes: Moniliales) as Free Conidia or Infection in Its Host, the Sawtoothed Grain Beetle, *Oryzaephilus surinamensis* (Coleoptera: Silvanidae)

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***Cephalonomia tarsalis*, an ectoparasitoid, and *Beauveria bassiana*, an entomopathogenic fungus, are potential biological control agents for the sawtoothed grain beetle, *Oryzaephilus surinamensis*. Several experiments were conducted to determine whether the two beneficial organisms are compatible. Wasps exhibited little avoidance behavior toward the fungus. Adult wasps oviposited on *B. bassiana*-infected larvae up to within 1 day of the host's death and the appearance of red fungal pigment. Wasp larvae are susceptible to the fungus and die within 1 day of oviposition on host larvae with mycosis. A 3-h exposure of adult wasps to 100 mg of *B. bassiana*/kg of wheat resulted in 52.7% mortality. Nevertheless, the wasps entered into grain containing *B. bassiana* conidia as freely as they entered into conidia-free grain. The mean prevalence of *B. bassiana* in 46 samples of pooled wheat representing 276 locations was 7.5 colony-forming units/g of wheat. Natural *C. tarsalis* exposure to *B. bassiana* in untreated stored wheat is likely to be below lethal quantities, and the introduction of the fungus in insecticidal quantities would have a negative impact on *C. tarsalis* populations.**

Key Words: *Cephalonomia tarsalis*; *Oryzaephilus surinamensis*; *Beauveria bassiana*; grain beetle; biological control; behavior; beneficial insects.

INTRODUCTION

Parasitoid wasps and entomopathogenic fungi are key components of integrated pest management programs in the stable environments of glasshouses (Fransen, 1993; Fransen and van Lenteren, 1993).

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They have been proposed for use in the similarly stable stored-product environments (Brower *et al.*, 1996; Moore *et al.*, 2000). Successful integration of the two control agents depends on absence of repellency by fungus-treated grain and minimal loss of parasitoids to fungal infection. The parasitoids should either be substantially less susceptible to the biocontrol fungus or have the ability to avoid contact with it.

Many Hymenoptera are physiologically susceptible to infection by *Beauveria bassiana* (Balsamo) Vuillemin. However, social Hymenoptera have behavioral mechanisms that may include grooming, nest hygiene, avoidance, secretion of antimicrobial compounds, and temperature regulation that avoid infection or minimize the effects of pathogens (Goettel *et al.*, 1990; Oi and Pereira, 1993). In contrast, parasitoid wasps are limited in such behaviors and have proven susceptible to direct *B. bassiana* application in laboratory environments (Danfa and van der Valk, 1999; de la Rosa *et al.*, 1997). Accordingly, parasitoids attack diseased hosts at their peril.

Pathogens of broad host range, such as *B. bassiana*, may not only kill the wasp offspring but also infect ovipositing female wasps. Even when the pathogen is not infectious for the wasps, the diseased hosts are generally unsuitable for parasitoid development. As a consequence, some parasitoids have evolved the ability to detect and avoid diseased hosts (Fransen and van Lenteren, 1993; Jones and Wraight, 1996).

Cephalonomia tarsalis (Ashmead) is both a predator and an ectoparasitoid of larval sawtoothed grain beetles, *Oryzaephilus surinamensis* (L.). The beetle larvae are also very susceptible to *B. bassiana*, a registered mycoinsecticide with excellent prospects for use against pests of grain in storage and processing. This work addresses the compatibility of the two biological control agents.

MATERIALS AND METHODS

Insects

C. tarsalis and *O. surinamensis* were collected from farm-stored wheat in Kansas and maintained in the laboratory for approximately 4 years. Beetles were reared to the last instar on rolled oats with brewer's yeast at $30 \pm 1^\circ\text{C}$. Wasps were maintained in quart jars with hard red winter wheat and excess host larvae at $30 \pm 1^\circ\text{C}$ and a 16:8 (L:D) h photoperiod. For experiments, emerging adult wasps were collected on 2 consecutive days and held jointly in jars of wheat with *O. surinamensis* larvae and 50% honey water on cotton at $30 \pm 1^\circ\text{C}$ and a 16:8 (L:D) h photoperiod for 3–5 days to allow mating before use. Chill-anesthesia was used for handling.

Fungus Treatment of Beetle Larvae

Commercially produced, unformulated conidia of *B. bassiana* GHA isolate were obtained from Mycotech Corp., Butte Montana. The conidia powder contained 6.3×10^{10} conidia/g. The germination rate after 18 h on Sabouraud dextrose agar was above 90% throughout the experiments. Approximately 200 2-week-old sawtoothed grain beetle larvae were placed in beakers with 5 mg of *B. bassiana* conidia mixed into 1 g of ground rolled oats that had been sieved through a 14 mesh (1.41-mm) sieve. The concentration is approximately $2 \times \text{LC}_{95}$ for sawtoothed grain beetle larvae (Lord, 2001). The beakers were covered with food wrap and placed in a 100% RH chamber at $26 \pm 1^\circ\text{C}$ for 24 h. Except for the 24-h fungus exposure periods, all larvae, including controls, were maintained on rolled oats in mesh-covered glass petri dishes at $26 \pm 1^\circ\text{C}$ and 75 \pm 1% RH over saturated NaCl.

No-Choice Oviposition

Oviposition and progeny survival of wasps with constant access to *B. bassiana*-treated and untreated beetle larvae were monitored over 4 days. Female wasps were placed individually in 12.5-cm ventilated tissue culture flasks with three fourth-instar beetles and a drop of honey water. The beetles were untreated or treated with 24-h exposure to *B. bassiana*, terminating 1 and 2 days before exposure to wasps. Incubation was at $26 \pm 1^\circ\text{C}$ and ca. 75 \pm 1% RH with a 13:11 h light:dark cycle with filtered, indirect light provided by a 3-W fluorescent bulb. To make daily counts without dislodging eggs, no grain was placed in the assay vessels. The presence and condition of wasp eggs and larvae and the condition of the hosts were monitored daily. There were 30 flasks per treatment in each of four replicated experiments conducted on different dates.

Choice Test for Oviposition

Beetle larvae were treated as for the no-choice test. Groups of three beetles were placed in 15 100-ml beakers

per treatment with 5 g of hard red winter wheat. All beakers were placed randomly in a 14 \times 36 \times 52-cm covered plastic bin, and 68–78 wasps were released in the center. Oviposition was assessed after 24 h of incubation under the test conditions described above. The data were classified as oviposition on untreated larvae, oviposition on treated larvae with red fungal pigment, and oviposition on healthy appearing treated larvae in which symptomatic red fungal pigment appeared after further incubation—i.e., diseased but not yet symptomatic larvae. Dried out larvae that were clearly unsuitable for oviposition were not included in the results. The experiment was replicated five times on different dates.

Choice Test for Entry into Fungus-Treated Grain

Female wasps were placed individually in the center of a 150-mm petri dish among three 60-mm plastic petri dishes, each containing 20 g of wheat with 0, 100, or 500 mg/kg *B. bassiana* conidia. Each 60-mm petri dish also contained four fourth-instar sawtoothed grain beetle larvae. The wasps were watched for 10 min and timed for residence in each treatment. A total of 108 wasps were observed entering the wheat.

*Mortality of Adult Wasps Exposed to *B. bassiana**

Groups of 20 female wasps were placed in shallow 250-ml plastic containers with 100 g of wheat kernels and 0, 100, or 500 mg/kg *B. bassiana* conidia for 3 h. They were then transferred to pint jars with 100 g of clean wheat with 40 fourth-instar sawtoothed grain beetles. The insects in jars with clean wheat were incubated for 8 days at $26 \pm 1^\circ\text{C}$ and 75 \pm 1% RH over saturated NaCl in plastic boxes and scored for survival. The test was replicated three times on separate dates.

*Prevalence of *B. bassiana* in Postharvest Wheat*

To determine the prevalence of *B. bassiana* in postharvest wheat, samples taken from terminal deliveries in six U.S. states were assessed. Each of 46 pooled wheat samples represented six within-state locations. The wheat varieties were mixed, but more than 90% was soft wheat. Fifty grams of wheat from each sample were washed with 25 ml of 0.05% Silwet L-77 (Loveland Industries, Greeley, CO), and 0.1 ml was spread on each of two 90-mm plates of wheat germ selective agar containing dodine and benomyl (Sneh, 1991). After incubation for 6 days at $26 \pm 1^\circ\text{C}$, the presence of characteristic *Beauveria* colonies was scored and confirmed microscopically.

Statistical Analysis

Analysis of variance was applied to untransformed data with StatView (SAS Institute, 1999). Differences among means were detected with the Student–Newman–Keuls test with $\alpha = 0.05$.

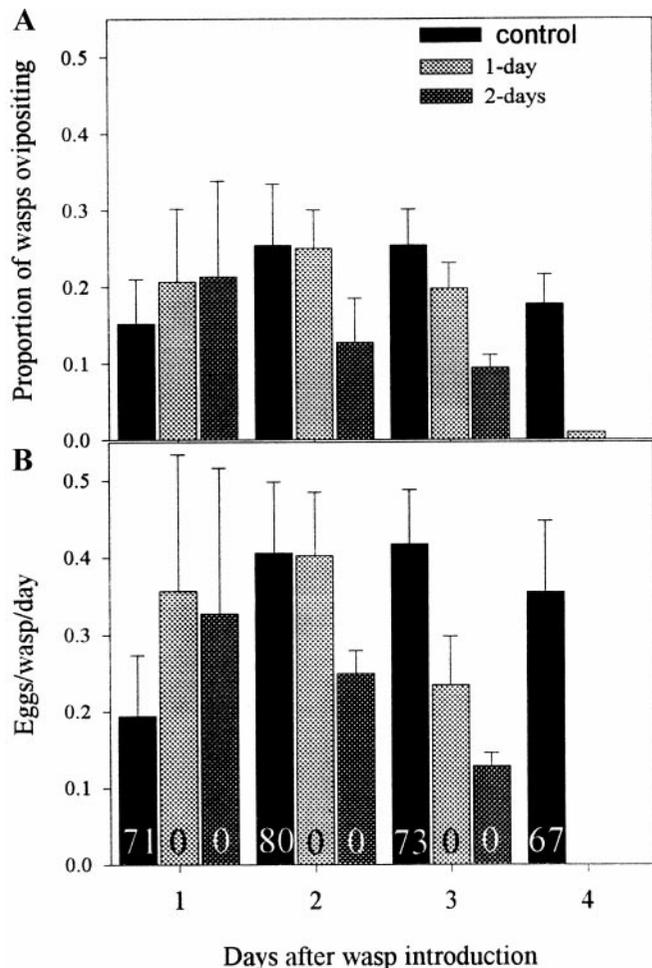


FIG. 1. No-choice oviposition and survival of *Cephalonomia tarsalis* on *Beauveria bassiana*-treated *Oryzaephilus surinamensis* larvae. (A) Cumulative proportion of wasps that deposited eggs. (B) Eggs deposited/wasp/day. The 1-day and 2-days refer to the interval between fungus exposure and exposure of beetle larvae to wasps. Bars are standard errors. The numbers at the bases of the bars are the percentages of the wasp's offspring that survived beyond 2 days postoviposition.

RESULTS

No-Choice Oviposition

Given no oviposition choice, female wasps deposited eggs on *B. bassiana*-infected sawtoothed grain beetle larvae for 3 or more days after the larvae were treated with fungus (Fig. 1). Oviposition was observed until within 1 day of host death and the appearance of the red pigment, oosporein. All of the wasp progeny that were deposited on fungus-infected hosts were dead and shriveled within 2 days of oviposition. Occasional observation of red pigmentation in wasp progeny prior to their deterioration indicated that at least some were killed by mycosis. The proportion of wasps that oviposited on *B. bassiana*-treated larvae was significantly lower than the proportion of wasps that oviposited on

untreated larvae by the 5th day after the fungus was applied, whether the fungus treatments were made 1 or 2 days before the wasps and beetle larvae were placed together. In other words, oviposition on beetles that were treated with fungus 2 days prior to wasp introduction was less than oviposition on control beetles at 3 days after wasp introduction ($F = 12.9$; $df = 2, 8$; $P = 0.003$), and oviposition on beetles that were treated with fungus 1 day prior to wasp introduction was less than oviposition on control beetles 4 days after wasp introduction ($F = 6.9$; $df = 2, 9$; $P = 0.015$). The number of eggs/host larva was significantly lower in both treatment groups of infected larvae at 3 days ($F = 6.9$; $df = 2, 9$; $P = 0.016$) after exposure to wasps, but not at 2 days ($F = 0.3$; $df = 2, 8$; $P = 0.74$) after exposure. On the 4th day after exposure of beetle larvae to wasps, all oviposition had ceased on infected hosts, whereas control oviposition remained.

Choice Oviposition

When the wasps were given a choice between *B. bassiana*-treated and untreated hosts, 23.5% (± 9.2 SD) of individuals oviposited on untreated hosts. Oviposition on *B. bassiana*-treated hosts was 21.5% (± 5.9) for treated hosts that did not show visible signs of mycosis and 18.1% (± 3.2) for hosts that had red pigment within the 24-h oviposition period. The differences were not statistically significant ($F = 1.5$; $df = 2, 15$; $P = 0.25$).

Choice Entry in Fungus-Treated Grain

The proportion of wasps that entered wheat containing 100 or 500 mg of *B. bassiana*/kg of wheat did not differ significantly from the proportion that entered untreated wheat ($F = 0.8$; $df = 2, 12$; $P = 0.46$) (Table 1). Likewise, the proportion of wasps that remained for at least 5 min in wheat that contained fungus did not differ significantly from the proportion that stayed in untreated wheat for at least 5 min ($F = 0.3$; $df = 2, 12$; $P = 0.72$).

TABLE 1

Mortality and Behavioral Responses of *Cephalonomia tarsalis* to *Beauveria bassiana* Conidia Mixed into Wheat

<i>Beauveria</i> concentration (mg/kg)	Percentage of wasps that remained 5 min (SD)	Percentage of wasps that entered (SD)	Percentage mortality (SD)
0	36.1 (8.5)a	31.0 (8.7)a	1.7 (2.5)a
100	29.7 (6.6)a	26.8 (3.1)a	52.7 (6.2)b
500	34.2 (8.8)a	30.5 (11.9)a	68.6 (7.8)c

Note. Behavior and mortality data were taken in separate series of experiments. Means followed by different letters in a column are significantly different (Student–Newman–Keuls, $\alpha = 0.05$).

TABLE 2

Colony-Forming Unit (CFU) Prevalence of *Beauveria bassiana* in Pooled Wheat Samples Taken from 6 States

State	Number of samples	Mean CFUs/g of wheat (SD)
North Carolina	7	31.8 (83.00)
Virginia	8	0.94 (2.65)
Illinois	8	1.25 (1.89)
Indiana	7	5.71 (14.05)
Missouri	8	1.56 (2.29)
Ohio	8	6.56 (9.16)
Total	46	7.50 (32.77)

Note. Each pooled sample comprised wheat taken from delivery terminals in six locations.

Mortality of Wasps Exposed to Fungus

Three-hour exposures to the 100 and 500 mg/kg test concentrations resulted in 52.5 and 68.6% mortality, respectively, contrasting with only 1.7% mortality among control wasps (Table 1). All three mortality responses differed significantly from one another ($F = 71.9$; $df = 2, 6$; $P < 0.0001$).

Fungus Prevalence in Postharvest Wheat

The mean number of colony-forming units (CFU) of *B. bassiana*/g of wheat was 7.5 (Table 2). Of 46 pooled wheat samples, only 1 produced more than 40 CFUs/g of wheat. If an assumption of less than 100 conidia/CFU is made, then the one high sample that produced 220 CFUs/g would have less than 1 mg of *B. bassiana* conidia per kg of grain, and the average sample would have less than 10 μ g of conidia per kg of wheat.

DISCUSSION

C. tarsalis oviposited on beetles with mycosis 4 to 5 days after their exposure to fungus. The decrease in oviposition thereafter can be attributed to the fact that the hosts were dead and desiccated and probably does not reflect detection of the specific disease. By 4 days after fungus exposure, the disease had progressed considerably, and some beetle larvae had died. The wasp did not exhibit the ability to avoid unsuitable hosts. Frequently, parasitoid eggs were present on larvae that had been dead for less than 24 h.

Many *B. bassiana* isolates produce the red dibenzoquinone pigment, oosporein (Vining *et al.*, 1962). Most, if not all, of the *O. surinamensis* larvae that developed *B. bassiana* infections became red near the time of death. In some cases, it was clear that *C. tarsalis* deposited eggs on hosts that had already turned red with fungal pigment even when apparently suitable hosts were available. Whether or not the pigment was present at the time of oviposition, all *C. tarsalis* prog-

eny on *B. bassiana*-infected hosts died within 2 days. Clearly, the wasp's ability to detect and avoid the infection in its host is poor.

More than half of *C. tarsalis* adults exposed to 100 mg of *B. bassiana* conidia/kg of wheat for 3 h succumbed within 8 days. Despite their susceptibility to the fungus, female wasps entered wheat treated with fungus conidia with nearly the same frequency that they did into untreated wheat, even at a concentration of 500 mg/kg, a concentration with visually detectable conidia dust. As is the case with vegetative *B. bassiana* in its host, the female wasps appear unable to detect and avoid lethal concentrations of free *B. bassiana* conidia on the surface of grain, their frequent habitat.

Most of the reported studies of insect-parasitoid-fungus interactions involve fungi that do not attack the parasitoid (e.g., Brobyn *et al.*, 1988; Powell *et al.*, 1986). In such cases, parasitoid-fungus interaction is competitive rather than pathogenic, and reports of antifungal mechanisms are few. One example is the rejection by *Encarsia formosa* Gahan of greenhouse whitefly larvae that had detectable hyphal bodies or mycelium of *Aschersonia aleyrodis* Webber in their hemolymph (Fransen and van Lenteren, 1993). There would be greater selection for antifungal mechanisms directed toward fungi of broad host range, such as *B. bassiana*, that can infect the parasitoid and the host. Jones and Wraight (1996) found that *Eretmocerus* sp. avoided oviposition on *Bemisia argentifolii* Bellows and Perring infected with *B. bassiana*. Production of antifungal compounds by both host and parasitoid has also been reported as a response to the presence of *B. bassiana*. Führer and El-Sufty (1979) reported that a material fungistatic for *B. bassiana* is produced by teratocytes in *Pieris brassicae* Raupen parasitized by *Apanteles glomeratus* L. Willers *et al.* (1982) reported antifungal anal secretions from larvae of an endoparasitoid of Lepidoptera, *Pimpla turionellae* L., that inhibited the growth of *B. bassiana*. Reported antagonism between *B. bassiana* and *Microplitis rufiventris* Kok. attacking *Spodoptera littoralis* (Boisduval) was not explained (El-Maghraby *et al.*, 1988). These associations involve organisms that are likely to encounter one another in nature, and the studies have addressed fungus effects on parasitoid larvae only.

There are surprisingly few data on the effects of *B. bassiana* on adult parasitoids. Direct applications onto adult *Cephalonomia stephanoderis* Betrem resulted in LC₅₀s in the range of 5.3×10^6 to 7.9×10^7 conidia/ml of spray (de la Rosa *et al.*, 1997). Indirect exposures of parasitoids to *B. bassiana* have shown mild effects. In a simulation of field exposure to *B. bassiana*, Geden *et al.* (1995) found less than 50% mortality of *Muscidi-furax raptor* Girault and Sanders from exposure to boards with up to 10^7 conidia/cm². Similarly, de la Rosa *et al.* (2000), working with the bethylid *Prorops nasuta* Waterston, reported only a 7% increase in mortality of

adults and no loss of F1 pupae when *B. bassiana* was applied by the dipping of coffee berries into water containing 3.5×10^8 conidia/ml.

C. tarsalis adult females exposed to 100 mg of *B. bassiana*/kg of wheat for 3 h resulted in 52.7% mortality. The median lethal dose for an 8-day constant exposure of sawtoothed grain beetles to *B. bassiana* GHA isolate in wheat is ca. 100 mg/kg (Lord, 2001). Accordingly, *C. tarsalis* females are at least as susceptible to the fungus as are their beetle hosts. Furthermore, wasp eggs that are deposited on hosts with mycosis do not survive. If *C. tarsalis* were subject to frequent encounter with *B. bassiana* at such high concentrations, there would be considerable selection pressure for development of recognition and avoidance or physiological defense. These natural antifungal mechanisms are lacking.

C. tarsalis has a well-developed ability to discriminate among hosts. Cuticular chemical cues, perceived through antennae, and movements by the host, once contacted, are major host recognition cues used by the parasitoid (Howard *et al.*, 1998). Vision is of little importance, and the pigment associated with *B. bassiana* mycosis is not an effective deterrent to oviposition. *C. tarsalis* apparently has little or no recognition capability for other potential deterrent cues from *B. bassiana*. *O. surinamensis* is the principal, if not the only, natural host for *C. tarsalis* (Powell, 1938). Consequently, stored grain is the wasp's principal habitat, and the prevalence of *B. bassiana* in stored grain may be taken as an index of the frequency of natural encounters between the wasp and the fungus. In 46 wheat samples from six U.S. states, there was an average of 7.5 CFUs/kg. This represents only a few micrograms of *B. bassiana*/kg of wheat and is well below concentrations that would pose a disease threat to insects. It seems reasonable to speculate that *C. tarsalis*'s susceptibility to *B. bassiana* and its inability to detect and avoid both free conidia and mycosis in host larvae are the results of infrequent natural encounters and lack of selection pressure.

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