Dietary Stress Increases the Susceptibility of *Tribolium castaneum* to *Beauveria bassiana*

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ABSTRACT Sanitation being an important component of chemical-free management of stored-products pests, the nutritional stress on insects that results from a clean environment may prove advantageous to the use of microbial controls. Dietary stress by food deprivation or suboptimal diet increased susceptibility of the red flour beetle, *Tribolium castaneum* (Herbst), larvae to *Beauveria bassiana* (Balsamo) Vuillemin. Fungus-related mortality increased linearly with the number of days of food deprivation. Rearing of larvae on a rice meal diet resulted in slower development and greater susceptibility to *B. bassiana* than rearing on whole wheat flour with brewer's yeast. Larvae that were fed for 24 h on flour with *B. bassiana* conidia consumed significantly less and weighed significantly less than those that were fed fungus-free flour. Thus, the presence of *B. bassiana* conidia is itself a cause of dietary stress.

KEY WORDS *Beauveria bassiana*, starvation, stress, diet, *Tribolium castaneum*

It is well established that stress can increase susceptibility to disease and contribute to the success of microbial insect control (Steinhaus 1960). Significantly altered pathogen susceptibility with dietary stress has been observed for many diseases and hosts, most notably viruses and Lepidoptera (Hoover et al. 1998, McVean et al. 2002, Noguchi and Yamaguchi 1984). Salama and Abdel-Razek (1992) reported that larvae of the Indianmeal moth, *Plodia interpunctella* (Hübner), and the Angoumois grain moth, *Sitotroga cerealella* (Olivier), were more susceptible to *Bacillus thuringiensis* Berliner toxin when fed on semisynthetic diet than when fed on whole or crushed maize, *Zea mays* L.

Published reports of the effect of diet on the efficacy of fungal pathogens of insects most often indicate that nutritional stress is significantly potentiating. Colorado potato beetle, *Leptinotarsa decemlineata* (Say), is more susceptible to *Beauveria bassiana* (Balsamo) Vuillemin when stressed with starvation (Furlong and Groden 2003), with a three-fold reduction in ED_{50}. Similarly, rearing *L. decemlineata* on less-preferred host plants resulted in significantly greater mortality of *B. bassiana*-treated larvae (Hare and Andreadis 1983). Donegan and Lighthart (1989) reported that starvation and nutritional stresses slightly but significantly increased the susceptibility of larval but not adult *Chrysoperla carnea* (Stephens) to *B. bassiana.* Boucias et al. (1984) found that for the velvetbean caterpillar, *Anticarsia gemmatalis* Hübner, low-quality diet increased the LC_{50} of *Nomuraea rileyi* (Farlow) Samson as much as 21-fold. Goettel et al. (1993) reported that leafcutter bees (Hymenoptera, Megachilidae) are more susceptible on artificial than natural diet. Contrary findings are not uncommon. For example, 24 h of starvation of adults of the aphid *Therioaphis maculata* (Buckton) resulted in reduced susceptibility to *Zoophthora radicans* (Brefeld) Batko (Milner and Soper 1981). James and Lighthart (1992) found that larval convergent lady beetle, *Hippodamia convergens* Guérin-Méneville, had a 150-fold greater LC_{50} for *Pseudomonas fluorescens* (Trevisan) when fed water only than when fed on aphids. Luz et al. (2003) found that starvation of *Rodnius prolixus* Stål did not affect nymphal susceptibility to *B. bassiana.* Interestingly, Altre and Vandenberg (2001) found that starving the larvae of the diamondback moth, *Plutella xylostella* (L.), increased susceptibility to one isolate of *Isaria fumosorosea* Wize (formerly *Paecilomyces fumosoroseus*) but not to another. Accordingly, assumptions regarding the relationship between dietary stress and susceptibility to fungus warrant testing.

Food deprivation through sanitation is the foundation of sound pest control in grain processing and storage facilities. Accordingly, there are existing practices that induce nutritional stress. Furthermore, the nutritional content of a commodity is a factor in pest development and may affect the ability of a pest to escape or succumb to both chemical and microbial controls.

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The red flour beetle, *Tribolium castaneum* (Herbst), is a major pest of stored grain and grain products, and it is a model organism, being the only beetle for which a published genome is currently available (Tribolium Genome Sequencing Consortium 2007). It is relatively tolerant of chemical controls (Arthur 2008) and among the most *B. bassiana*-tolerant of stored-products pests (Akbar et al. 2004, Lord 2007). The only report of a dietary effect on *T. castaneum* disease susceptibility is that of George (1971), who reported that protein or cholesterol deficiency increased the mortality of beetles that were infected with *Paranosema whitei* (Weiser), a primitive fungus. The objective of the study described here was to determine whether the tolerance to *B. bassiana* can be reduced by the introduction of dietary stress.

Materials and Methods

All of the experiments were conducted with *T. castaneum* from a laboratory colony of Kansas City origin that was started in 2005 from >100 individuals and is much less susceptible to *B. bassiana* than the older *T. castaneum* colony at our laboratory. (Larval mortality was 7% for this strain versus 37% for an inbred strain when exposed to 100 mg conidia/kg diet in an unpublished study.) The *B. bassiana* was commercially produced, unformulated conidia of strain GHA (Laverlam, Butte, MT) that contained 9.4×10^{10} conidia per g. To confirm viability, the conidia were spread on Sabouraud dextrose agar with a cotton swab, incubated for 18 h at 26°C, and examined microscopically for the presence of germ tubes. Germination rates were at least 85% throughout the study.

Food Deprivation. *T. castaneum* larvae were used 14-d postoviposition. Half (160) were placed in 118-ml (4-oz.) glass jars having filter paper in the lids for gas exchange with 20 g of 100 mg/kg *B. bassiana* in whole wheat kernels (11.7% moisture), and half were placed in jars with 20 g of untreated wheat. After 4 h, the larvae were removed and placed in individual 2-cm^3 wells of assay trays (C-D International, Pitman, NJ). Each replicate unit consisted of a 32-well group. Initially, 100 mg of all-purpose wheat flour with 5% brewer's yeast were added to one group of wells with control larvae and one group with treated larvae. At 2, 4, 6, and 8 d, diet was added to one more group each of controls and treated larvae. Counts and removal of dead larvae were done daily from 3 to 10 d after treatment. Red pigment was noted to confirm infection, and frass and exuvia were removed from starved larvae with a vacuum probe. Incubation was at 30°C and 60–75% RH. The experiment was carried out three times (replicates). All experiments were completely random design.

Diet Quality. Beetle larvae were reared to 11 d from oviposition in 118-ml glass jars on 20 g of either rice meal made from unenriched basmati rice or whole wheat flour with 5% brewer's yeast that passed through a 50 mesh sieve. Basmati rice was used because it is the only locally available rice that is not fortified. It is 7% protein and 78% carbohydrate. Whole wheat flour is 14% protein and 72% carbohydrate. For treatment, the larvae were transferred to rice meal or whole wheat flour that was untreated or contained 10% *B. bassiana* conidia and held for 24 h before transfer to untreated diet. After treatment, they were weighed in groups of 20 and placed in 20 g of the diet on which they were reared. Daily, from days 4–8 after treatment, larvae from a jar of each treatment were scored for mortality and held for final recount at the termination of sampling at 14 d posttreatment. Accordingly, all samples were independent except for the final recount. Incubation was at 30°C and 60–75% RH. There were four temporal replicates.

Feeding Response to Fungus. Because the results of diet quality experiment suggested reduced weight gain when the diet contained *B. bassiana* conidia, a test of diet consumption was carried out. Beetle larvae were reared on all-purpose wheat flour for 11 d from oviposition. The larvae were then divided into groups of 20, weighed, and placed in 30-ml plastic cups with ≈250 mg of moisture-adjusted, weighed flour that contained 0, 1, 10, or 100 mg of *B. bassiana* conidia per g. Incubation was limited to 24 h to avoid the confounding effects of infection. The insects and flour were reweighed to determine the amount of diet consumed and weight gain. Initially, there were 13 replicates. Those in which larvae died during the exposure were discarded resulting in 9, 9, 7, and 12 replicates for 0, 1, 10, or 100 mg/g treatments, respectively. Incubation was at 30°C and 55% RH.

Statistics. StatView (SAS Institute, Cary, NC) was used for repeated measures analysis of variance (ANOVA) of the food deprivation data. Other ANOVAs, regression analysis of food deprivation data, and *t*-tests were done with SigmaStat 3.1 (Systat Software, Inc., Point Richmond, CA). Differences among means were detected with Fisher's protected least significant difference (LSD) test. Mortality was adjusted for control mortality according to Abbott (1925).

Results

Food Deprivation. Food deprivation for 4, 6, or 8 d after exposure to *B. bassiana* resulted in significant increases in the mortality of fungus-treated larvae beginning at 8 d posttreatment ($F = 3.67$; df = 4, 10; $P = 0.04$) (Fig. 1). The corrected mortality of fungus-treated larvae was greater for those that were food deprived for 8 d than for all other groups except those that were food deprived for 6 d. There was a significant interaction of days of food deprivation and interval posttreatment ($F = 5.98$, df = 36, $P < 0.01$). Fungus-related mortality at 10 d posttreatment increased linearly with the number of days of food deprivation (mortality = 6.6 + 5.0*days, $r^2 = 0.996$, $P < 0.01$). Mortality among control larvae (those that were starved but not exposed to fungus) exceeded 1.1% only with 8 d of food deprivation, wherein there was a mean of 16.7% (SD = 1.8).

Diet Quality. When beetles were reared in diets of different nutritional value, the adjusted mortality of *B. bassiana*-treated larvae reared in rice meal was signif-

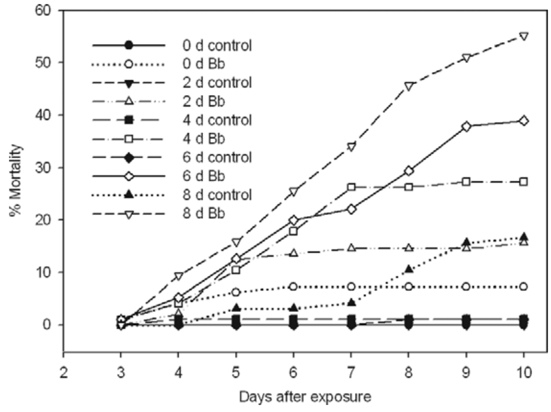

Fig. 1. Mortality of *T. castaneum* larvae after exposure to *B. bassiana* with 0, 2, 4, 6, or 8 d of food deprivation.

icantly greater than those reared in whole wheat flour with yeast beginning at 7 d posttreatment (t = 2.46, df = 6, P = 0.049) (Table 1). There was no control mortality among wheat-reared larvae, whereas the average control mortality of rice-reared larvae was 2.84% (SD = 0.65). The rice-reared larvae weighed significantly less than wheat-reared larvae (F = 7.49; df = 3, 12; P < 0.01) (Table 2). At the final assay scorings 14 d posttreatment, 90.4% of the wheat-fed beetles and only 3.2% of the rice-fed beetles had emerged as adults.

Feeding Response to Fungus. There was significantly less diet consumed (F = 6.44; df = 3, 33; P < 0.01) and weight gain (F = 5.33; df = 3, 33; P < 0.01) when conidia were mixed into the diet at all three tested concentrations (Table 3). There was no significant difference among the concentrations.

Discussion

Dietary stress affects diverse aspects of an insect's physiology and ability to maintain homeostasis. Food deprivation increases the efficacy of chemical insecticides and the physical insecticide diatomaceous earth for *T. castaneum* (Arthur 2000a,b) and could be expected to do likewise with susceptibility to disease.

Table 1. Adjusted mortality of *B. bassiana*-treated *T. castaneum* larvae reared on basmati rice meal or whole wheat flour with brewer's yeast

Days posttreatment	% mortality[a] (SD)	
	Rice meal-reared	Wheat flour-yeast-reared
4	10.1a (5.7)	3.7a (2.5)
5	20.1a (10.9)	9.4a (4.7)
6	28.7a (12.5)	13.3a (5.8)
7	32.3a (12.9)	15.1b (5.4)
8	34.6a (11.8)	16.0b (5.8)
14	46.3a (14.4)	24.0b (10.7)

[a] Means within a row with different letters are significantly different by Student's t-test (P < 0.05). There were four replicates of 20 larvae.

Table 2. Weight (milligrams) of *T. castaneum* larvae reared on whole wheat flour and brewer's yeast or basmati rice meal with or without exposure to *B. bassiana* conidia

	Mean[a] (SD)
Wheat-yeast	0.80a (0.16)
Wheat-yeast and *B. bassiana*	0.72a (0.16)
Rice	0.54b (0.04)
Rice and *B. bassiana*	0.47b (0.03)

[a] Means within a column followed by different letters are significantly different by Fisher's protected LSD (P < 0.05). There were four replicates of 20 larvae.

Among the factors that may contribute to increased fungus effect is extended intermolt period that allows greater contact time for the fungus to germinate and penetrate before being cast off with exuvia. Dietary stress caused lower weights and delayed adult emergence in these assays, indicating prolonged intermolt intervals. In addition, immune compromise including reduced encapsulation and production of antimicrobial peptides may be operative. Siva-Jothy and Thompson (2002) found that short-term nutrient deprivation of the yellow mealworm, *Tenebrio molitor* L., a species that is closely related to *T. castaneum*, caused diminished immune function as measured by phenoloxidase activity and encapsulation of nylon monofilament. In contrast, Rantala et al. (2003) reported that the nutritional condition of male *T. molitor* affected phenoloxidase activity but not encapsulation.

There is great divergence in the effect of various stresses on *B. bassiana* efficacy for *T. castaneum* (Lord 2007, 2009), and there may be overlap in causation. Shakoori et al. (1988) reported that starved *T. castaneum* larvae experienced water loss. Desiccation increases *T. castaneum*'s susceptibility to *B. bassiana* (Lord 2007). Perhaps desiccation stress is a contributing factor in the enhancement of *B. bassiana*'s efficacy for *T. castaneum* with starvation.

Reduced feeding on diet that contained conidia was a somewhat surprising finding from this study. There are numerous reports of avoidance of Hypocreales fungi by insects, especially termites (Baverstock et al. 2010). In general, *M. anisopliae* is more repellent than *B. bassiana.* Mburu et al. (2009) found that >1 g was needed to demonstrate *B. bassiana* repellency for termites in Y-tube tests, in contrast to *M. anisopliae,* which required <0.1 g for the more virulent isolates.

Table 3. Diet consumed per larva and percentage weight change of *T. castaneum* larvae reared on wheat flour with or without *B. bassiana* conidia

Conidia concn (mg/g)	Mean[a] (SD)	
	Diet consumed (mg)	% wt gain
0	0.39a (0.06)	49.1a (7.13)
1	0.29b (0.05)	34.4b (7.65)
10	0.28b (0.11)	34.9b (13.92)
100	0.24b (0.08)	33.9b (9.74)

[a] Means within column followed by different letters are significantly different by Fisher's protected LSD (P < 0.05).

There is only one previous record of *B. bassiana* repellency or antifeedancy for beetles. Ormond (2007) found that the sevenspotted lady beetle *Coccinella septempunctata* L. avoided *B. bassiana* on leaves. The results presented here demonstrate an antifeedant effect that may or may not correspond to repellency, because the insects were immersed in the treatments and unable to avoid contact with the conidia. Regardless, the results of this study have implications for the proposed use of *B. bassiana* in baits (Geden and Steinkraus 2003) and merit further investigation with other insects.

Fungal infections have been linked to feeding reductions (Hajek 1989, Arthurs and Thomas 2000), but the brevity of incubation period would render infection negligible as a factor in the feeding reduction. The conidia would require most of the exposure time for germination even under optimal germination conditions, such as on agar media, and penetration of host cuticle would require additional time. The time to 50% germination for four *B. bassiana* isolates tested by Liu et al. (2003) ranged from 15 to 18 h at 28°C. It is not likely that 24 h would be sufficient to establish infections. It seems that the presence of *B. bassiana* conidia itself can be a nutritional stress factor through feeding deterrence.

The low fungus-induced mortalities in this study are the result of using a fungus-tolerant strain of a fungus-tolerant insect. In addition to its selection as a model insect, this challenging target was selected partly because of its tolerance and apparently robust defense mechanisms and partly because enhancement of fungus activity is more easily detected. Also, because it is a major pest control problem for grain processing facilities, it is a target for which fungal efficacy must be improved if mycoinsecticides are to be used by the managers of those facilities. Biological controls are most likely to be used by managers who are committed to pesticide reduction and are likely to be conscientious about sanitation practices, which impose nutritional stress on pests. Thus, there are practices in place that favor *B. bassiana* efficacy for *T. castaneum.*

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