Enhanced Efficacy of *Beauveria bassiana* for Red Flour Beetle with Reduced Moisture

JEFFREY C. LORD[1]

Grain Marketing and Production Research Center, USDA–ARS, 1515 College Avenue, Manhattan, KS 66502

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ABSTRACT Red flour beetle, *Tribolium castaneum* (Herbst), is a major pest of stored and processed grains that is tolerant of *Beauveria bassiana* (Balsamo) Vuillemin under most conditions. Laboratory assays demonstrated that desiccating conditions improved the efficacy of the fungus. For *T. castaneum* larvae exposed to *B. bassiana* at different vapor pressure deficits (VPDs), the LC_{50} potency ratios were 2.3 for 2.42 kPa and 3.9 for 3.31 kPa compared with 1.06 kPa. There were significant effects of moisture on *B. bassiana*-associated mortality even with brief exposure to low VPD before or at the beginning of exposure to fungus. When *T. castaneum* larvae were held at 3.31 kPa 1 d before exposure to *B. bassiana* and/or in the first 1 or 2 d of exposure to the fungus, there was significantly greater mortality for all of the low moisture treatments than occurred with continuous incubation at 1.06 kPa. *B. bassiana* efficacy for adult *T. castaneum* was significantly better for VPDs of 3.56 or 3.78 than 1.06 kPa. Weight gain over 4 d of incubation with moisture held constant was significantly less at a VDP of 3.31 kPa than at 1.87 or 1.06 kPa. Treatment with fungus did not affect weight gain significantly. There was no significant difference in larval water content among treatments.

KEY WORDS *Beauveria bassiana*, *Tribolium castaneum*, desiccation, humidity, flour beetle

Red flour beetle, *Tribolium castaneum* (Herbst), is perhaps the most problematic beetle pest of stored grain and grain products, and it is particularly troublesome in flour mills. It has a propensity to develop resistance to most widely used insecticides (Beeman 1983, Collins 1998), and there are no microbial insecticides available for this beetle. The most promising candidate for beetle pests of stored grain materials, *Beauveria bassiana* (Balsamo) Vuillemin, does not have commercially adequate efficacy for *T. castaneum* under most test conditions (Padin et al. 1997, Rice and Cogburn 1999, Akbar et al. 2004).

It is widely thought that entomopathogenic fungi are dependent on high ambient moisture for efficacy. Many studies have demonstrated that this is often not the case, and ambient humidity has been found to have little impact on *B. bassiana* efficacy in some insect systems. For example, Ramoska (1984) reported that *B. bassiana* conidia were infective against the chinch bug, *Blissus leucopterus* (Say), at all relative humidities tested, ranging from 30 to 100% RH. Marcandier and Khachatourians (1987) also reported no significant effects of relative humidity on *B. bassiana*-related mortality of the grasshopper *Melanoplus sanguinipes* (F.) in the range from 12 to 100%. The preceding reports and our results differ from others, such as Huafeng et al. (1998) working with the bark beetle *Dendrolimus punctatus* Walker, and Luz and Fargues (1999) working with the kissing bug, *Rhodnius prolixus* Stål, who reported dependence on high humidity for the germination as well as infection by *B. bassiana.* These differences in the effects of relative humidity on *B. bassiana* efficacy may have occurred because of differences in the microclimate on the cuticle or possibly to differences in cuticular chemical composition of different hosts.

We reported previously that efficacy of *B. bassiana* for *Rhyzopertha dominica* (F.) was significantly enhanced by dry conditions (Lord 2005) and that mortality of *T. castaneum* was not significantly different at 56 or 75% RH, but the trend was for higher mortality at the lower relative humidities (Akbar et al. 2004). The objective of this study was to confirm that trend for *T. castaneum* larvae, determine its applicability to adults, and confirm the role of stress in the phenomenon by measuring weight gain effects.

Materials and Methods

Fungus and Insects. All of the experiments were conducted with *T. castaneum* from a laboratory colony that has been maintained on a wheat flour diet since 1958 and has an eastern Kansas origin. The *B. bassiana* was commercially produced, unformulated conidia of strain GHA (Laverlam, Butte, MT) that contained

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[1] Corresponding author-e-mail: lord@gmprc.ksu.edu.

9.4×10^{10}conidia per g. To confirm viability, the conidia were spread on Sabouraud dextrose agar with a cotton swab and incubated for 18 h at 26°C. Germination rates were at least 90% throughout the study. The diluent for conidia was the insect diet of crimped hard red winter wheat, *Triticum aestivm* L.

Bioassays. The humidity for incubation was maintained throughout the assays by the use of plastic storage containers with saturated salt solutions (Winston and Bates 1960, Greenspan 1977). All assays were incubated at 30°C with 3.78-kPa vapor pressure deficit (VPD) corresponding to 11% RH and 5.9 ± 0.1% grain moisture, 3.56-kPa VPD corresponding to16% RH and 6.5 ± 0.1% grain moisture, 3.31-kPa VPD corresponding to 22% RH and 7.9 ± 0.1% grain moisture, 1.87-kPa VPD corresponding to 56% RH and 11.0 ± 0.1% grain moisture, and 1.06-kPa VPD corresponding to 75% RH and 13.2 ± 0.1% grain moisture. All grain was preconditioned to the target moisture. The humidities were confirmed with BK Precision 625 (BK Precision, Yorba Linda, CA) and Vaisala HmI41 (Vaisala Oyj, Helsinki) hygrometers. The final moisture content was measured with a Dickey-John GAC 2000, or, when out of the meter's range, by weight loss after heating to 120°C until weight stabilized. Beetle larvae were used 15 d after collection of eggs from overnight oviposition. Adults were of mixed sexes, and they were used 7–14 d after emergence. Assay vessels were 118-ml (4-oz.) glass jars with filter paper inserted into the lid rims for gas exchange. Fifty grams of crimped wheat was placed in each vessel. All replicates were temporal to avoid pseudoreplication.

Assays to assess the effect of moisture on larval dose–mortality response were carried out with three VPDs, namely, 1.06, 1.87, and 3.31 kPa. The doses were seven two-fold dilutions from 2,400 to 18.75 mg of conidia per kg of grain. Mortality was assessed after 8 d of incubation. The assay was conducted three times with replicates of 30 beetles in glass jars with 50 g of crimped wheat that was equilibrated to the desired moisture.

To test the effect of brief exposure to dry conditions before or at the beginning of exposure to *B. bassiana,* larvae were held at 3.31 kPa VPD for 24 or 48 h and then transferred to 1.06-kPa VPD for the duration of the incubation period. Treated insects were exposed to 200 mg of *B. bassiana* conidia per kg. The 3.31-kPa incubation times relative to the start of fungal exposure were 1) 1 d prior, 2) 1 d prior and first day, 3) first day only, 4) first 2 d, and 5) no 3.31-kPa incubation (1.06 pKa only). All beetles, including those that were not subject to moisture change, were transferred to fresh grain at the time of each change in incubation. Controls were subject to the same transfers and moisture conditions with no *B. bassiana* exposure. Mortality was assessed after 30 d of incubation when the survivors had emerged as adults. There were seven temporal replicates of 30 beetles.

Adults were assayed by 10 d of continuous exposure to a single dose of 500 mg of conidia per kg of crimped wheat that had been equilibrated to 13.2, 7.9, 6.5, or 5.9% moisture, corresponding to VPDs of 1.06, 3.31, 3.56, and 3.87, respectively. In a preliminary test, incubation over desiccant with wheat of 2.2% moisture was lethal to all of the tested adult beetles. There were five temporal replicates of 30 adult beetles.

Table 1. Probit analysis of *B. bassiana* for *T. castaneum* larvae at three humidities ($n = 90$)

VPD	Slope ± SE	LD50 (95% FL)	χ^2
		(mg conidia/kg grain)	
1.06	0.97 ± 0.08	259.6 (173.9–399.7)	56.3
1.87	0.84 ± 0.07	111.6 (61.6–180.2)	62.2
3.31	0.84 ± 0.08	66.4 (34.1–107.2)	55.4

Weight Change. The effect of moisture and *B. bassiana* on larval weight change was measured after 4 d at 1.06, 1.87, and 3.31 kPa VPD with or without 300 mg/kg *B. bassiana* conidia. Larvae were aged 15 d postegging and reared at 75% RH and 30°C. The larvae were weighed at the start and termination of the incubation. They were then dried at 65°C for 4 d and reweighed to determine water content. There were five replicates of 10 larvae in 89-ml plastic cups with 5 g of moisture-equilibrated, crimped wheat.

Statistics. POLO (LeOra Software, Berkeley, CA) was used for probit analysis of dose–mortality data. SigmaStat 3.1 (Systat Software, Inc., Point Richmond, CA) was used for analysis of variance (ANOVA). Differences among means were detected with Fisher least significant difference (LSD) test.

Results

Bioassays. The *T. castaneum* larval dose–mortality response to *B. bassiana* was affected by moisture conditions (Table 1). The dose–response log-probit lines were parallel ($\chi^2 = 4.8$, df = 2, $P = 0.09$) and unequal ($\chi^2 = 50.8$, df = 4, $P < 0.01$). The confidence limits overlapped for 1.06 and 1.97 kPa and for 1.87 and 3.31 kPa but not for 1.06 and 3.31 kPa. The LC_{50} potency ratios compared with VPD of 1.06 kPa were 2.3 (95% CI, 1.6–3.4) for 2.42 kPa and 3.9 (95% CI, 2.6–5.9) for 3.31 kPa. There was no control mortality.

There were significant effects of moisture on *B. bassiana*-associated mortality even with brief exposure to low VPD before or at the beginning of exposure to fungus. When *T. castaneum* larvae were held at 3.31 kPa 1 d before *B. bassiana* exposure and/or in the first 1 or 2 d of exposure to the fungus, all of the low moisture treatments resulted in significantly greater mortality than occurred with continuous incubation at 1.06 kPa ($F = 4.46$; df = 30, 4; $P = 0.006$). The mortality did not significantly differ by Fisher LSD among the low moisture treatments (Fig. 1). There was no mortality in the any of the *B. bassiana*-free controls.

The mortality of *B. bassiana*-treated adult *T. castaneum,* as adjusted with Abbott's formula (Abbott 1925) for mortality of the controls at the corresponding VPDs, also was affected significantly by moisture ($F = 16.8$; df = 3, 16; $P < 0.001$) (Fig. 2). The mean mortality for each VPD differed significantly from all other treatments except that the mortality at 3.56 kPa did not differ from that for 3.31 kPa and the mortality

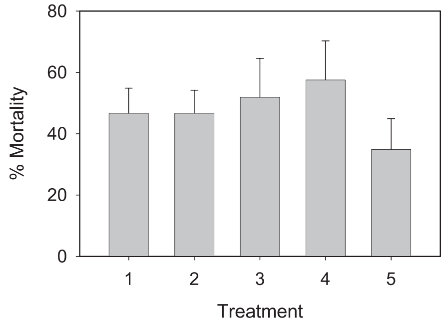

Fig. 1. Mortality of *T. castaneum* larvae with 7 d of exposure to 100 mg/kg *B. bassiana* with various periods of 3.31-kPa VPD incubation before incubation at 1.06 kPa. Treatments: the 3.31-kPa VPD incubation times relative to fungus exposure were 1) 1 d prior, 2) 1 d prior and first day, 3) first day only, 4) first 2 d, and 5) no 3.31-kPa incubation. Bars are SD.

at 3.31 kPa did not differ from that at 1.06 kPa. There was no adult control mortality at the reference VPD of 1.06 kPa, but there was successively greater control mortality of each higher VPD.

Weight Change and Water Content. Weight gain over 4 d of incubation with moisture held constant was significantly less at a VPD of 3.31 kPa than at 1.87 or 1.07 kPa ($F = 9.8$; df = 2, 12; $P < 0.01$). Treatment with fungus did not affect weight gain significantly ($F = 0.5$; df = 1, 12; $P = 0.49$) (Fig. 3). There was no significant interaction of fungal treatment and moisture ($F = 0.1$; df = 2, 12; $P = 0.89$). Larvae that were exposed to *B. bassiana* at 3.31 kPa experienced an average weight loss of 0.04 mg.

There was no significant difference in larval water content among treatments ($F = 1.4$; df = 5, 45; $P = 0.26$). The moisture percentage ranged from 57.1 (SD = 1.1) for 3.31 kPa without *B. bassiana* to 58.1 (SD = 1.2) for 3.31 kPa with *B. bassiana.*

Discussion

Although *B. bassiana* isolates reportedly vary in their water requirement for conidial germination

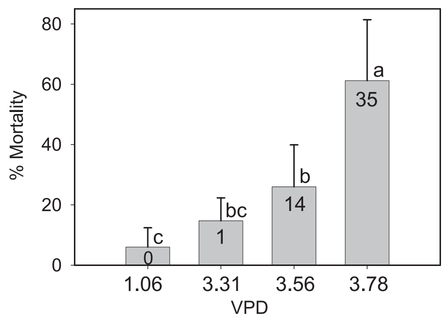

Fig. 2. Mortality of *T. castaneum* adults exposed to 500 mg of *B. bassiana* per kg of grain at four VPDs. Control mortality is listed on the bars. Means with the same number are not significantly different by Fisher LSD. Bars are SD.

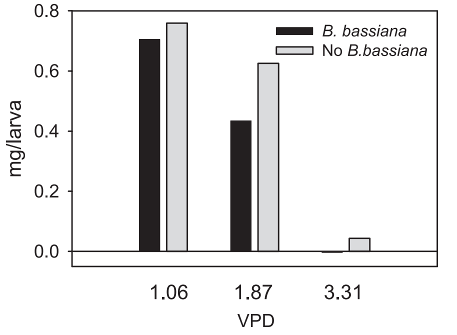

Fig. 3. Weight gain of *T. castaneum* larvae after 4 d of incubation with and without 300 mg/kg *B. bassiana.*

(Devi et al. 2005), like all fungal conidia, they require liquid water or a high relative humidity for the germination. Most isolates have low water content, and hydration is an essential first step in the germination process, which has led to the widely held opinion that *B. bassiana* efficacy is directly moisture related. One benefit of low ambient moisture is enhanced longevity of *B. bassiana* conidia (Hong et al. 1997, Lord 2005). This study implicates insect desiccation stress as another benefit. The involvement of insect stress in enhancement of *B. bassiana* efficacy with desiccation is confirmed by a key observation in this study. There was greater mortality of *T. castaneum* larvae that were exposed to desiccating conditions before, but not during, their exposure to *B. bassiana* than those that were not exposed to desiccation. The phenomenon of greater mortality of *B. bassiana*-treated beetles that are under desiccation stress in combination with the benefit of improved residual activity with dryness indicates that emphasis on moist conditions for the use of the fungi that have dry conidia in insect control is perhaps misguided. Among the many published reports of efficacy of entomopathogenic fungi in dry environments, I have found none other than my previous report on experiments with *R. dominica* (Lord 2005) that implicate desiccation stress as an enhancing factor for the efficacy of an entomopathogenic fungus.

Adult *T. castaneum* Are Highly Tolerant of Both Desiccation and *B. bassiana*. The species classification as tolerant of low relative humidity (Howe 1965) seems well justified, because the VPDs that resulted in enhanced *B. bassiana* efficacy for adults would be lethal to most stored-product beetles. Nevertheless, the control mortality that did occur at low moisture levels was an indication of stress. In our previous work, there was no *B. bassiana* dose–mortality response of *T. castaneum* adults when assays were restricted to a salubrious moisture level of 75% RH, and the mortality did not exceed 10% in spite of doses of up to 2,000 mg of conidia per kg of diet (Akbar et al. 2004). The observation of 61% mortality at a VDP of 3.78 and a dose of 500 mg/kg offers encouragement for improved *B. bassiana* efficacy for red flour beetle adults.

The results presented here provide a partial explanation for the previously observed synergism between diatomaceous earth and *B. bassiana* (Lord 2001, Akbar et al. 2004). Diatomaceous earth is a desiccating agent (Ebeling 1971); thus, it may have some of the same physiological effects as incubating insects in a stressfully dry environment. Joint action in causing water loss is a possible interaction that was explored in this study. Water loss in the tick *Amblyomma americanum* (L.) is reported to result from treatment with *Metarhizium anisopliae* (Metschnikoff) Sorokin (Yoder et al. 2006). There was no indication of such a phenomenon in the results presented here, because the water content of larvae was not affected by either fungal treatment or VPD.

We have been exploring possible means to improve the efficacy of *B. bassiana* for *T. castaneum* and other stored-product insects to make it commercially viable for use in alternative pest control. This study advances that purpose by introducing the benefits of a strategy that might be used to take advantage of existing dry conditions or to include moisture reduction as a component of the strategy. Stored grain materials are especially suited to such a strategy. The moisture conditions of stored-product facilities are suboptimal for most of the insect pests that frequent them. Accordingly, there may be an adventitious boost to fungal efficacy through desiccation stress. Because grains and grain products are the sole sources of water for most stored-product insects, it may be expected that the low dietary water in dry grain would contribute to the desiccation due to high atmospheric VDP.

The results presented here provide a basis for further study of desiccation and other stresses for improving mycoinsecticide efficacy and other insect controls. We want to understand what physiological parameters are changed in response to desiccation and other stresses and how they affect insect susceptibility to *B. bassiana.* With the recent availability of its genome sequence (www.hgsc.bcm.tmc.edu/projects/tribolium/), *T. castaneum* is an excellent model insect for such studies.

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