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Low humidity, moderate temperature, and desiccant dust favor efficacy of *Beauveria bassiana* (Hyphomycetes: Moniliales) for the lesser grain borer, *Rhyzopertha dominica* (Coleoptera: Bruchidae) ☆

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Abstract

Entomopathogenic fungi are often considered to be efficacious only with high ambient moisture. When adult female *Rhyzopertha dominica* were introduced onto wheat kernels with 200 mg/kg of *Beauveria bassiana* and/or 100 mg/kg of diatomaceous earth (DE) and incubated at 26, 30, 32.5, or 34 °C, there was significantly lower emergence of adult progeny at 43% RH than at 75% RH. On grain that was treated with both the fungus and DE, there were 95–97% reductions in adult progeny vs. controls at 43% RH and 22–91% reductions at 75% RH. On grain that was treated with fungus alone, there were 82–90% reductions in progeny adults at 43% RH and only 8–76% reductions at 75% RH. When adult beetles were exposed to *B. bassiana* at 30 °C and RHs of 43, 56, 75, or 85%, the mortality means were not significantly different among humidities, but when the beetles were exposed to *B. bassiana* from egg to adult under the same regimes, the number that survived was lower for *B. bassiana*-treated beetles than for control beetles at 43, 56, or 85% RH, but did not differ significantly at 75% RH. A temperature of 34 °C had a negative impact on efficacy at 75% RH, but not at 43% RH. Preincubation of conidia at 30 °C and 75% RH, but not 43% RH, for 5 days resulted in reduced mortality of adult beetles. The germination rates of *B. bassiana* conidia declined more rapidly at the greater RHs and when mixed with wheat than when unmixed, thereby reducing residual activity. The lower progeny adult production at 43% RH than at 75% RH, suggests that stress contributes to the greater fungus and DE effects at the lower humidity.
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Keywords: *Beauveria bassiana*; *Rhyzopertha dominica*; Diatomaceous earth; Lesser grain borer; Desiccation; Temperature; Stress

1. Introduction

The lesser grain borer, *Rhyzopertha dominica* (F.), is a major cosmopolitan pest of stored wheat. *Beauveria bassiana* (Balsamo) Vuillemin has shown potential for controlling *R. dominica*, especially in combination with desiccant dusts (Lord, 2001). A perception that its efficacy is dependent on environments of high ambient moisture is an impediment to its adoption for stored-product pest management. The validity of that perception is unclear. The reported influence of ambient moisture on *B. bassiana* infectivity for insects varies from no effect (e.g., Ferron, 1977; Marcandier and Khachatourians, 1987) to a direct correlation between moisture and efficacy (e.g., Haraprasad et al., 2001; Ramoska, 1984). Similarly, temperature effects on *B.*

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bassiana's infectivity, development, and survival are key components of its use for insect control and have been the subject of many studies.

Temperature and humidity conditions in the stored grain environment fluctuate within a relatively narrow range when the grain mass is large, but grain storage temperatures often exceed the temperature optima for entomopathogenic fungi. Warm season temperatures in the range of 27–34 °C, which coincide with maximum insect activity, have been recorded in the top meter of farm-stored wheat in Kansas (Hagstrum, 1987). The upper end of that range is near the upper limit for growth of many *B. bassiana* isolates, including the commercial isolate used in this study, GHA (Fargues et al., 1997). Accordingly, a key determinant of *B. bassiana*'s potential for pest management in grain is the temperature limits for efficacy against the primary target pests. Grain moisture is adjusted to compromise between loss of profit from low test weight and loss of quality to fungal and insect infestation. The moisture content of stored wheat in North America is mainly in the range of 10.5–13.5% (Hagstrum, 1987; Reed and Pan, 2000), which corresponds to ca. 45–60% RH at 25 °C in hard red winter wheat.

To address concerns about *B. bassiana*'s efficacy in stored grain under operational conditions and to better develop strategies for its use, laboratory assays were conducted with *R. dominica* under selected temperature and humidity conditions and in combination with insecticidal desiccant dust.

2. Materials and methods

2.1. Insects and treatments

The *R. dominica* were from a colony that has been maintained at the USDA Grain Marketing and Production Research Center, Manhattan, KS, for several years and has eastern Kansas origin. Unformulated *B. bassiana* strain GHA was obtained from Mycotech Corporation, Butte, MT. The stock contained 6.3×10^{10} *B. bassiana* conidia per gram. The germination rate of the stock conidia was determined periodically through completion of the experiments. Conidia were spread on Sabouraud dextrose agar (SDA) and incubated for 18 h at 26 °C, and 200 conidia were scored for the presence of visible germ tubes. The germination rate was at least 93% for all assays. The use rate was 200 mg of conidia/kg of wheat kernels in all assays. The diatomaceous earth (DE) was formulated with 10% silica gel (Protect-It, Hedley Technologies, Blaine, WA). It was used at a rate of 100 mg/kg of wheat. Hard red winter wheat was used throughout and 10% was crimped to facilitate feeding by neonates.

2.2. Rhyzopertha dominica progeny production when exposed to B. bassiana and DE under different temperature and humidity regimes

The initial experiment was a test of temperature and humidity effects on the efficacy of *B. bassiana* and diatomaceous earth individually and in combination. On the second day after adult emergence, female *R. dominica* were selected by exposing ovipositors when applying pressure to the abdomen. Males mate aggressively (Crombie, 1941), and insemination was assumed to be complete. They were held on whole kernel wheat at 30 ± 1 °C and 57 ± 5% RH for 1–2 weeks before use. Each experimental unit consisted of 12 randomly selected females in 118 ml (4 oz) wide-mouth glass jars with 100 g of wheat and filter paper inserted into the lid rims. The wheat was untreated or treated with conidia, DE, or both conidia and DE. Incubation was at 43 ± 1% RH and 26, 30, 32.5, or 34 ± 1 °C, corresponding to vapor pressure deficits of 1.95, 2.41, 2.82, and 2.97 kPa, respectively or at 75 ± 1% RH and 26, 30, 32.5, or 34 ± 1 °C, corresponding to vapor pressure deficits of 0.87, 1.07, 1.11, and 1.32 kPa, respectively. Treatment vessels were placed on grids in 15 × 30 × 50 cm incubation boxes with substratums of saturated K_2CO_3 or NaCl for 43 and 75% RH, respectively (Greenspan, 1977). The grain was conditioned before the assays by incubation for several weeks over the appropriate salt solution until the moisture contents had stabilized within 0.1%. The parental females were removed from the jars after 4 days of incubation. Thus, the data indicate effects on progeny production from oviposition through emergence. The number of progeny that survived to adulthood was scored weekly beginning at 6 weeks post-treatment until emergence ceased. There were four temporal replicates that were set up at intervals of at least 1 week with different *R. dominica* cohorts. Temperature and humidity in the head space of incubation boxes were monitored with HOBO data loggers (Onset, Pocasset, MA).

2.3. Effect of humidity on B. bassiana efficacy for R. dominica larvae and adults

The assay vessels were as described above but with 50 g of wheat kernels whose moisture content was equilibrated over K_2CO_3 for 43% RH, NaBr for 56% RH, NaCl for 75% RH or KCl for 85% RH (Greenspan, 1977). The respective corresponding wheat moisture contents were 10.9–11.1, 11.7–11.9, 14.3, and 16.2–16.4 and vapor pressure deficits were 2.41, 2.33, 1.07, and 0.68 kPa. The wheat moisture contents were measured with Dickey–John GAC 2000 grain moisture meter. For larval assays, 20 eggs, 3 days post-oviposition, were placed in each vessel and scored for adult emergence after 6 weeks, allowing completion under