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Authors: Thompson, Brian M., and Reddy, Gadi V. P.

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# Effect of temperature on two bio-insecticides for the control of confused flour beetle (Coleoptera: Tenebrionidae)

Brian M. Thompson and Gadi V. P. Reddy\*

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## Abstract

Stored product pests account for as much as 10% of food loss worldwide. Insects such as the confused flour beetle, *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae), are major pests of small grain in storage bins and grain elevators in Montana. Cold winters and dry summers have traditionally helped reduce levels of *T. confusum* in storage bins. However, with change in winter temperatures, this pest has become harder to manage using physical measures such as desiccation. The alternative, the chemical insecticide aluminum phosphide, is effective at controlling *T. confusum* in grain stores but is highly toxic to mammals, and recently there has been development of resistance in grain pests. Newly approved biologically based insecticides can provide appreciable levels of control without the drawbacks of aluminum phosphide. In this study, we tested the short-term effectiveness of spinosad (extracts of *Saccharopolyspora spinosa* Mertz and Yao; Actinomycetales: Pseudonocardiales) and *Beauveria bassiana* (Bals.-Criv.) Vuill. (Hypocreales: Cordycipitaceae) (a fungus-based insecticide) for their effectiveness against *T. confusum* under various temperature regimes. Insects exposed to low temperature (8 °C) sustained high rates of mortality (about 80% for the control and both treatments). When held at 16 °C, mortality was low for both biologically based insecticide treatments and the control. At 22 °C, spinosad induced high mortality, whereas at 25 °C, *B. bassiana* induced mortality. Overall, our results indicate that temperature affected the effectiveness of these biologically based insecticides against the confused flour beetle and may be important when considering implementation of control programs in grain stores.

Key Words: *Tribolium confusum*; *Saccharopolyspora spinosa*; *Beauveria bassiana*; climate change; stored product pest

## Resumen

Plagas de productos almacenados representan hasta el 10% de las pérdidas de alimentos en todo el mundo. Los insectos como el escarabajo confundido de la harina, *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae), es una de las principales plagas de granos pequeños en compartimentos de almacenamiento y elevadores de granos en Montana. Los inviernos fríos y veranos secos tradicionalmente han ayudado a reducir los niveles de *T. confusum* en compartimentos de almacenamiento. Sin embargo, con el cambio en las temperaturas invernales, esta plaga se ha vuelto más difícil de manejar utilizando las medidas físicas tales como la desecación. La alternativa, el insecticida químico fosforo de aluminio, es eficaz en el control de *T. confusum* en granos almacenados, pero es altamente tóxico para los mamíferos, y recientemente se ha encontrado el desarrollo de resistencia en las plagas de granos. Insecticidas con base biológica recién aprobados pueden proveer un nivel apreciable de control sin los inconvenientes de fosforo de aluminio. En este estudio, hemos probado la eficacia a corto plazo de spinosad (extractos de *Saccharopolyspora spinosa* Mertz y Yao; Actinomycetales: Pseudonocardiales) y *Beauveria bassiana* (Bals.-Criv.) Vuill. (Hypocreales: Cordycipitaceae) (un insecticida a base de hongos), por su eficacia contra *T. confusum* bajo diferentes regímenes de temperatura. Insectos expuestos a baja temperatura (8 °C) sostuvieron altas tasas de mortalidad (aproximadamente el 80% para el control y ambos tratamientos). Cuando fueron mantenidos a 16 °C, la mortalidad fue baja para ambos tratamientos insecticidas con base biológica y el control. A los 22 °C, spinosad indujo una mortalidad elevada, mientras que a 25 °C, *B. bassiana* indujo la mortalidad. En general, nuestros resultados indican que la temperatura afecta a la eficacia de estos insecticidas con base biológica contra el escarabajo confuso de la harina y puede ser importante cuando se considera la implementación de programas de control en granos almacenados

Palabras Clave: *Tribolium confusum*; *Saccharopolyspora spinosa*; *Beauveria bassiana*; cambio climático; plagas de productos almacenados

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Globally, 10 to 20% of all grain produced is lost due to stored product pests before it reaches the consumer (Phillips & Throne 2010). Climate plays an important role in grain storage as it interacts with the grain and the pests that consume it (Smith 1970; Fields 1992; Vayias et al. 2009). Bacteria, fungi, and insects, the primary pests of stored grain, are highly responsive to changes in climate. Climate affects the growth rate, reproduction, mortality, and geographic distribution of pests (Magan et al. 2003). The interconnectedness of climate and biology has predicted changes in distribution, phenology, and abundance of many animals, and stored grain pests are likewise expected to adapt to their changing surroundings (Estay et al. 2009). Changes in climate

may also alter current and future control strategies for stored grain pests as changes in temperature, rainfall, and crops all adjust to new climate paradigms (Mora et al. 2015).

At high latitudes, seasonally cold ambient air temperatures are conducive to physical control of grain pests through desiccation and freezing (Fields 1992). Cold air is continuously pumped through the grain mass in storage using fans. A continuous supply of cold dry air desiccates and freezes flour beetle larvae and adults of the confused flour beetle, *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae). Heated or cooled air treatments allow rapid alterations of the internal environment of the grain bin, which usually is well buffered from external

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Western Triangle Agricultural Research Center, Montana State University, 9546 Old Shelby Rd., P. O. Box 656, Conrad, Montana 59425, USA

\*Corresponding author; E-mail: reddy@montana.edu

temperature (Smith 1970). Physical control using heated or cooled air is only applied when outside air temperature provides a natural source of conditioned air. Under a warming climate, forced air circulation may lose efficacy, especially at higher latitudes and higher elevations where winter temperatures are expected to moderate over the coming century.

The development of new pesticides that reduce the risk of non-target effects (e.g., development of resistance, residue problems, etc.) is badly needed in grain production (Herrman 1998). Aluminum phosphide is currently the best control measure for almost all stored product species, but is highly toxic to humans and animals, and resistance has developed within *T. confusum* populations (Dieterich et al. 1967; Klimmer 1969; Benhalima et al. 2004; Collins et al. 2005; Pimentel et al. 2008). Alternative strategies for the control of stored grain pests are needed. Biologically based insecticides are an alternative that is gaining traction for their adaptability and safety compared with aluminum phosphide, but the effectiveness of biologically based control measures is reliant on their ability to function under various climate regimes.

Biologically based insecticides are an attractive alternative to physical control and aluminum phosphide insecticide. Spinosad is a bioinsecticide produced from the bacterium *Saccharopolyspora spinosa* Mertz and Yao (Actinomycetales: Pseudonocardiales) that is increasingly used against stored grain pests (Hertlein et al. 2011). It consists of metabolites that are toxic via contact or ingestion (Thompson et al. 2000; Nayak et al. 2005). Once inside the insect body, spinosad excites the nervous system, causing paralysis and eventually death. Spinosad ingested with the stored grain is 5 to 10 times more active than spinosad encountered through surface contact (Spinosad Technical Bulletin 2001).

Entomopathogenic fungi such as *Beauveria bassiana* (Bals.-Criv.) Vuill. (Hypocreales: Cordycipitaceae), in contrast, work primarily on surface contact. Spores of *B. bassiana* attach to the insect cuticle when the insect brushes against the spores (Jaronski 2013). Binding of active sites on the infective spore with the insect cuticle initiates germination and the start of the infection process. Entomopathogenic fungi may germinate in grain storage conditions through favorable microclimates on the insect's body, where moisture levels are high, to grow and infect. Oral, anal, and respiratory orifices are moist microhabitats where fungal pathogens may enter the insect body and initiate infection (Akbar et al. 2004; Mukawa et al. 2011). Although *B. bassiana* often displays host specialization (Zimmermann 1986), it can also be a generalist pathogen capable of infecting many insect species (Wu et al. 2014). *Beauveria bassiana* is reported to be effective for managing both *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) (Stephou et al. 2012; Zamani et al. 2013; Golshan et al. 2014) and *T. confusum* (Wakefield 2006).

The differing nature of these biologically based insecticides for controlling insects at differing temperatures has not been examined. Changes in temperature can affect the reproduction, development, and behavior of insects and their pathogens (Régnière et al. 2012; Krechmer & Foerster 2015). Growth and reproduction of many stored grain pests is optimal at 25 to 33 °C (Fields 1992). We tested the effectiveness of *B. bassiana* and spinosad against *T. confusum*, which is one of the major stored grain pests in the Golden Triangle grain-growing region of Montana, USA, when exposed to various temperatures.

## Materials and Methods

### SOURCE AND REARING OF INSECTS

Adults of *T. confusum* were purchased from Carolina Biological Supply Company, Burlington, North Carolina, and reared on a 1:1 mixture of whole-wheat Durham grain and flour grown and processed at the

Western Triangle Agricultural Research Center, Conrad, Montana. Laboratory cultures were maintained for 1 mo prior to use in experimental trials at which time all life stages were present within the colony. Rearing containers were clear plastic storage containers (30 cm long × 9 cm wide × 5 cm deep) with <1 mm diameter aeration holes in the lid. Colonies were held in environmental chambers (Shel Lab, Cornelius, Oregon) at 20 °C and 50% RH under complete darkness until needed.

### TEST ARENA

Tests were conducted in chambers modified from 60 mL sample vials (SecurTainer II™, Simport Scientific, Quebec, Canada). Container lid centers were removed, and lids were screwed down over the top of sterilized vellum cloth for ventilation. Between tests, all test vials and covers were sterilized with 70% ethanol and UV light for 24 h before use. Each vial received 5.0 g of equal parts wheat flour and whole-wheat seeds identical to the rearing mixture. The test arena volume precluded microclimate variance, and wheat moisture was not measured during the experiment.

### LARVAL RESPONSE TO TEMPERATURE AND INSECTICIDAL ACTIVITY

Because the larvae are the most damaging life stage of *T. confusum*, this stage was chosen for all trials. Larvae in the last instar (9th) were selected from the rearing colony for use in the experiment. The larval age was determined based on the descriptions given in Park (1934). Ninth instar larvae are brown in color, 6.0 mm long, 0.69 mm broad across the head, and weigh 2.4 mg. Late instar larvae are very active feeders (Park 1934). We tested 2 biologically based pesticide treatments, namely, spinosad 80% (Entrust® WP; Dow Agro Sciences, Indianapolis, Indiana) and *B. bassiana* (BotaniGard® 22WP; Laverlam International, Butte, Montana) at 8, 16, 22, and 25 °C. Each temperature used in this study is typical of temperatures experienced in the local grain production area (central Montana) during grain harvest and storage. Temperatures outside this range are known to be detrimental to *T. confusum* survival (Boina et al. 2008).

Each vial with 5 larvae (treatment × temperature combination) was replicated 5 times (for a total of 25 test larvae per pesticide × temperature combination). Biologically based insecticides were mixed with wheat grain and flour prior to adding test larvae. *Beauveria bassiana* was added at the label rate of  $2 \times 10^{13}$  spores per 0.45 kg of grain as dry material, so as not to alter moisture levels in the grain. Spinosad was added at the label rate of 1 part per million (ppm) dry powder. Test vials were held in the dark at 1 of the 4 experimental temperatures and at constant relative humidity of 52% in a Panasonic MLR-352H-PE plant growth chamber. Control insects were held under identical experimental conditions minus the addition of the biologically based insecticides. Larvae were extracted from the wheat-flour mixture once a week to determine mortality or survival. Trials were continued until the larvae had either died or pupated (12 to 45 d depending on temperature treatment).

### RESPONSE OF ADULTS OF *T. CONFUSUM* TO *B. BASSIANA*

Adult flour beetles were held at one temperature (22 °C) in the presence of *B. bassiana* under exposed conditions and in grain. Both assays took place in containers identical to those used for larvae. The grain mixture and *B. bassiana* concentrations were also the same as in the larval experiment. "Exposed" assays consisted of placing adult beetles on sterile filter paper in SecurTainers™ without grain. *Beauveria bassiana* treatments received  $2 \times 10^{13}$  spores per 0.45 kg of grain or, for the exposed containers that had no grain, this same quantity of spores but in vials without grain. The spores were counted under the microscope. Beetles were held in the dark and monitored every other day for mortality. Mortality was assessed by gently squeezing the beetles with forceps to look

for movement. After 28 d, mortality was evaluated for all assessment times. Ten beetles were tested in each replicate container. There were 5 containers for each treatment. Dead beetles were placed in Petri dishes with sterile filter paper moistened with 100  $\mu$ L sterile water, and fungal growth was monitored for the presence of *B. bassiana*.

## STATISTICAL ANALYSES

The percentage of mortality was calculated for each replicate as the number of dead individuals out of the original number placed in each replicate container. Percentages were arcsine transformed before statistical analysis to correct for the assumption of normality and percentage data. Analysis of covariance was used to determine whether or not treatments affected survival across the time period using the aov command in R for the model at each temperature (R Core Team 2013). Post-hoc analysis was conducted with Tukey's HSD test for multiple comparisons. Statistical significance is reported where appropriate at a  $P$ -value  $\leq 0.05$  with the Holm-Bonferroni adjustment for multiple comparisons.

## Results

### LARVAL RESPONSE TO TEMPERATURE AND INSECTICIDAL ACTIVITY

Temperature affected the effects of spinosad and *B. bassiana* on survival of *T. confusum* larvae. Insects exposed to low temperature

(8 °C) sustained high rates of mortality (about 80% for control and both treatments) (Fig. 1). Biological control treatments were not significantly different from the control treatment at any time period during the experiment ( $F = 1.713$ ;  $df = 2$ ;  $P = 0.18$ ). Mortality in the control was statistically equivalent to those with biological control agents throughout the 45 d of treatment. During this time, very few (<10%) larvae reached the pupal stage.

Mortality at the intermediate temperature of 16 °C did not differ significantly between treatments ( $F = 0.658$ ;  $df = 2$ ;  $P = 0.52$ ). Mortality slowly increased at the same rate across treatments over the course of exposure at this temperature (Fig. 1). Most larvae (>60%) entered pupation by the end of 15 d, at which time the study was concluded.

Larvae held at 22 °C experienced a high rate of mortality when exposed to spinosad ( $F = 62.53$ ;  $df = 2$ ;  $P < 0.001$ ). After 14 d, larval mortality on spinosad approached 88% (Fig. 1). Spinosad was significantly different from both *B. bassiana* and the control treatment ( $P < 0.001$ ) as examined using Tukey's HSD test for multiple comparisons. *Beauveria bassiana* and the control were not significantly different by post-hoc Tukey's test. After 14 d, the majority (>80%) of surviving larvae had entered the pupal stage.

At the highest temperature tested (25 °C), mortality was significantly higher in the *B. bassiana* treatment compared with the control or spinosad ( $F = 24.21$ ;  $df = 2$ ;  $P < 0.001$ ) (Fig. 1). The *B. bassiana* treatment resulted in over 80% mortality compared with <10% mortality observed for the control and spinosad treatments. The experiment was allowed to run until all larvae had either died or pupated. More than 80% of surviving larvae pupated in the first 2 wk of this temperature

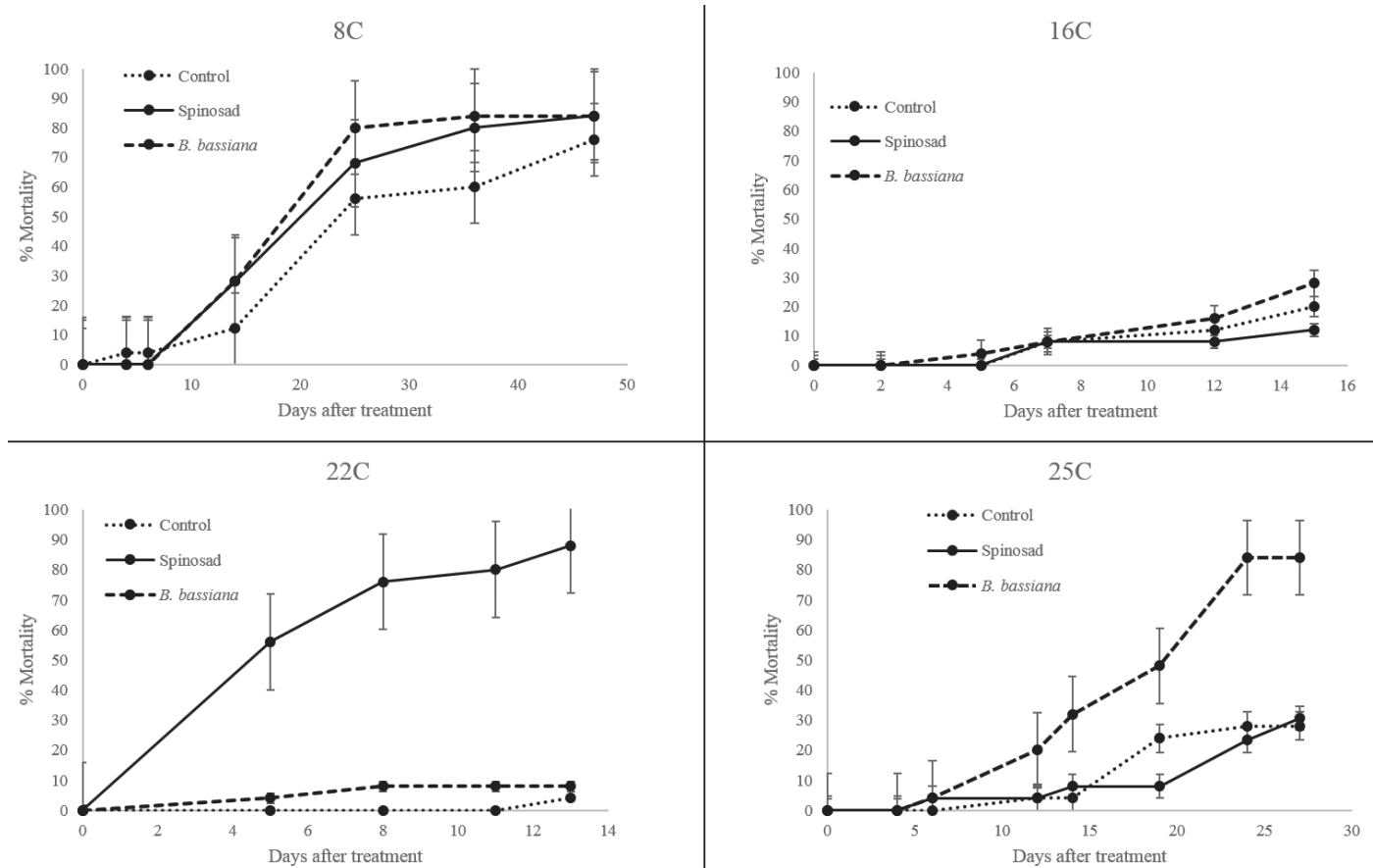


Fig. 1. Mortality of *Tribolium confusum* larvae exposed to *Beauveria bassiana* (large dash), spinosad (solid line), and control (small dash) at 8, 16, 22, and 25 °C. Vertical error bars depict the residual SE of the mean.

treatment. All the dead larvae killed in the *B. bassiana* treatment yielded fungal hyphae indicative of *B. bassiana* when placed in chambers with high humidity to encourage fungal growth.

#### ADULT *T. CONFUSUM* RESPONSE TO *B. BASSIANA*

There were significant differences in adult response (Fig. 2) to *B. bassiana* under exposed and concealed environments ( $F = 5.035$ ;  $df = 3$ ;  $P = 0.002$ ) at 22 °C. Beetles exposed to *B. bassiana* without grain were subject to high mortality ( $84 \pm 8\%$ ), whereas beetles in the treatment that included grain showed similar mortality to the control (about  $30 \pm 10\%$ ) ( $F = 1.312$ ;  $df = 1$ ;  $P = 0.254$ ). All the dead beetles displayed fungal infection.

## Discussion

Forced air circulation may alter pest diversity and population dynamics but may also alter the effectiveness of particular control strategies. Pumping ambient air into grain bins to desiccate pest insects is a standard practice in northern climates. This technique is effective but relies on an inexpensive source of ambient air. In the absence of cold winter temperatures for prolonged periods providing inexpensive cold air, as is predicted with current climate forecasts, insecticide fumigants are the only alternative. Aluminum phosphide is costly and hazardous to use in large operations because of its risk to humans (Gupta &

Ahlawat 1995). Biologically based materials are a potential alternative because of their safety and ease of use. The use of biologically based insecticides would also remove the need to halt operations during grain treatment. However, the level of efficacy of these materials may depend on temperature (Wu et al. 2014).

Mortality rates of *T. confusum* larvae varied significantly with temperature in this study. At the lowest temperature (8 °C), mortality rates were high but similar among treatments, suggesting that mortality was primarily due to the temperature regime. As temperatures rose, insect behavior was possibly an important factor in mortality in the treatments with spinosad or *B. bassiana*. Insects feeding or entering diapause or pupal stages increased or decreased their chances of mortality depending on their exposure risk and the optimal efficacy of the treatments (Rees 1995). Spinosad is more toxic through ingestion than via contact (Athanassiou & Kavallieratos 2014). At higher temperatures, larvae rapidly pupated, and this shortened feeding period may have limited overall activity, resulting in less exposure to the spinosad biological control agent but possibly more susceptibility to *B. bassiana* as temperatures were held at the high end of optimal growth temperature for the beetles (Athanassiou et al. 2010; Wu et al. 2014). Likewise, at colder temperatures, larval activity slowed and mortality was predictably lower at first, despite longer exposure times. At optimal temperatures, insects fed more and ingested more spinosad. This presumably explains the high mortality at 22 °C for the spinosad treatment.

Dry conditions within the grain are not ideal for fungal pathogen growth, but microsites on the insect body have high moisture conducive to spore germination. Spores of *B. bassiana* stick to the cuticle of passing insects before infection. Germination in microclimates until conditions are favorable may explain the higher mortality in the higher range of tested temperatures. Greater stress caused by high temperature has a negative impact on flour beetle immune systems (Mahroof et al. 2003). Immunocompromised beetles are more likely to succumb to fungal pathogens. The results of this study agree with previous results for *B. bassiana* on this and other stored product pests as effective method for control (Vassilakos et al. 2006) and adds the biologically relevant thermal component that affects activity.

Our results suggest that temperature plays a role in biological control of *T. confusum*. Spinosad and *B. bassiana* are promising insecticides for control of stored grain pests such as *T. confusum*, but temperature should not be ignored when implementing either control strategy. However, additional studies are required on the effect of microclimatic factors, relative humidity, etc. Possibly the use of mixtures of biological control agents could result in improved control of *T. confusum* in stored grain because the optimal control by each product was maximized at different temperatures. This study also suggests that with a changing climate, attention needs to be paid to the interaction of temperature with the effectiveness of insecticides against stored grain pests.

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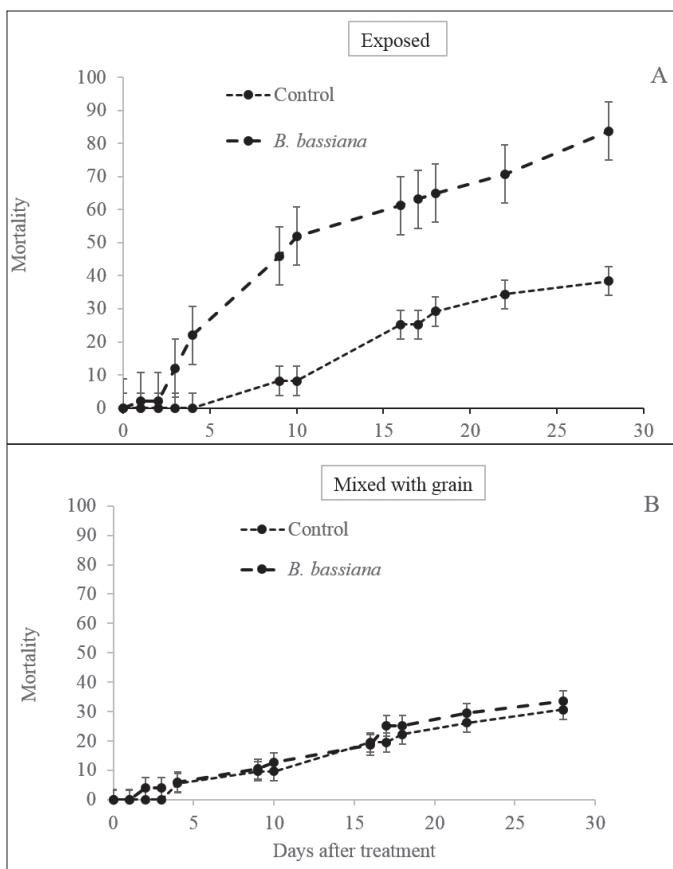


Fig. 2. Mortality of *Tribolium confusum* adults with exposure to *Beauveria bassiana* (large dash line A) on paper and B) in grain compared with controls not exposed (small dash line). Vertical error bars depict the residual SE of the mean.



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