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Development of a Bioassay System for the Predator, *Xylocoris flavipes* (Heteroptera: Anthocoridae), and Its Use in Subchronic Toxicity/Pathogenicity Studies of *Beauveria bassiana* Strain GHA

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ABSTRACT Microbial biocontrol agents are useful commercially only if they do not harm other natural biocontrol organisms, at recommended use rate in the environment where the microorganism is being used. To test the hypothesis that the predatory warehouse pirate bug, *Xylocoris flavipes* (Reuter), is not adversely affected by the entomopathogen, *Beauveria bassiana* (Balsamo) Vuillemin strain GHA, we developed a method using individually confined bugs during a 10-d feeding regime. Three concentrations of a conidial suspension were applied to assay surfaces (filter paper disks) to achieve 2.7×10^7, 2.6×10^6, and 2.6×10^5 conidia per cm^2, representing 100X, 10X, and a field rate of 2.6×10^{13} conidia per ha (±10%), respectively. Fifth instar *X. flavipes* suffered 0% infection at the field rate when confined to treated filter paper for 10 d (16% and 42% infection, respectively, at 10X and 100X the field rate). Second instar migratory grasshoppers, *Melanoplus sanguinipes* (F.), exposed to the same doses suffered 97, 92, and 100% mortality at the three respective doses 10 d after exposure. These data indicate that *B. bassiana* can be used safely at recommended application levels without significant effect on fifth instar populations of *X. flavipes*.

KEY WORDS mycoinsecticide, nontarget organism, biological control, *Melanoplus sanguinipes*, stored product insects, predators

Beauveria bassiana (BALSAMO) Vuillemin is one of three species of fungus with a broad host range that is currently registered for use as insect control agents. An ideal biocontrol agent fungus is one with a restricted host range (Goettel et al. 1990). Because the various isolates of *B. bassiana* have a broad host range of >700 species (Li 1987), it is possible that some isolates of this fungus are infectious to nontarget invertebrates. After isolates of candidate fungi have been shown efficacious against a target insect, it is important to test their safety for nontarget invertebrates. Coccinellid predators tested have shown only slight susceptibility to *B. bassiana* under conditions reflective of field exposure at recommended field rates for target insects (Magalhães et al. 1988, Inglis et al. 1997, Jaronski and Goettel 1997). Some isolates of *B. bassiana*, however, have virulence for both pestiferous and beneficial Heteroptera, for example, Reduviidae, specifically Triatominae (Luz et al. 1998), Rhopalidae (Reinert et al. 1999), and Miridae (Bilewicz-Pawinka and Bajan 1973, Bidochka et al. 1993, Jaronski et al. 1998, Noma and Strickler 1999). Strain GHA of *B. bassiana* is presently being produced on a commercial scale by Emerald BioAgriculture (formerly Mycotech Corp., Butte, MT) and has been registered by the U.S. EPA as a microbial insecticide for use against a variety of Orthoptera, thrips, aphids, whiteflies, and chrysomelid beetles. Strain GHA also has some field efficacy against the diamondback moth, *Plutella xylostella* (L.) (Shelton et al. 1998, Vandenburg et al. 1998). Effectiveness of this strain against *Lygus hesperus* Knight and *Lygus lineolaris* (Palisot de Beauvois) in the laboratory and field, respectively, was demonstrated by Noma and Strickler (1999) and others (Steinkraus 1996, Steinkraus and Tugwell 1997).

The Anthocoridae (Heteroptera) or flowerbugs, are important beneficial insects found throughout the world, in temperate and tropical areas. This group of small insects, usually <4 mm as adults, is predaceous in all instars and as adults. Prey include such pests as aphids (Rakauskas 1984), European corn borer eggs (Reid 1991) and larvae (Isenhour et al. 1989), corn earworm eggs (Reid 1991) and larvae (Isenhour et al. 1989), boll weevils (Wright 1993), psyllids (Herard and Chen 1985, Barrion et al. 1987, Hagen and Dreistadt 1990), whiteflies (Dolling 1991), mites (Heitmans et al. 1986, McCaffrey and Horsburgh 1986a), thrips (Kajita 1986, Saucedo-Gonzales and Reyes-Villanueva 1987), scales (Dolling 1991), and pine bark beetle larvae (Schmitt and Boyer 1983).

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These prey can cause serious economic damage throughout the world by decreasing yield in many crops such as: sorghum (Steward et al. 1991), apples (McCaffrey and Horsburgh 1986b), pears (Vilajeliu 1989), berries (Rakauskas 1984), soybeans (Isenhour and Marston 1981), tea (Carayon 1982), corn (Reid 1991), and cotton (Elov 1984). Potential prey can also cause economic loss in postharvest commodities such as grain (Donnelly and Phillips 2001), dry edible beans (Sing 1997), almonds (Brower and Press 1988, Press 1989), peanuts (Press et al. 1979, Keever et al. 1986, Brower and Mullen 1990) and processed food (Kraszpulski and Davis 1988).

Some anthocorids are presently sold commercially, for example, the insidious flower bug, *Orius insidiosus* (Say), and the minute flower bug *O. tristicolor* (White) (Planet Natural 2001), *O. tristicolor* and *Xylocoris flavipes* (Reuter) (Biofac, Inc. 2001) to control agricultural pests in alfalfa, greenhouses, and stored products. *X. flavipes* has been approved for application on stored grain in the U.S. (Anon. 1992). *Montantoniola moraguezi* (Puton) has been introduced from the Philippines and Hawaii to control thrips in California (Henry 1988). *Tetraphleps abdulghani* Ghauri and *T. raoi* Ghauri have been introduced from India and Pakistan into British Columbia and Nova Scotia without apparent establishment to control aphids (Henry 1988). Some anthocorid species are cosmopolitan because of their relationship with postharvest commodities (Miller 1971, LeCato and Davis 1973, Dunkel and Ivie 1994, Yao et al. 1998).

The anthocorid we selected for our study is the warehouse pirate bug, *X. flavipes.* This pirate bug is cosmopolitan in distribution, and found wherever storage insects are located, in prairie areas, agricultural areas, and in wooded areas. The specific microhabitat typical for this anthocorid is decaying vegetation and rotting seeds. Although four of the five studies of the impact of conventional residual pesticides (Press et al. 1978, Niemycczyk 1973, Parker and Nilakhe 1990, Baker and Arbogast 1995) and fumigants (Press and Flaherty 1978) on anthocorids used *X. flavipes,* no studies have reported virulence of entomopathogenic fungi to any anthocorid, including *X. flavipes.* These chemical pesticide studies also used grouped predators, without access to prey, over a 4–5 d period. Cannibalism (up to 100%) is well known in *X. flavipes* (Arbogast 1979). Therefore, the test animals in previous studies were subject to cannibalism or starvation during the reported bioassays. Evaluating entomopathogenic fungi, however, requires a prolonged observation period, typically 7–10 d, because of the latent and patent periods of these fungi.

We designed a bioassay procedure that provided isolation of individual insects and optimum prey availability during a 10-d observation period. Using this new bioassay design, we hypothesized that the Anthocoridae, specifically *X. flavipes,* are not significantly adversely affected by *B. bassiana* Mycotech strain GHA (hereafter referred to as Strain GHA) at the recommended field rate (Inglis et al. 1997, Jaronski and Goettel 1997) and one and two orders of magnitude greater (concentrations fatal for the migratory grasshopper, *Melanoplus sanguinipes* [F.]). For the test, we developed a Strain GHA-treated surface that would mimic exposure to a leaf surface over a 10-d period. This bioassay regime is considered subchronic because exposure to the substance (*B. bassiana*) was conducted over an extended period of time (≈20% of the organism's lifetime 56 d [Sing 1997; EPA, personal communication]) and included two developmental stages, the last immature instar and the adult. These studies were used to support registration of this mycoinsecticide.

Materials and Methods

***B. bassiana* Strain GHA.** Technical powder having 1.43×10^{11} viable Strain GHA conidia per g residual culture solids (Mycotech Lot #930210GHA.A) was suspended in 0.05% Tween 80 in deionized water with a Potter-Elvejhem cell homogenizer. This resulted in a stock suspension of 1.77×10^{10} conidia per ml, which was verified by hemocytometer counts. This concentration was within 10% of the target concentration, 1.7×10^{10} conidia per ml.

The entire stock solution was then serially diluted with deionized water to yield suspensions with 1.77×10^9, 1.72×10^8, and 1.76×10^7 conidia per ml (determined by hemocytometer count). These dilutions were applied at the rate of 20 μl to each filter paper disk (1.18 cm in diameter). These inocula yielded 2.7×10^7, 2.6×10^6, and 2.6×10^5 conidia per cm^2, which are 100, 10, and 1X (±10%) a field rate of 2.5×10^{13} conidia per ha, respectively. The recommended U.S. field rate is 2.5×10^{13} conidia per ha, (Inglis et al. 1997, Jaronski and Goettel 1997).

Heat-killed Strain GHA was prepared by autoclaving conidia at 121°C (103.4 kilopoise [KPa] = 15 psi) for 25 min. The nonviability of the resulting conidia was confirmed by plating diluted spore suspensions on Sabouraud Dextrose agar plus 1% yeast extract and Sabouraud Dextrose agar and incubating the plates at 28°C for 16–20 h. The agar surface was then examined by phase contrast microscopy at 400×, and none of a minimum of 400 observed conidia had germinated. The heat-killed Strain GHA was suspended and diluted as described earlier for the normal test substance to prepare the three concentrations of spore suspensions.

Bioassay Chamber for *X. flavipes*. Disks were prepared for the assay from Whatman No. 1 filter paper by a metal punch. Care was taken to handle these disks with sterilized forceps only. The control disks were treated with deionized distilled water. Active Strain GHA, heat-killed Strain GHA, and control solutions were delivered to the disks by a micropipettor after the disks were placed in the bioassay chamber. The bioassay chamber we designed and used for these experiments was a 1 dram glass vial (5 cm long, 1.5-cm diameter) with the filter paper disk placed squarely on the inside bottom of the vial, similar to the design used in previous survivorship studies (Arbogast et al. 1977).

Thirty vials were used per treatment. Each set of vials was placed on two 100-mm diameter glass petri dish (15 vials per dish) and covered with a glass petri dish.

Xylocoris flavipes. A single fifth-instar *X. flavipes* was placed in each vial for a total of 30 insects per treatment. This immature stage was chosen as the test animal because the U.S. EPA prefers subadults for efficacy and nontarget testing (U.S.-EPA 1989). Fifth instar *X. flavipes* were obtained from stock cultures reared in the Stored Product Insect Laboratory, Montana State University. The stock *X. flavipes* colony was maintained on the red flour beetle, *Tribolium castaneum* (Herbst). The original colony of *X. flavipes* was obtained from the USDA Stored Product Insect Laboratory, Savannah, GA, USA. The original colony of *T. castaneum* was collected from corn in St. Paul, MN, USA. The *T. castaneum* were grown on organic wholewheat flour with 10% wt:wt brewer's yeast. In contrast to insect colonies consisting of a single species, *X. flavipes* were not placed on fresh media every month. Fifth instars were removed when needed, approximately every 3–8 wk. Predator-prey colonies were maintained in balance with additions of fresh media for the flour beetles every 6 mo. The mass rearing and the bioassay were conducted under similar environmental conditions 27 ± 1°C and 65 ± 5% R.H. 12:12 (L:D).

After the first 72 h of exposure, each *X. flavipes* was fed daily with a 1–3 mm long *T. castaneum* larva. *T. castaneum* larvae were separated from the media, exuviae, frass, and other debris with a fine hairbrush and moved into a glass petri dish. One larva was dropped into each vial containing a live *X. flavipes.* The behavioral response of *X. flavipes* was assessed every 24 h for 10 d. A *X. flavipes* was considered moribund if it did not ambulate and could not right itself within 20 s after the vial (open end up) was inclined at a 45° angle to the horizontal, yet still moved its legs or antennae. A *X. flavipes* was considered dead if it showed no movement, even after it was disturbed with a metal probe or small paintbrush.

Each dead *X. flavipes* was individually incubated in plastic petri dishes at ≥95% RH and room temperature to confirm the presence of *B. bassiana.* A cotton pad saturated with water was placed in each petri dish containing one or more *X. flavipes* cadavers and the plate was wrapped in plastic to maintain high humidity. Cadavers were incubated 5 d at 27 ± 1°C and observed daily. At 5 d, the frequency of cadavers developing the white sporulating mycelium typical of *B. bassiana* was recorded. The identity of all fungi emerging from cadavers was verified by morphology observed under microscopic examination.

Melanoplus sanguinipes. *M. sanguinipes* were continuously reared in the USDA-ARS Rangeland Insects Laboratory, Bozeman MT. This nondiapausing colony was previously found to be 15 times more susceptible to *B. bassiana* GHA than diapausing, field-derived *M. sanguinipes* (S.T.J., unpublished data). Rearing diet consisted of washed, organically grown romaine lettuce plus wheat bran. Grasshopper exposure chambers consisted of sterile glass petri dishes (9-cm diameter) with bottoms covered by a Whatman No. 1 filter paper. Before introduction of *M. sanguinipes* to the petri dish, filter papers were treated with the *B. bassiana* spore suspension. Each spore suspension was agitated 30 s before 0.9 ml were pipetted onto the paper surface. Treated filter papers were air-dried 35 min in each petri dish, the assay chamber for *M. sanguinipes,* on the lab table. After filters were dry, three 2.5 cm^2 pieces of washed organic romaine lettuce and 1 g of wheat bran were added to each dish. With untreated controls the filter paper was wetted with 0.9 ml deionized water. To prevent cross-contamination during this set up and the subsequent examination and feeding, the laboratory table was washed with 0.26% sodium hypochlorite, and fresh paper towels were laid on it before observations/manipulations with each treatment group. Each treatment also had its own equipment for observation, including separate forceps, and *M. sanguinipes* catching apparatus. Observations were made in the following order: untreated control, heat-treated 1X, heat-treated 10X, heat-treated 100X, active 1X, active 10X, and active 100X. For each treatment there were six replicates each with 5 second-instar *M. sanguinipes.* Bioassays were conducted in an environmental chamber that provided conditions of 28 ± 0.5°C and 68 ± 5% R.H. 12:12(L:D) h. The health of each *M. sanguinipes* was evaluated at the same time each day for 10 d. Health evaluation was based on behavioral observations. Insects were classified normal if they were moving with antennae up; moribund if they were not moving or slightly moving, antennae down, or they were sitting on their legs; dead if they were lying on one side with no movement whatsoever; missing if they were not in the dish; killed if they were dead from the handling process. Cadavers were removed after observations and incubated using the same method as described for *X. flavipes.* Daily, after mortality observations, *M. sanguinipes* were fed three pieces of lettuce equal in quantity to the previous feeding, lettuce from previous feeding was removed, and bran eaten was replaced.

Statistical Analysis. Mortality data were tabulated and summarized as trends in cumulative mortality, corrected for control mortality (Abbott 1925), for each dose of test substance, both active and heat-killed. Statistical analyses of the percent mortality on days 7 and 10 were performed using Fisher Exact Test within COSTAT, version five (Cohort Software, Minneapolis MN). Probit analysis of bioassay data were performed when possible using data from day 5, 7, and 10 of the assay, and the LC_{50}s with associated statistics were calculated for each interval. Probit analyses were performed using PC-Probit [Borland International, Scotts Valley, CA, (Borland International 1987)].

Results

X. flavipes. There was no mortality at recommended field rates of Strain GHA after seven or 10 d, but higher concentrations were detrimental to *X. flavipes.* Fisher Exact Test (Table 1) indicated there was no significant *X. flavipes* mortality at the field rate (2.5×10^{13}

Table 1. Day 7 and day 10 cumulative mortalities and mycosis of the warehouse pirate bug, *X. flavipes*, with results of Fisher's Exact Test comparing control with active or heat-killed *B. bassiana* at three rates of application, 1X (2.7×10^{13} conidia per ha, or 2.6×10^{5} conidia/cm^2), 10X (2.6×10^{6} /cm^2), and 100X (2.6×10^{7} /cm^2)

Days of exposure to *B. bassiana*	Treatment	*n*	Mortality	*P* value	Mycosis as percent of dead
7	Control	30	6.7%	—	0%
	1X Active	30	0%	0.38	—
	10X Active	29	13.7%	0.09	100%
	100X Active	30	26.7%	0.009	100%
	1X Heat-killed	30	6.7%	1.00	0%
	10X Heat-killed	30	0%	1.00	—
	100X Heat-killed	30	0%	0.24	—
10	Control	30	10.0%	—	0%
	1X Active	30	0%	0.12	—
	10X Active	29	20.7%	0.10	100%
	100X Active	30	40.0%	<0.001	100%
	1X Heat-killed	30	0%	1.00	—
	10X Heat-killed	30	0%	1.00	—
	100X Heat-killed	30	10.0%	0.23	0%

conidia/h) of the active preparation of *B. bassiana* (Tables 1 and 2; Fig. 1). All *X. flavipes* molted to the adult stage during the bioassay. There was significant mortality in excess of control mortality by day 7 ($P = 0.009$) and day 10 ($P < 0.001$) with the active *B. bassiana* preparation at 100X the field rate (Tables 1 and 2; Fig. 1). Control mortality was 7% at day 7 and 10% at day 10, whereas the 10X preparation had 14 and 21% mortality, respectively. There was no significant mortality with any of the heat-treated *B. bassiana* on either day 7 or day 10 (Table 1). All surviving anthocorids in controls, active conidia, and heat-killed conidia treatments successfully molted to adults. 100% of *X. flavipes* that were classified moribund in the bioassay procedure died within 48 h of initial classification. Only one individual (heat treated 100X rate) was at any time classified as moribund. This insect died within 48 h of classification and never molted to the adult stage.

B. bassiana was observed to grow from and sporulate on 100% of the cadavers from the active *B. bassiana* treatments. Cadavers from the 10X and 100X rates of heat-killed Strain GHA also had 100% incidence of *B. bassiana*. *B. bassiana* grew and sporulated on two of the three cadavers from the untreated control, but neither of the two cadavers for the 1X heat-killed Strain GHA treatment exhibited *B. bassiana* under identical environmental conditions. There was contamination of the cadavers in the control and heat-killed Strain GHA treatments, but it did not affect the basic validity and integrity of the study. We have since altered our procedure of introducing the prey on a daily basis to prevent contamination of the heat-treated and untreated controls. In subsequent studies incorporating a sterile transfer technique developed by the authors (unpublished data), there was no contamination with *B. bassiana*.

Probit analysis of the day 10 mortality data (Table 2) indicated an estimated LC_{50} of 2.84×10^{15} conidia per ha (155 times the recommended use rate). The estimated LC_{90} was determined to be 8.2×10^{16} conidia per ha or 3,300 times the recommended use rate. [NB: both of these are really extrapolations because they exceed the 100X high dose.]

M. sanguinipes. There was significant mortality in excess of control mortality by day 5 at 100X field rate, day 8 at 1X and 10X field rates, with the active *B. bassiana* preparation (Fig. 2; Table 3). Lack of sufficient dose–response separation on any day of the test precluded acceptable probit analysis. All *M. sanguinipes* that died in the active concentrations had the typical appearance of *B. bassiana* infection, namely the typical emergent mycelium and sporulation, while those dying from heat-treated spores did not. By day 10 the final corrected mortality was 97% at 1X field rate, 92% at 10X field rate, and 100% at 100% recommended field rate of *B. bassiana* strain GHA. Because probit analysis was rejected because of excessive heterogeneity, the LC_{50} values could not be calculated.

Table 2. Probit analyses of cumulative mortality in *X. flavipes* because of exposure to *B. bassiana* for 5, 7, and 10 days after application to the substrate

Days since treatment	*n*	Slope ± SE	Estimates of LC_{50}[1,2]	95% Confidence limits of LC_{50}[2]	Estimates of LC_{90}[2]
5	119	0.72 ± 0.22	1.93×10^{16}	3.45×10^{15} – 1.80×10^{20}	1.19×10^{18}
7	119	0.78 ± 0.68	1.22×10^{16}	2.96×10^{15} – 5.19×10^{18}	5.43×10^{17}
10	119	0.90 ± 0.21	3.83×10^{15}	1.56×10^{15} – 3.21×10^{16}	8.15×10^{16}

[1] Mortalities were first corrected for the respective control mortalities and then subjected to maximum likelihood regression probit analysis using PC-Probit.

[2] Expressed as conidia per hectare where 2.5×10^{13} conidia/ha represent 1X field rate; generated by PC Probit.

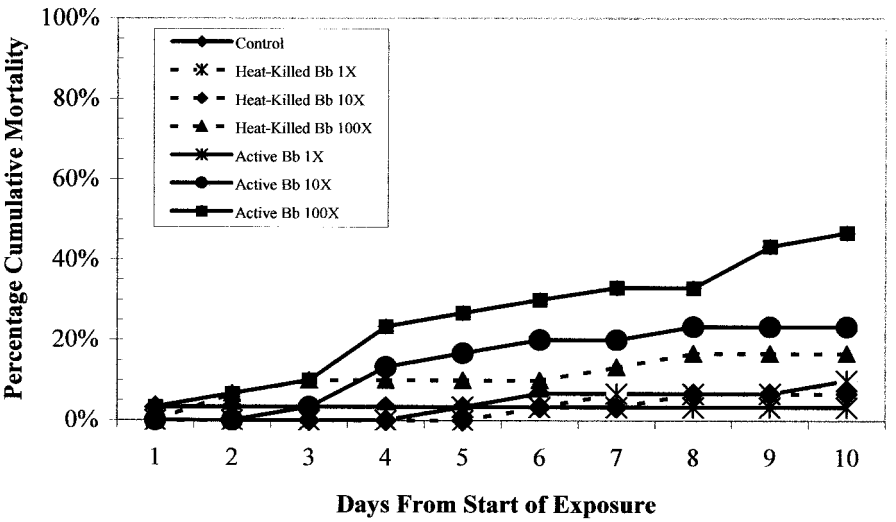

Fig. 1. Trends in cumulative mortality[1] of fifth instars of *X. flavipes* exposed to Strain GHA conidia of *B. bassiana*. 1X = the field rate 1X (2.7×10^{13} conidia per ha, or 2.6×10^5 conidia/cm^2) 10X (2.6×10^6/cm^2) and 100X (2.6×10^7/cm^2).

Discussion

The experimental procedure and bioassay chamber developed provided an adequate analysis of *X. flavipes* confined to a treated surface for 10 d. The assay procedure provided a useful method for assessing morbidity because each individual classified as moribund did not recover, but proceeded to die within a short period. This bioassay design also provided for a test of successful development to the adult stage and a period of exposure of the young adult, also a stage relatively vulnerable to the test substance, conidia of an entomopathogenic fungus.

Anthocorids in general occupy a variety of habitats, including those habitats occupied by grasshoppers, migratory locusts, Mormon crickets, and whiteflies. Areas where a mycoinsecticide might be applied and

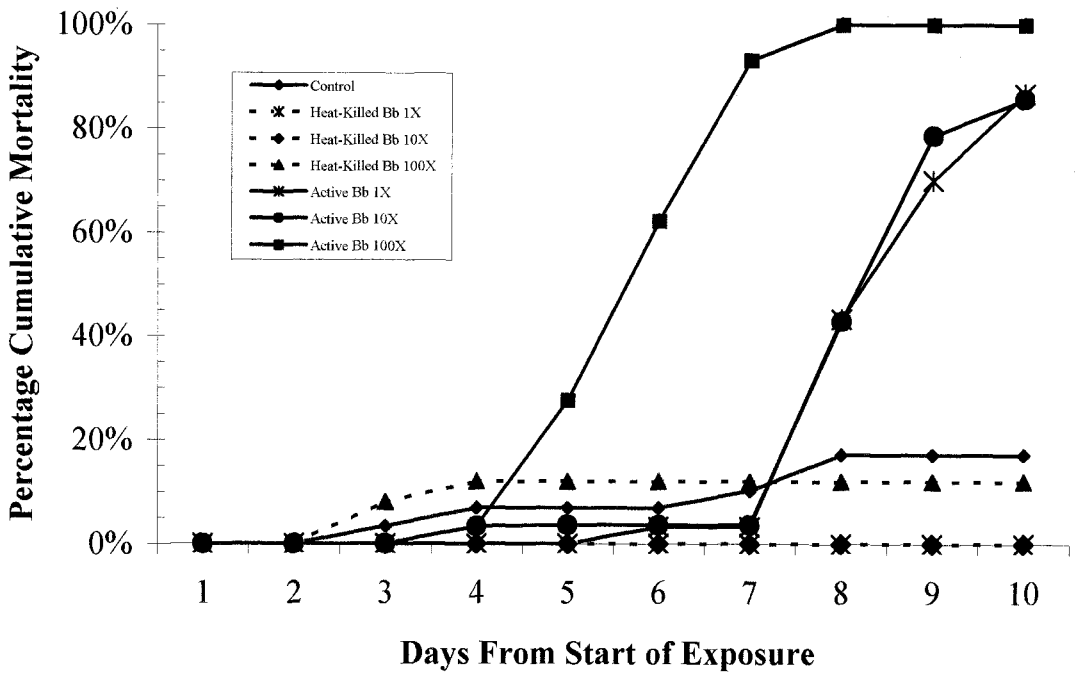

Fig. 2. Trends in cumulative mortality of second instar *M. sanguinipes* exposed to strain GHA conidia of *B. bassiana* under the same conditions as *X. flavipes* 1X = the field rate (2.7×10^{13} conidia per ha, or 2.6×10^5 conidia/cm^2), 10X (2.6×10^6/cm^2) and 100X (2.6×10^7/cm^2).

Table 3. Day 7 and day 10 cumulative mortalities and mycosis of second-instar *M. sanguinipes*, with results of Fisher's Exact Test comparing control and active or heat-killed *B. bassiana* at three rates of application, 1X (2.7×10^{13} conidia per ha, or 2.6×10^{5} conidia/cm^2 10X (2.6×10^{6} /cm^2) and 100X (2.6×10^{7} / cm^2)

Days of exposure	Treatment	*n*	Mortality	Fisher's Exact Test Significance	Mycosis as percent of dead
7	Control	30	10.3%	—	0%
	1X Active	30	10.0%	0.33	100%
	10X Active	28	10.7%	0.33	100%
	100X Active	29	100%	<0.001	100%
	1X Heat-killed	30	0%	0.29	0%
	10X Heat-killed	29	0%	0.16	0%
	100X Heat-killed	29	0%	0.33	0%
10	Control	30	17.9%	—	0%
	1X Active	30	86.7%	<0.001	100%
	10X Active	28	85.7%	<0.001	100%
	100X Active	29	100%	<0.001	100%
	1X Heat-killed	30	0%	0.52	0%
	10X Heat-killed	29	0%	0.80	0
	100X Heat-killed	29	10.3%	0.88	0%

where pirate bugs occur include shrubs, trees, herbaceous vegetation both before and during flowering, and decaying vegetation (Dolling 1991). In the current study, we observed *X. flavipes* was not affected by a rate of *B. bassiana* GHA that represented the manufacturer's recommended rate for this isolate against grasshoppers in the U.S. (2.5×10^{13} spores/ha), a rate that effectively killed *M. sanguinipes* in the bioassay. For an estimated 50% mortality (LC_{50} is only an estimate because the dose–responses did not bracket 50% mortality) among fifth-instar and young adult anthocorids, 155 times the recommended field rate for grasshoppers and crickets would be required. Ten times and 100 times the field rate of *B. bassiana* GHA did cause some *X. flavipes* mortality, especially the latter dose, but these are not concentrations that would be used unless application directions for the product are wildly ignored. Absence of mycosis in cadavers from the heat-killed *B. bassiana* treatments indicate that mortality was not because of infection by the fungus. We, therefore, accepted our hypothesis that at the field rate of application, which effectively suppresses the grasshopper, *M. sanguinipes*, *X. flavipes* is unaffected by strain GHA. Strain GHA is compatible in laboratory assays with populations of *X. flavipes* and probably in field use with other species of predaceous anthocorids.

The actual impact of *B. bassiana* GHA on *X. flavipes* is probably much lower in the field than that observed in this study. Jaronski et al. (1998) observed during a field evaluation of GHA against nontarget insects in cotton that a single spray of 1.5×10^{13} spores/ha had no significant adverse effect on *Orius*, *Nabis*, *Geocoris*, and miscellaneous reduviids (none of these insects were identified to species). Thus, Strain GHA would seem to be compatible with field populations of at least other species of predaceous anthocorids, if not other Heteroptera. The present data resulted from continuous exposure of *X. flavipes* to *B. bassiana.* In the field, short-term, acute exposure would be more likely to occur than the continuous 10-d exposure in the assay system. While a direct spray might reach anthocorids present in the sprayed crop and inflict fatal infection, the mobility of these insects is such that acquisition of spores from sprayed substrate is the more likely route, especially among insects migrating into the treated field from outside areas.

The half-life of *B. bassiana* conidia in the field is short. Müller-Kogler (1965) demonstrated considerable reduction in *B. bassiana* conidial germination after 2 h exposure to natural sunlight. Ignoffo and Garcia (1992) reported an 88–41% reduction of *B. bassiana* germination after 2 h exposure to simulated sunlight. Physical stability studies by Jaronski (1997) indicate the half-life of *B. bassiana* GHA conidia to be 1.5-d exposure to direct, natural sunlight and 5–11 d on the undersides of leaves protected from direct sunlight. Rainfall can further remove *B. bassiana* conidia from foliage, dropping conidial counts 2–3 orders of magnitude during a heavy rainfall (Inglis et al. 1995, 2000). Furthermore, the rate measurements of *B. bassiana* conidia per ha in the study we are here reporting were based on a flat surface and do not reflect the increased surface area created by the three dimensional form of foliage in a hectare of natural habitat.

Interestingly, Steinkraus and Tugwell (1997) reported that an herbivorous heteropteran, *L. lineolaris*, was susceptible to strain GHA in a field trial. There, 91.5% and 84.7% of *L. lineolaris* individuals caged on sprayed canola succumbed to GHA infection when the fungus was applied at 1.2×10^{13} or 5×10^{13} conidia/ha, respectively. Similarly, Noma and Strickler (1999) observed that *L. hesperus* was quite susceptible to GHA at the equivalent of 2.5×10^{13} conidia/ha in laboratory assays. Why there should be such a difference between Miridae and Anthocoridae is not clear. Sosa-Gomez et al. (1997) observed that the pentatomid *Nezara viridula* (L.) is highly resistant to infection by many isolates of *B. bassiana* and *Metarhizium anisopliae* (Metschnikov) Sorokin. This resistance seems to be because of fungistatic compounds among the insect's cuticular lipids. There may be similar mechanisms at work among the Anthocoridae.

Our data imply that *B. bassiana* will be compatible with application of the warehouse pirate bug, *X. flavipes*, to manage insects in stored commodities. Re-

cent studies indicate *B. bassiana* is efficacious versus the rice weevil, *Sitophilus oryzae* (L.) in durum wheat (Padin et al. 2002). Other studies with the same strain that we tested (GHA) also found GHA efficacious for stored grain insects (Lord 2001). *X. flavipes* is an effective predator at both low and high prey densities for insects that feed inside kernels of grain, such as, larvae of the lesser grain borer, *Rhyzopertha dominica* (F.). (Donnelly and Phillips 2001), larvae of several species of bruchids (Sing 1997), and larvae of the Angoumois grain moth, *Sitotroga cerealella* (Oliver) (LeCato and Arbogast 1979). *X. flavipes* is also an effective predator for stored product insect species that do not feed hidden within a grain kernel or legume seed, such as, the red flour beetle, *T. castaneum*, the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.) and the Indian mealmoth, *Plodia interpunctella* (Hübner) (Donnelly and Phillips 2001). The combined efficacy of *X. flavipes* and *B. bassiana* still needs to be tested in a real-scale storage system.

Whereas previous studies of effects of toxic agents on *X. flavipes* did not exceed the established 4-d limit of nymph and adult survival without feeding, our studies provided a method by which this assay time can be extended. An extension of this assay time is particularly important for studies with entomopathogenic fungi that require 5–10 d after exposure for significant mortality. With the modified sterile transfer of prey, this experimental design represents the first reported microscale toxicity test for contact agents developed for Anthocoridae that prevents cannibalism and provides prey over a ten day period. Furthermore, this is a good system for testing other microbial insecticides, especially fungi, versus predaceous Heteroptera.

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