Eur. J. Entomol. **105**: 455–460, 2008
http://www.eje.cz/scripts/viewabstract.php?abstract=1351
ISSN 1210-5759 (print), 1802-8829 (online)

Susceptibility of endemic and exotic North American ladybirds (Coleoptera: Coccinellidae) to endemic fungal entomopathogens

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Key words. Coccinellidae, *Beauveria bassiana*, *Olla v-nigrum*, *Coleomegilla maculata*, *Hippodamia convergens*, *Cycloneda munda*, *Harmonia axyridis*, *Coccinella septempunctata*, entomopathogen, endemic

Abstract. We tested the laboratory susceptibility of four endemic and two exotic species of North American Coccinellidae to a single rate (2.5×10^5 conidia/ml) of *Beauveria bassiana* derived from different sources. The endemic species *Olla v-nigrum*, *Cycloneda munda* and *Hippodamia convergens* were susceptible to *B. bassiana* Isolate B which was originally isolated from *O. v-nigrum* and the rate assayed was known to represent the LC_{50} against *O. v-nigrum.* However, neither the endemic *C. maculata* nor the exotic species *Harmonia axyridis* and *Coccinella septempunctata* were susceptible to this isolate at this rate. Additionally, all species of endemic and exotic Coccinellidae used in these assays were resistant to *B. bassiana* strain GHA and BbAR1 at that rate. We discuss the implications of these results in regard to the establishment of exotic species in new habitats.

INTRODUCTION

A dearth of information exists regarding the relative susceptibility of different species of predaceous Coccinellidae to entomopathogens under natural conditions. What is known about entomopathogens attacking coccinellids generally involves one of four situations: (1) rates of infection by nematodes or protozoa (Ceryngier & Hodek, 1996; Shapiro-Ilan & Cottrell, 2004); (2) mortality of overwintering populations (Iperti, 1966a, b; Ceryngier, 2000); (3) male-killing bacteria (Hurst & Jiggins, 2000); or (4) the effect of entomopathogens on non-target coccinellids (Magalhaes et al., 1988; Giroux et al., 1994; James & Lighthart, 1994; Todorova et al., 1994; James et al., 1995, 1998; Pingel & Lewis, 1996; Poprawski et al., 1998; Cagáň & Uhlík, 1999; Smith & Krischik, 2000; Todorova et al., 2000; Pell & Vandenberg, 2002).

Given that many coccinellid species may occupy the same habitat, both spatially and temporally, it is curious that so little information exists regarding relative susceptibility among species of predaceous Coccinellidae to the same strain or isolate of an entomopathogen. Within the context of infection between species of Coccinellidae, Cottrell & Shapiro-Ilan (2003) reported that field-collected *Olla v-nigrum* (Mulsant) was commonly found infected by *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales) but *Harmonia axyridis* (Pallas) was never found infected by *B. bassiana* nor did any field-collected beetle exhibit mycosis after death. They also showed that *B. bassiana*, from naturally-infected *O. v-nigrum*, was pathogenic to *O. v-nigrum* but not *H. axyridis*. In this instance, *O. v-nigrum* is an endemic species in North America where *H. axyridis* is a newly established exotic species. Lack of adaptation by the pathogen to the newly-arrived *H. axyridis* or higher resistance by this exotic species could have provided protection. Likewise, Shapiro-Ilan & Cottrell (2004) showed that the entomopathogenic nematode *Steinernema carpocapsae* (Weiser) caused significantly less mortality in the exotic *H. axyridis* than in the endemic species *Coleomegilla maculata* (Degeer) or *O. v-nigrum* one day after treatment and also significantly less mortality compared with those same two species and the exotic *Coccinella septempunctata* L. two days after treatment. These examples show that differential susceptibility to entomopathogens exists between coccinellid species that occupy the same habitat and provides insight regarding a species' potential to become established in new territories. In addition to resistance to endemic entomopathogens by invading species contributing to their successful establishment, higher susceptibility of endemic competitors to endemic entomopathogens could further contribute to the establishment of introduced species.

In this study, our primary objective was to determine the differential susceptibility of endemic and exotic species of North American Coccinellidae, known to overlap within the same habitats, to a low dosage of different sources of *B. bassiana*. Here we examine the susceptibility of these species to *B. bassiana* as derived from (1) a naturally-infected *O. v-nigrum* adult; (2) a commercial product and (3) the susceptibility of only the native species to *B. bassiana* from the soil of a pecan orchard.

MATERIAL AND METHODS

Insects

Species used in these studies included four coccinellids endemic to North America (*O. v-nigrum*, *C. maculata*, *Cycloneda munda* (Say) and *Hippodamia convergens* Guérin-Méneville) and two coccinellid species that have been introduced into North America (*H. axyridis* and *C. septempunctata*) (Gordon, 1985). The laboratory colonies of *O. v-nigrum* and *H. axyridis* originated from adult beetles collected from pecan

orchards at the USDA, Agricultural Research Service, Southeastern Fruit & Tree Nut Research Laboratory at Byron, GA, USA. The *C. maculata* colony originated from overwintering adult beetles collected near Lexington, KY, USA. The *C. munda* colony originated from adults collected near Bonnieville, KY, USA. The *H. convergens* colony originated from adults purchased from Gardens Alive!® (Lawrenceburg, IN). Adult *C. septempunctata* were field collected at the USDA, Agricultural Research Service, Southeastern Fruit & Tree Nut Research Laboratory at Byron, GA, USA for use in assays. Field-collected adults of laboratory-reared species, except for *H. convergens*, from the USDA laboratory at Byron, GA, were added intermittently to the colonies. Each species was housed in 9-cm-diameter petri dishes in an environmental chamber at 25 ± 1°C and a photoperiod of 14L : 10D. Except for *C. septempunctata* which was field-collected, all lady beetle species were maintained on frozen *Ephestia kuehniella* Keller (Lepidoptera: Pyralidae) eggs, supplemented with a beef-based diet (Beneficial Insectary, Redding CA, USA) and water was provided with a moistened cotton dental wick (Cottrell, 2005). Adult beetles used in assays were from 10–20 days old, except field-collected *C. septempunctata* adults where age was not known.

Fungus

Beauveria bassiana used in this study was obtained from three sources: (1) the GHA strain of *B. bassiana* (derived from the commercial product Mycotrol® ES [Mycotech, Butte, MT]); (2) soil from a pecan orchard near West Helena, AR, USA, (hereafter referred as the "BbAR1" isolate) and (3) Isolate "B" (as used by Cottrell & Shapiro-Ilan, 2003) originally recovered from a naturally-infected *O. v-nigrum* adult in a pecan orchard in Byron, GA, USA. To obtain conidia for these assays, all isolates were cultured on SDAY agar for 14 d at 25 ± 1°C (Goettel & Inglis, 1997) and used immediately or stored at 4°C for 3–4 days. Conidia were harvested by scraping the surface of agar plates and suspending spores in 8 ml sterile, dH_2O using a vortex. For each assay, a hemacytometer was used to determine the concentration of conidia in the mixture and subsequent dilutions were made from this mixture (Goettel & Inglis, 1997). A surfactant was not used when suspending *B. bassiana* conidia in water because Goettel & Inglis (1997) state that surfactants may interfere with propagule adherence to the insect. Although hydrophobicity of aerial conidia can cause difficulty when suspending *B. bassiana* conidia (stored as dry technical powders) in water (Wraight et al., 2001), conidia used in this study were scraped directly from agar plates for preparation and thorough mechanical agitation was maintained through the assay procedure. Additionally, any *B. bassiana* treatment applied to each species was from the same original mixture of conidia. Therefore all species were treated with the same rate of conidia (2.5×10^5 conidia/ml).

Bioassay

Procedures for conducting the bioassay were done as presented by Cottrell & Shapiro-Ilan (2003). The day before an assay began, beetles were fed ad libitum. The day of the experiment, beetles were placed singly into glass culture tubes (12 × 75 mm), tubes were capped with parafilm (to prevent escape) and placed in a refrigerator at 6 ± 2°C for approximately 30 min to decrease beetle activity for application of treatments. All bioassays were done with adult beetles using a modified dip method. Beetles were removed from the refrigerator and 1 ml of treatment, from a continuously stirred source, was pipetted into the glass culture tube for 5 s. The application procedure consisted of a tube being held in one hand and the other hand used to apply the treatment and then agitate by tapping the bottom of the tube with a finger. This agitation insured that the beetle was entirely immersed in the treatment. After 5 s, the treatment was pipetted out of the tube, discarded and the beetle gently tapped out of the tube onto a sterile paper towel that absorbed any droplets remaining on the beetle. The beetle was then transferred into a sterile 9-cm-diameter petri dish (1 beetle/dish) provisioned with a moistened cotton dental wick. Petri dishes (1 beetle/dish) with beetles receiving the same treatment were grouped together. Groups of beetles receiving the same treatment were grouped on trays and placed, according to the experimental design, in an environmental chamber at 25°C and 14L : 10D and near 88% relative humidity. Beetles were checked daily for mortality over the next 9 days. Any beetle found dead remained in the petri dish and was examined daily for visual signs of mycosis (Goettel & Inglis, 1997). These dead beetles were observed until visual signs of mycosis appeared or for 14 days after treatments were applied.

TABLE 1. Coccinellid species and *B. bassiana* isolates/strains, and their sources, assayed. Each *B. bassiana* isolate/strain was tested against each coccinellid species along with a water control treatment in each experiment.

Experiment	Coccinellid spp.	*B. bassiana* Isolates/Strains	Source
1	*O. v-nigrum* *C. munda* *C. maculata* *H. convergens*	Isolate B GHA	*O. v-nigrum* Mycotrol® ES
2	*O. v-nigrum* *C. munda* *C. maculata* *H. convergens*	Isolate B GHA BbAR1	*O. v-nigrum* Mycotrol® ES Arkansas pecan orchard
3	*O. v-nigrum* *C. munda* *C. maculata* *H. convergens* *H. axyridis* *C. septempunctata*	Isolate B GHA	*O. v-nigrum* Mycotrol® ES

Susceptibility of coccinellids to *B. bassiana*

Three experiments assaying the susceptibility of coccinellids to *B. bassiana* were done (Table 1) and each experiment included a water-only control treatment. Briefly, the first experiment tested two sources of *B. bassiana* (Isolate B and GHA) against the four endemic North American species, the second experiment consisted of two separate identical trials done concurrently and tested three sources of *B. bassiana* (Isolate B, GHA and BbAR1) against the four endemic North American species and the third experiment tested *B. bassiana* from the two sources used in the first experiment against the four endemic and the two exotic species, *H. axyridis* and *C. septempunctata*.

Beauveria bassiana (Isolates B, BbAR1 and strain GHA) was assayed at a rate of 2.5×10^5 conidia per ml. Beetles used in experimental assays were fed *E. kuhniella* eggs every other day starting 24 h after treatments were applied. We used a randomized complete block design and blocked by shelf within the environmental chamber. Treatments within blocks were randomly assigned to positions. Assays used from 3 to 4 treatments per species and 4 blocks. Within each block, eight insects (n = 32) were used in experiment 2 and ten insects (n = 40) were used in experiments 1 and 3. Cumulative percentage mortality for each treatment was calculated.

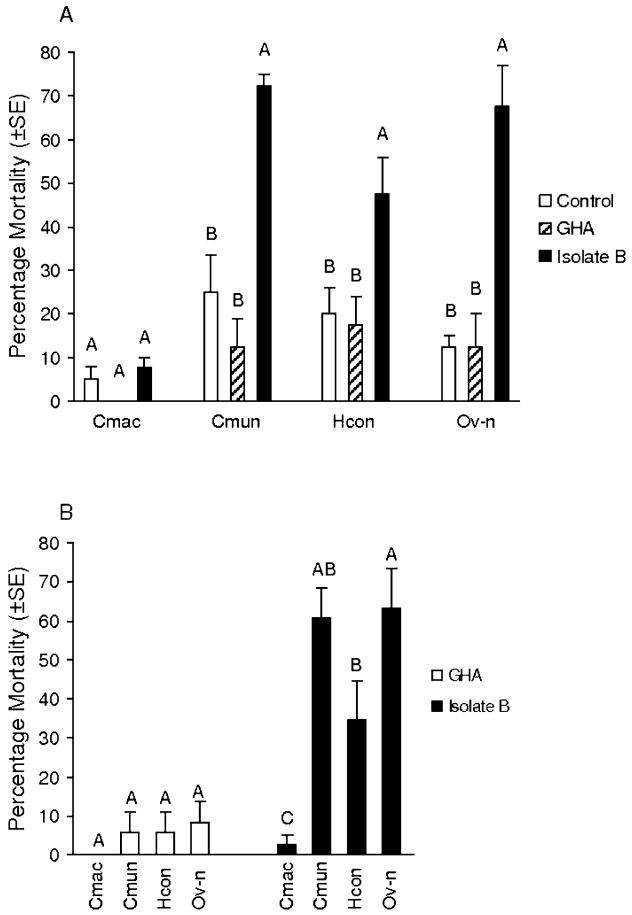

Fig. 1. A – percentage mortality (± SEM), by species, nine days after adult coccinellids were treated with *Beauveria bassiana* (derived from a naturally-infected *Olla v-nigrum* adult [Isolate B] and from a commercial product [GHA strain]) and a water control. B – percentage mortality of those same adult coccinellids, by fungus treatment, after correcting for control mortality. Within each coccinellid species (A), or within each fungus treatment (B), an unlike letter above each column indicates a significant treatment effect ($P < 0.05$). Ov-n – *O. v-nigrum*; Cmac – *C. maculata*; Cmun – *C. munda*; Hcon – *H. convergens*.

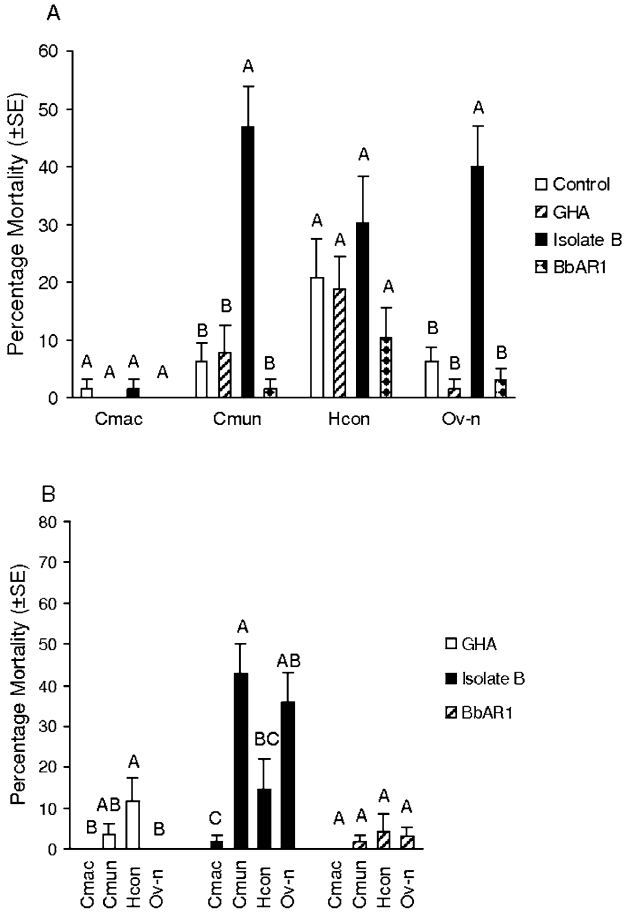

Fig. 2. A – percentage mortality (± SEM), by species, nine days after adult coccinellids were treated with *Beauveria bassiana* (derived from a naturally-infected *Olla v-nigrum* adult [Isolate B], soil from an Arkansas pecan orchard [BbAR1], and from a commercial product [GHA strain]). B – percentage mortality of those same adult coccinellids, by fungus treatment, after correcting for control mortality. Within each coccinellid species (A), or within each fungus treatment (B), an unlike letter above each column indicates a significant treatment effect ($P < 0.05$). Ov-n – *O. v-nigrum*; Cmac – *C. maculata*; Cmun – *C. munda*; Hcon – *H. convergens*.

Data analysis

Due to a lack of independence, main effects (i.e., insect species and fungus treatment) in the factorial experiment addressing susceptibility were analyzed separately through one-way analysis of variance (ANOVA) (Cochran & Cox 1957; JMP, 2002). For each experiment, the fungal treatment effect was analyzed within each insect species by comparing mortality observed in the *B. bassiana* treatments to each other and in the control. The insect species effect was analyzed for each *B. bassiana* source; in this case, however, to avoid potential bias caused by unequal control mortality, Abbott's formula (Abbott, 1925) was applied to the data before analysis. When a significant treatment effect was detected (i.e., $P < 0.05$), mean separation was accomplished using the Tukey-Kramer Honestly Significant Difference (HSD) test (JMP, 2002). Following death, beetles remained in the petri dish and the cotton wick continued to be moistened every 48 h. Daily observations of dead beetles for external mycelia were continued until 14 days after treatment.

RESULTS

In experiment one, the interaction of the main effects insect species × fungus strain was significant ($F = 5.11$; df = 6, 33; $P < 0.001$). Susceptibility varied significantly among *B. bassiana* sources within the insect species *C. munda*, *H. convergens* and *O. v-nigrum* ($F = 21.54$; df = 2, 6; $P < 0.05$, $F = 10.23$; df = 2, 6; $P < 0.05$ and $F = 15.13$; df = 2, 6; $P < 0.05$, respectively) but not in *C. maculata* ($F = 4.20$; df = 2, 6; $P > 0.05$) (Fig. 1A). Mean separation revealed that the highest mortality was achieved using Isolate B against *C. munda*, *H. convergens* and *O. v-nigrum*; whereas mortality in the GHA treatment was never different from each species' control mortality (Fig. 1A). Additionally, within each fungus treatment, the mortality across insect species was similarly low for the GHA strain ($F = 0.78$; df = 3, 9; $P > 0.05$) but varied significantly for Isolate B ($F = 20.01$; df = 3, 9; $P < 0.05$) (Fig. 1B) by being lowest for *C. macu-*

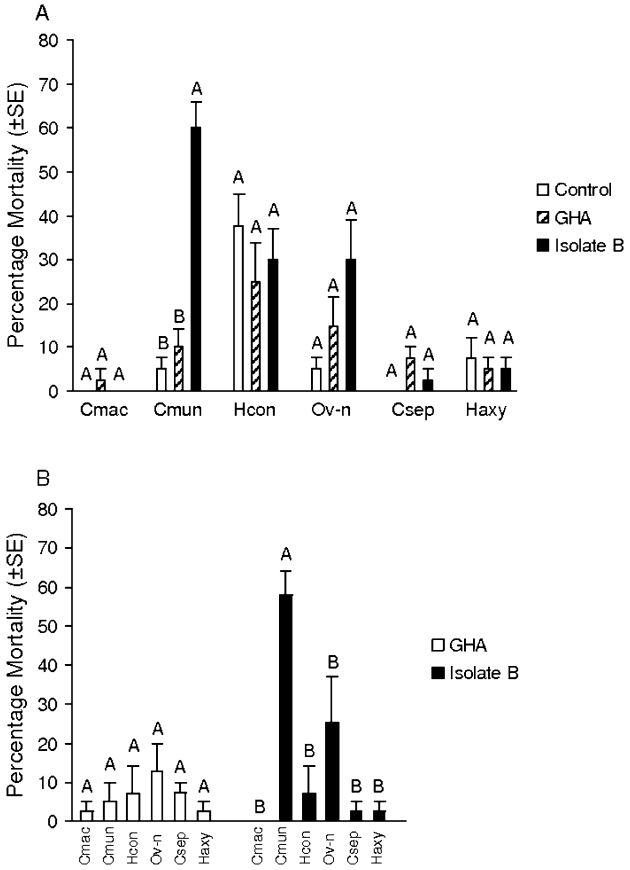

Fig. 3. A – percentage mortality (± SEM), by species, of four native (Ov-n, Cmac, Cmun and Hcon) and two exotic (Csep and Haxy) coccinellids nine days after treatment with *Beauveria bassiana* (derived from a naturally-infected *Olla v-nigrum* adult [Isolate B] and from a commercial product [GHA strain]) and a water control. B – percentage mortality of those same adult coccinellids, by fungus treatment, after correcting for control mortality. Within each coccinellid species (A), or within each fungus treatment (B), an unlike letter above each column indicates a significant treatment effect ($P < 0.05$). Ov-n – *O. v-nigrum*; Cmac – *C. maculata*; Cmun – *C. munda*; Hcon – *H. convergens*; Csep – *C. septempunctata*; Haxy – *H. axyridis*.

lata and similarly high for *O. v-nigrum* and *C. munda*. No mycosis was detected on any insect species receiving the control and GHA treatments for experiment one. However, mycosis was detected on 26% (21 of 82) of dead *C. munda*, *H. convergens* and *O. v-nigrum*, respectively, when treated with Isolate B but never on *C. maculata*.

In experiment two no interaction was detected between the trial and *B. bassiana* treatment effects ($F = 1.90$; df = 3, 111; $P > 0.05$) or trial and insect species effects ($F = 0.13$; df = 3, 111; $P > 0.05$) thus we combined data from these two trials for analyses. In this experiment, susceptibility varied significantly among *B. bassiana* sources within *C. munda* and *O. v-nigrum* ($F = 18.66$; df = 3, 25; $P < 0.05$ and $F = 20.63$; df = 3, 25; $P < 0.05$, respectively) but not in *C. maculata* and *H. convergens* ($F = 0.64$; df = 3, 25; $P > 0.05$ and $F = 1.58$; df = 3, 25; $P > 0.05$, respectively) (Fig. 2A). Within both *C. munda* and *O. v-nigrum*, Isolate B resulted in the highest mortality; whereas, GHA and BbAR1 mortality was similar to control mortality (Fig. 2A). Within each fungus strain, significant differences in mortality across insect species were detected for both GHA and Isolate B ($F = 3.50$; df = 3, 25; $P < 0.05$ and $F = 9.57$; df = 3, 25; $P < 0.05$) but not BbAR1 ($F = 0.57$; df = 3, 25; $P > 0.05$). Through mean separation, GHA strain caused higher mortality against *H. convergens* than against *C. maculata* and *O. v-nigrum* (Fig. 2B). Isolate B resulted in higher mortality of *C. munda* and *O. v-nigrum* than *C. maculata*; *H. convergens* mortality was intermediate (Fig. 2B). No dead beetles from the control or GHA treatments developed mycosis. However, when treated with Isolate B, 76% (48 of 63) of dead *C. munda*, *H. convergens* and *O. v-nigrum*, respectively, developed visible signs of mycosis. The only species to develop mycosis when treated with BbAR1 was *O. v-nigrum* (1 of 2).

In experiment three, where we included the introduced species *C. septempunctata* and *H. axyridis*, susceptibility among the fungus strains only varied significantly for *C. munda* ($F = 66.6$; df = 2, 6; $P < 0.05$). For all other species (i.e., *C. maculata*, *H. convergens*, *O. v-nigrum*, *C. septempunctata* and *H. axyridis*) mortality among fungus strains within each species did not vary significantly ($F = 1.00$; df = 2, 6; $P > 0.05$, $F = 0.74$; df = 2, 6; $P > 0.05$, $F = 3.35$; df = 2, 6; $P > 0.05$, $F = 4.2$; df = 2, 6; $P > 0.05$ and $F = 0.16$; df = 2, 6; $P > 0.05$, respectively) (Fig. 3A). Within fungus strains, mortality across species was similar for GHA ($F = 0.56$; df = 5, 15; $P > 0.05$) but different across species for Isolate B ($F = 11.20$; df = 5, 15; $P < 0.05$). Mean separation revealed that mortality of *C. munda* treated with Isolate B was higher than mortality of any other species treated with Isolate B (Fig. 3B). In experiment three, no dead beetles of any species from the control treatment developed mycosis and only *C. septempunctata* (1 of 3) developed mycosis when treated with GHA. When treated with Isolate B, however, 45% (23 of 51) dead *C. munda*, *H. convergens*, *O. v-nigrum* and *C. septempunctata* developed visible signs of mycosis. No dead *C. maculata* or *H. axyridis* ever developed signs of visible mycosis.

DISCUSSION

Regarding those coccinellid species used in these experiments, the endemic *O. v-nigrum*, *C. munda* and *H. convergens* and the exotic *C. septempunctata* are primarily aphidophagous, whereas the endemic *C. maculata* and exotic *H. axyridis* are highly polyphagous (Gordon, 1985; Hodek & Honěk, 1996). Although their food preferences may differ, all of the endemic and exotic species used in this study can generally be found to overlap spatially and temporally in various habitats (Rhoades, 1996; Colunga-Garcia, 1997; Wells et al., 2001; Nault & Kennedy, 2003; Mizell, 2007). Thus, results from this laboratory study do have implications for the interaction of these species in natural settings.

The *B. bassiana* treatments used in this study were selected based upon: (1) the wide-range of susceptible insects, i.e, GHA; (2) the known susceptibility of an endemic species, i.e, Isolate B; and (3) the unknown viru-

lence of a *B. bassiana* source from a habitat that would encompass all of the endemic coccinellids tested, yet was distant to the area from which Isolate B was collected, i.e., BbAR1. The BbAR1 strain was pathogenic to insects as Shapiro-Ilan et al. (2003) collected this isolate from soil through insect baiting with *Galleria melonella* (L.) (Lepidoptera: Pyralidae).

In this study we have shown that differential susceptibility to the entomopathogen *B. bassiana* exists among species of endemic and exotic Coccinellidae found in North America. Moreover, we have shown that different sources of inoculum result in differential rates of mortality among the endemic beetles. These experiments were done using *B. bassiana* conidia at the LC_{50} rate as determined by Cottrell & Shapiro-Ilan (2003) for Isolate B against *O. v-nigrum*. This rate is quite low compared with rates of conidia used in many studies examining coccinellid mortality via *B. bassiana* (James et al., 1995, 1998; Cagáň & Uhlík, 1999; Rajendran & Gopalan, 1999; Smith & Krischik, 2000) and highlights the increased virulence of this isolate to certain endemic species of North American coccinellids.

Given that the coccinellid species tested here overlap both temporally and spatially, it stands to reason that the likelihood of transmission and successful infection by Isolate B of *B. bassiana*, and possibly other virulent isolates, will be higher among susceptible endemic coccinellid species than successfully-established exotic species. Due to the fact that *B. bassiana* derived from non-coccinellid sources typically required higher rates to achieve mortality (James et al., 1995, 1998; Rajendran & Gopalan, 1999; Cagáň & Uhlík, 1999; Smith & Krischik, 2000), it is likely that the general incidence of *B. bassiana* isolates that are highly virulent to exotic coccinellids, i.e., *H. axyridis* and *C. septempunctata*, will be rather low. This strengthens our argument that successfully-established exotic species benefit from both their innate immunity to endemic pathogens and decreased competition from endemic species that are susceptible to those same endemic pathogens.

In the current study, *B. bassiana* derived from an infected *O. v-nigrum* adult, i.e., Isolate B, consistently resulted in the highest mortality. Mortality of all species treated with GHA and BbAR1 was similar to control mortality. Higher mortality of *O. v-nigrum* and *C. munda* when treated with Isolate B showed that these two endemic species are quite susceptible to this isolate of *B. bassiana*. In stark contrast, the endemic *C. maculata* was resistant to all *B. bassiana* treatments we applied, as were the exotic *C. septempunctata* and *H. axyridis*. *Hippodamia convergens* appeared poorly suited for survival under our rearing methodology and hence was a poor test specimen suffering comparable mortality for the control and *B. bassiana* treatments in two of three experiments. For those endemic beetles that developed visible signs of mycosis after death, the vast majority had been treated with Isolate B, including *H. convergens*. Overall, *C. maculata* averaged < 2% mortality in all treatments and never developed visible signs of mycosis.

Although our assays determined that differential susceptibility to *B. bassiana* exists among the coccinellid species assayed, our data does not provide a reason for this difference. Whether this difference in susceptibility is widespread among coccinellids in North America is beyond the scope of our results. However, it is quite intriguing that two highly successful exotic species demonstrated resistance to all tested sources of *B. bassiana*, whereas only one endemic species did so, i.e., *C. maculata*. Thus, further experimentation assaying *B. bassiana* and other coccinellid species from more sources (e.g., from the new and native ranges of the introduced species) is necessary to gain a holistic understanding of the relation between pathogen resistance and establishment of exotic species. At present, very little is known regarding the susceptibility of exotic coccinellids to diseases in their native range. In keeping with this thought, this current study could be used to infer that the highly-polyphagous and *Beauveria*-resistant *C. maculata* would possibly be a species to successfully establish if introduced to new regions of suitable climate. As such, bioassays could play a key role in predicting which species would be likely to establish regardless of whether the species was intentionally or accidentally introduced.

In agreement with Cottrell & Shapiro-Ilan (2003), we found the exotic *H. axyridis* to be resistant to both the Isolate B and GHA treatments, as was the exotic *C. septempunctata*. Yet more research should be done to address the issue of resistance of exotic species to pathogens from their native regions and thus further explore the enemy release hypothesis as applied to successfully-established, exotic coccinellids. Additionally, we would be remiss not to report that *H. axyridis*, but not *C. septempunctata*, in North America is commonly collected in the field infected by the ectoparasitic fungus *Hesperomyces virescens* (Laboulbeniales: Laboulbeniaceae) (Riddick & Schaefer, 2005; Nalepa & Weir, 2007) as is the endemic *O. v-nigrum* (T. Cottrell, unpubl. data). Of the endemic species tested here, it would appear that the more-susceptible *O. v-nigrum* and *C. munda* would be more likely to face higher levels of interspecific competition with invading competitors such as *H. axyridis*. These endemic species, and possibly others, must confront both the higher rates of infection and interspecific competition (see Cottrell 2004, 2005, 2007) from the invading species, whereas an endemic species such as *C. maculata* is relatively immune to the pathogen.

ACKNOWLEDGMENTS. The authors wish to thank A. Amis, K. Halat, R. Laster & W. Evans (USDA-ARS Southeastern Fruit & Tree Nut Research Laboratory, Byron, GA) for providing technical assistance and two anonymous reviewers for critically reviewing and improving this manuscript.

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Received September 18, 2007; revised and accepted December 12, 2007