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Susceptibility of a native and an exotic lady beetle (Coleoptera: Coccinellidae) to *Beauveria bassiana*

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Abstract

The exotic multicolored Asian lady beetle, *Harmonia axyridis*, became established and recently spread across much of North America and southern Canada. In a habitat now used by both the invading *H. axyridis* and a native lady beetle, *Olla v-nigrum*, we discovered that the native lady beetle was commonly infected by *Beauveria bassiana*; whereas, the exotic *H. axyridis*, was not. Laboratory assays revealed that *B. bassiana* isolates collected from naturally infected *O. v-nigrum* were pathogenic to adult *O. v-nigrum* but not to adult *H. axyridis*. In contrast, the GHA strain of *B. bassiana* was not significantly pathogenic to *O. v-nigrum* nor *H. axyridis*. Late-season field collections revealed significantly higher *B. bassiana* infection of *O. v-nigrum* than *H. axyridis*. Our results lead us to hypothesize that low susceptibility of *H. axyridis* to *B. bassiana* (found to infect *O. v-nigrum*) may provide an intraguild advantage to *H. axyridis* over *O. v-nigrum*; this may also occur with other species of native lady beetles and other endemic entomopathogens in different habitats and regions.
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1. Introduction

The multicolored Asian lady beetle, *Harmonia axyridis* (Coleoptera: Coccinellidae), was recently established within the US. Intentional releases of this species were first documented in 1916 with multiple introductions, both accidental and intentional, occurring at multiple sites in the US through the 1980s (Gordon, 1985; Tedders and Schaefer, 1994). It now occurs throughout much of the US (Chapin and Brou, 1991; Colunga-Garcia and Gage, 1998; Cottrell and Yeargan, 1999; Dreistadt et al., 1995; Hesler et al., 2001; Krafsur et al., 1997; LaMana and Miller, 1996; Michaud, 2002; Tedders and Schaefer, 1994) and southern Canada (Coderre et al., 1995). *Harmonia axyridis* is highly polyphagous and survives in a variety of habitats ranging from coniferous and deciduous forests to plantings of annual row crops (Colunga-Garcia and Gage, 1998; Tedders and Schaefer, 1994; Wallace and Hain, 2000). In fact, this exotic species has become so abundant that it is now one of the predominant lady beetle species in various habitats such as pecan orchards (TEC, unpublished data), apple orchards (Brown and Miller, 1998), citrus groves (Michaud, 2002), tobacco (Wells and McPherson, 1999), and sweet corn (Cottrell and Yeargan, 1999). Ironically, *H. axyridis* adults seeking to overwinter within home interiors can reach such high numbers that they are regarded as pests in these situations (Coderre et al., 1995; Nalepa et al., 1996). Brown and Miller (1998) first documented *H. axyridis* in West Virginia in 1994 and by 1996 it represented 89% of all Coccinellini collected in apple orchards.

Traits that suggest why *H. axyridis* has apparently outcompeted native coccinellid species in some habitats include its polyphagous diet, high fecundity (Michaud, 2002), aggressive behavior (Cottrell and Yeargan, 1999; Michaud, 2002), high mobility (With et al., 2002), and large body size (Cottrell and Yeargan, 1999; Michaud, 2002). Another factor influencing populations of native

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and exotic species may involve attack by parasites. Obrycki (1989) reported on differential susceptibility of certain native and exotic coccinellid species to the parasitoid *Dinocampus coccinellae* (Hymenoptera: Braconidae). Following this trend, Hoogendorn and Heimpel (2002) showed that successful parasitism of *H. axyridis* by *D. coccinellae* was lower than parasitism of the native *Coleomegilla maculata* (Coleoptera: Coccinellidae) even though the proportion of each species attacked was similar. But surprisingly, there is no information regarding the impact of endemic entomopathogens upon establishment of introduced natural enemies and relatively little information exists with regard to how endemic entomopathogens affect populations of native or exotic natural enemies (especially with regard to the natural enemies' viability for use in biological control programs; Vinson, 1990). When data concerning the effect of entomopathogens upon natural enemies has been presented, most concerned the potential impact of biorational products upon natural enemies (Laird et al., 1990). With regard to lady beetles, so frequently mentioned in biological control literature, no study has examined the susceptibility of native versus exotic predaceous lady beetles to endemic entomopathogens (see Ceryngier and Hodek, 1996 for a review of entomopathogens known to attack some species of Coccinellidae). Studies that have addressed entomopathogens and their impact upon predaceous lady beetles typically focused on one of four situations: rates of infection by nematodes or protozoans (Ceryngier and Hodek, 1996), mortality of overwintering populations (Ceryngier, 2000; Iperti, 1966a,b), effect of biorational pesticides on non-target lady beetles (Cagáň and Uhlík, 1999; Giroux et al., 1994; James and Lighthart, 1994; James et al., 1998; James et al., 1995; Magalhaes et al., 1988; Pell and Vandenberg, 2002; Pingel and Lewis, 1996; Poprawski et al., 1998; Smith and Krischik, 2000; Todorova et al., 1994; Todorova et al., 2000), and male-killing bacteria (Hurst and Jiggins, 2000).

We became intrigued with the impact of entomopathogens upon predaceous lady beetles when we discovered that some adults of the native *Olla v-nigrum* (Coleoptera: Coccinellidae) overwintering under bark on trees in pecan orchards were infected with *Beauveria bassiana*. Unlike *H. axyridis*, which can overwinter in large aggregations with thousands of beetles, *O. v-nigrum* adults typically overwinter in small clusters (generally <25 beetles) under tree bark. The dead infected *O. v-nigrum* adults had typical visible external *B. bassiana* mycelia (T.E. Cottrell, personal observation). Some live *O. v-nigrum* adults collected from pecan bark and held in the laboratory (provided aphids and water) died and also exhibited mycosis. Although *H. axyridis* and *O. v-nigrum* share similar life histories with regard to sites for oviposition, larval development, and pupation and co-occur in various habitats, we had never observed mycosis of *H. axyridis*. This was true whether *H. axyridis* were from overwintering aggregations in buildings or from field collections (from various habitats including pecan orchards).

Thus, our objective for this study was to examine mortality of the native *O. v-nigrum* and the exotic *H. axyridis* when assayed with *B. bassiana* isolates collected from *O. v-nigrum* and a commercially available *B. bassiana* strain. In addition, mycosis of field-collected *O. v-nigrum* and field-collected *H. axyridis* were compared.

2. Materials and methods

2.1. Insect colonies

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 and Tree Nut Research Laboratory at Byron, GA. The colonies were housed in 9-cm-diameter petri dishes in an environmental chamber at 25 ± 1 °C and a photoperiod of 14:10 (L:D) h. Beetles used in laboratory assays were reared in 9-cm-diameter petri dishes and fed live aphids (blackmargined aphids [*Monellia caryella*] and yellow pecan aphids [*Monelliopsis pecanis*] [Homoptera: Aphididae]), frozen lepidopteran eggs (*Helicoverpa zea* [Lepidoptera: Noctuidae], and *Ephestia kuhniella* [Lepidoptera: Pyralidae]), supplemented with a ground beef-beef liver diet (Cohen, 1985) and water provided with a moistened cotton dental wick. Aphids were reared on foliage of green-house grown seedling pecans (Cottrell et al., 2001). Beetles of both species were from 10 to 20 days old when used in assays.

2.2. Fungus

Three collections of *B. bassiana* were made from field-collected *O. v-nigrum*. These adult *O. v-nigrum* were collected from overwintering sites beneath pecan bark, taken to the laboratory, and held singly in petri dishes at 25 ± 1 °C and a photoperiod of 14:10 (L:D) h with food and water. Three beetles (from separate trees spaced at least 20 m apart within the orchard) that died after removal to the laboratory and showed typical *B. bassiana* mycosis were used as sources of field-collected *B. bassiana*. We refer to these collections of *B. bassiana* as Isolates A, B, and C. All isolates were cultured on SDAY agar for 14 days at 25 ± 1 °C (Goettel and 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 and Inglis, 1997). A surfactant was not

used when suspending *B. bassiana* conidia in water because Goettel and 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 both *O. v-nigrum* and *H. axyridis* was from the same original mixture of conidia. Therefore same rates of conidia were applied to both species.

2.3. Bioassay

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 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 in a 3.8 L covered, plastic container ($30.5 \times 16.5 \times 8.9$ cm). Containers were placed, according to the experimental design, in an environmental chamber at 25 °C and 14:10 (L:D) h photoperiod and 88% relative humidity.

2.4. LC_{50} and LT_{50} determination

We arbitrarily chose Isolate B to determine the LC_{50} and LT_{50} against *O. v-nigrum*. Adult beetles were tested against a water control and Isolate B at rates of 10^4, 10^5, 10^6, 10^7, and 10^8 conidia per ml using 25 insects per concentration. After correcting for control mortality (Abbott, 1925), probit analysis was used to estimate the LC_{50} based on the cumulative percentage mortality for the five concentrations of conidia 8 days after treatment. Probit analysis also was used to estimate the LT_{50} for those *O. v-nigrum* adults receiving the higher rates of conidia (10^6, 10^7, and 10^8 conidia per ml) (SAS, 1996).

2.5. Susceptibility of *O. v-nigrum* and *H. axyridis* to *B. bassiana*

Three trials, each done on separate dates, were used to compare susceptibility of adult *O. v-nigrum* and adult *H. axyridis* to *B. bassiana*. *B. bassiana* (Isolates A, B, C and strain GHA derived from Mycotrol ES [Mycotech, Butte, MT]) and a water control were assayed concurrently against laboratory-reared *O. v-nigrum* and *H. axyridis*. All *B. bassiana* were assayed at a rate of 2.5×10^5 conidia per ml. Beetles in trials 1 and 2 were fed live aphids, on pecan leaves, every other day starting 24 h after treatments were applied. Beetles in trial 3 were fed using the same schedule but *E. kuhniella* eggs were provided as the food source. We used a randomized complete block design and blocked by shelf within the environmental chamber. Treatments within blocks were randomly assigned to positions. Each trial had five treatments per species (i.e., *O. v-nigrum* and *H. axyridis*) and three blocks for trials 1 and 2 but with four blocks for trial 3. Ten insects were used per treatment. Mortality data were recorded daily for 8 days. Cumulative percentage mortality for each treatment was calculated as was mean survival time for those beetles that died during the experiment. In each trial, linear contrasts were used to compare species' mortality for each treatment with its respective control. This was done using a one-tailed test to determine if treatment mortality was significantly greater than control mortality when $\alpha = 0.05$ (SAS, 1996). Orthogonality of these contrasts was not determined because Kuehl (1994) states that construction of contrasts must not be dictated by their orthogonality but rather to answer research questions. 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 for 2 weeks following date of death. Mean days from death until occurrence of visible mycelia was analyzed by trial separately for each species using ANOVA (SAS, 1996).

2.6. High rate of *B. bassiana* conidia against *H. axyridis*

We tested higher rates of conidia from Isolate B against only *H. axyridis* in two separate trials both set up as RCBD using three and four blocks, respectively. First, we tested a water control and conidia (3.9×10^6, 3.9×10^7, and 3.9×10^8 conidia per ml) against laboratory-reared adult *H. axyridis* using six or seven beetles per treatment. Assay procedures were as previously described except that mortality was recorded every 48 h for 2 weeks with observations of dead beetles continuing for 2 weeks after death. For the second trial, only one rate of *B. bassiana* (1×10^8 conidia per ml) in addition to the water control was assayed against 10 insects per treatment. Mortality data from each trial was separately analyzed using ANOVA (SAS, 1996).

2.7. Beauveria bassiana infection of field-collected insects

Fifty adult *O. v-nigrum* adults were collected from the field in late April 2002 and held singly in petri dishes with food and water, as previously described, within an environmental chamber at 25 ± 1 °C and a photoperiod of 14:10 (L:D) h. Beetles were held for 30 days and checked every 2–3 days for *B. bassiana* mycosis. On two other separate occasions, one during September and the other during October, 2002, about 100 active adults each of *O. v-nigrum* and *H. axyridis* were collected singly from pecan orchards and held in the laboratory, as previously described, for 2 weeks to record mycosis. Any beetle that died within the 2-week interval was held for 2 weeks following date of death. Mycoses data from the two late-season collections were separately analyzed in a 2×2 contingency table using Haber's correction for continuity (Zar, 1999).

3. Results

3.1. LC_{50} and LT_{50} determination

Eight days after treating *O. v-nigrum* with Isolate B, probit analyis was used to estimate a LC_{50} (95% fiducial limits) of 2.5×10^5 (9.2×10^4 to 6.4×10^5) conidia per ml ($\chi^2 = 4.57$; df = 3; $P = 0.21$; slope $\pm$ SE $= 0.77 \pm 0.03$). Treatment mortality of lady beetles increased with higher conidia doses (10^4 to 10^8 conidia per ml) and ranged from 36 to 100% while control mortality was 12%. The estimated LT_{50} (95% fiducial limits) in days was 9.3 (7.8–13.5) ($\chi^2 = 1.17$; df = 6; $P = 0.98$; slope $= -0.27 \pm 0.01$), 7.5 (6.6–9.2) ($\chi^2 = 3.91$; df = 6; $P = 0.69$; slope $= -0.35 \pm 0.01$), and 5.4 (4.5–8.1) ($\chi^2 = 12.1$; df = 6; $P = 0.06$; slope $= -0.49 \pm 0.02$) for the higher rates of conidia tested (i.e., 10^6, 10^7, and 10^8 conidia per ml, respectively).

3.2. Susceptibility of O. v-nigrum and H. axyridis to B. bassiana

Mean survival time (days $\pm$ SE) for *H. axyridis* that received *B. bassiana* treatments and died during the study ranged from 2.7 ± 0.6 to 6.0 ± 0.7 days across treatments (number of dead beetles for treatments ranged from 5 to 8) while *H. axyridis* control survival averaged 4.9 ± 0.7 days ($n = 9$) (data combined from all trials). Mean survival of *O. v-nigrum* that received *B. bassiana* treatments and died ranged from 5.9 ± 0.5 to 6.8 ± 0.2 days (number of dead beetles for treatments ranged from 20 to 57) and mean survival in the control was 5.6 ± 0.5 days ($n = 19$) (data combined from all trials).

When analyzing mortality, we did not combine data sets and use 'trial' as a blocking factor due to a significant trial × treatment × species interaction ($P < 0.05$). When *H. axyridis* treatment mortality was separately contrasted with *H. axyridis* control mortality in each trial, no *B. bassiana* treatment in any trial resulted in mortality significantly higher ($P > 0.05$) than its corresponding control mortality (Fig. 1). This was not the case for treatments applied to *O. v-nigrum*, especially for *B. bassiana* isolates collected from *O. v-nigrum.* In each successive trial, Isolate A caused significantly higher

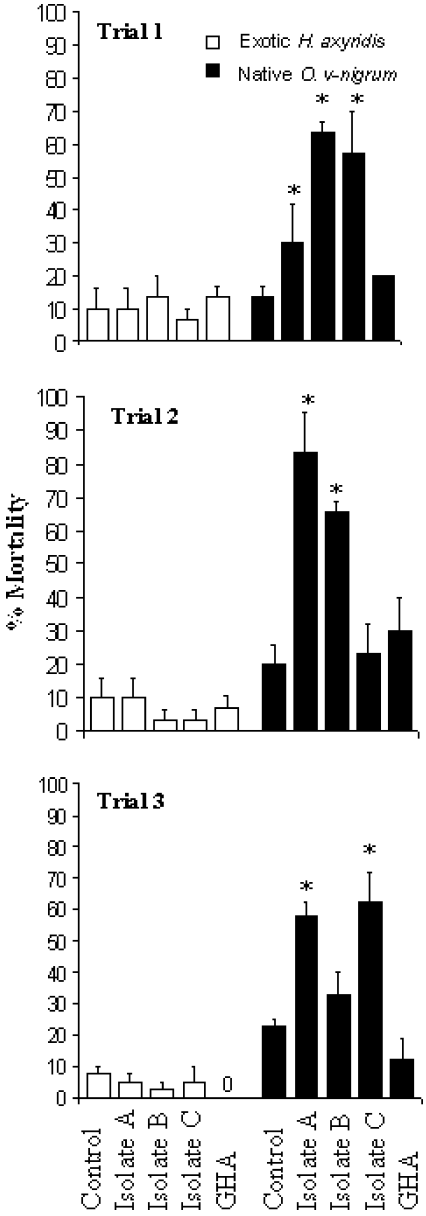

Fig. 1. Results from three separate trials showing mortality of *Hormonia axyridis* and *Olla v-nigrum* 8 days after treatment with *B. bassiana* Isolates A, B, and C (from field-collected *O. v-nigrum*) and the *B. bassiana* GHA strain from Mycotrol. All treatments were applied at the rate of 2.5×10^5 conidia per ml. An asterisk above a column inticates that mortality due to that treatment was significantly higher ($P < 0.05$) compared with control mortality for that same species in that same trial.

Table 1
Mortality of field-collected *O. v-nigrum* and *H. axyridis*

Month	Species	Number collected	Number dead in 14 days	Number with external mycelia
September	*H. axyridis*	100	5	0
	O. v-nigrum	100	12	4
October	*H. axyridis*	100	6	0*
	O. v-nigrum	99	47	38*

* Significant difference in numbers of *H. axyridis* and *O. v-nigrum* (collected during the same month) exhibiting external mycosis.

mortality of *O. v-nigrum* when contrasted with control mortality ($t_1 = 1.77$, $t_2 = 6.50$, $t_3 = 5.73$; $df_{1,2} = 16$, $df_3 = 24$; $P < 0.05$) (Fig. 1). Isolate B also resulted in significantly higher mortality when contrasted with the control in trials 1 and 2 ($t_1 = 5.30$, $t_2 = 4.68$; $df = 16$; $P < 0.05$) and approached statistical significance in trial 3 ($t = 1.64$; $df = 24$; $P = 0.0573$) (Fig. 1). Isolate C resulted in significantly higher mortality when contrasted with the control in trials 1 and 3 ($t_1 = 4.60$, $t_3 = 6.55$; $df_1 = 16$, $df_3 = 24$; $P < 0.05$) but not in trial 2 (Fig. 1). Mortality of *O. v-nigrum* treated with the GHA strain of *B. bassiana* was not significantly higher than the control mortality in any trial (Fig. 1).

Mortality of *H. axyridis* never exceeded 13.3% for any treatment in any trial and no *H. axyridis* adults that died during trial 1 or 3 were observed to develop visible external mycelia; only one *H. axyridis* that died during trial 2 developed mycelia. In contrast, percentage mycosis of those *O. v-nigrum* that died in *B. bassiana* treatments, from all trials, ranged from 21.0 to 90.5%. No mycosis of *O. v-nigrum* from controls was detected.

The number of days from death until visible signs of external mycelial growth on *O. v-nigrum* (±SE) was not significantly different between *B. bassiana* treatments by trial ($P > 0.05$); mean days to mycelial detection were 3.8 ± 1.2, 1.9 ± 0.4, 4.1 ± 1.1 and 2.5 ± 1.4 days for Isolates A, B, and C and the GHA strain, respectively (data combined from all trials).

3.3. High rate of B. bassiana conidia against H. axyridis

High concentrations of conidia, 3.9×10^6, 3.9×10^7, and 3.9×10^8, from Isolate B did not significantly increase percentage mortality (±SE) of *H. axyridis* (4.8 ± 4.8, 10.3 ± 5.2, and 31.8 ± 17.5, respectively) compared with control mortality (11.1 ± 11.1) ($F = 1.72$; $df = 3,6$; $P = 0.2624$). Nine beetles ($n = 60$), from all rates of conidia assayed, died during this experiment but only one developed visible mycelia. When a second test was done using Isolate B at 1×10^8 conidia per ml, no significant difference in percentage mortality was detected between treated (30.0 ± 7.1) and control beetles (10.0 ± 5.8) ($F = 2.67$; $df = 1,3$; $P = 0.2010$). A total of 12 beetles ($n = 40$) treated with the high rate of Isolate B died during this second trial but only one of those 12 dead beetles developed visible mycelia.

3.4. Beauveria bassiana infection of field-collected insects

Four percent of *O. v-nigrum* (collected from peach foliage during the spring) that died within 2 weeks of being collected exhibited typical *B. bassiana* mycosis. Numbers of active *O. v-nigrum* and *H. axyridis* adults (collected during September) that died in the laboratory within 2 weeks and exhibited mycosis were not significantly different ($\chi^2 = 1.57$; $df = 1$; $\alpha = 0.05$). However, the same test done in October did result in significantly more active *O. v-nigrum* adults being infected as compared with active *H. axyridis* adults ($\chi^2 = 13.37$; $df = 1$; $\alpha = 0.05$) (Table 1).

4. Discussion

The impact of entomopathogens upon arthropod natural enemies, specifically the predaceous Coccinellidae, is poorly understood and scarcely has been studied (Ceryngier and Hodek, 1996; Gilkeson, 1997). Selection of biological control agents for pest management programs typically is done with regard to parameters such as host range, geographic range, fecundity, and voltinism but never with regard to resistance to endemic diseases in the new habitat. It is interesting that establishment of introduced arthropod pest species has been attributed, in part, to their separation from natural control agents that attacked them in their native range (Ehler, 1998; Williamson, 1996). There are also reports that exotic pathogens can attack native species and that the pathogen's impact on either the new association species (Crooks et al., 2001) or on a native pest (Grewal et al., 2002) is greater than on old association species or exotic pests. It should be expected that intentionally introduced arthropod natural enemies should initially be free from their own natural enemies because removal of parasites from predators and hyperparasites from parasites is necessary before release into a new environment and culturing of insects before release can free them from entomopathogens (Simmonds, 1966). But available literature on disease of arthropod natural enemies most often concerns how an entomopathogen or biorational product for use against arthropod pest species might affect non-target natural enemies and does not address the susceptibility of introduced natural enemies to endemic pathogens. The data we presented, though limited

to two species within a specific area, provide insight on another possible mechanism that may have allowed an invading species to be competitive against a native species. We hypothesize that lower susceptibility by *H. axyridis* to endemic entomopathogens that attack native lady beetles may occur across a larger geographic region. Similarly, Torchin et al. (2001) reported on lower parasite infections of the European green crab, *Carcinus maenas* (L.), in introduced regions of the world compared with those in its native European range. This may also explain, in part, similar success of other introduced Coccinellidae, i.e., *Coccinella septempuncta*, that spread rapidly across the US (Elliot et al., 1996; Phoofolo and Obrycki, 1995).

Although coccinellids are susceptible to various entomopathogenic fungi (Ceryngier, 2000; Iperti, 1966a,b; Tillemans et al., 1990; Todorova et al., 1994), our data is the first account of differential susceptibility of a native and an exotic coccinellid species to an entomopathogenic fungus that was isolated from the native species. When assaying *B. bassiana* treatments at the established LC_{50}, we found that control mortality of *H. axyridis*, in any trial, was never higher than 10% and no *B. bassiana* treatment resulted in significantly higher mortality than the control. On the other hand, *O. v-nigrum* control mortality ranged from 13.3 to 22.5% (across all trials) and at least two *B. bassiana* isolates collected from *O. v-nigrum* still resulted in significantly higher mortality in each of the three trials. This was at first surprising given the relatedness of these species (i.e., both in tribe Coccinellini) and contrasts with Magalhaes et al. (1988) whom showed similar high mortality for adults of two coccinellid species (i.e., *C. maculata* and *Eriopis connexa*) when treated with the same *B. bassiana* isolate (i.e., ARSEF 731). But Todorova et al. (1994) tested two *B. bassiana* isolates and found that ARSEF 2991 was significantly more pathogenic to *C. maculata* larvae than ATCC 44860. Both, however, were highly pathogenic to the Colorado potato beetle (*Leptinotarsa decemlineata* [Coleoptera: Chrysomelidae]). Previous studies and the data we presented are not directly comparable due to differences in lady beetle species, insect stages, treatment application methods, and doses which sometimes reached rates of 10^8 conidia per ml; whereas, we used a much lower rate (i.e., 10^5 conidia per ml). Nonetheless our results with higher rates of conidia (up to 3.9×10^8) still did not significantly affect mortality of *H. axyridis*.

Geographic distribution maps for *O. v-nigrum* in the US (Gordon, 1985) and the recent widespread dispersal of *H. axyridis* across the US (Chapin and Brou, 1991; Colunga-Garcia and Gage, 1998; Hesler et al., 2001; LaMana and Miller, 1996; Tedders and Schaefer, 1994), indicate that these species co-occur in various habitats. Thus there has been ample opportunity for *H. axyridis* and *O. v-nigrum* to interact and, quite possibly, there would have existed favorable conditions for conidia from *B. bassiana*-infected *O. v-nigrum* to come into contact with *H. axyridis*. *O. v-nigrum* adults are commonly found overwintering on pecan tree trunks under bark (Mizell and Schiffhauer, 1987) and some of these adults are found dead and infected with *B. bassiana*. Adults and larvae of both *O. v-nigrum* and *H. axyridis* commonly traverse pecan trunks (T. E. Cottrell, personal observation). However, no field-collected *H. axyridis* adults exhibited mycosis when collected from the same pecan orchards and even from the same trees that yielded *O. v-nigrum* with mycosis thus corroborating our laboratory data. During October, field-collected *O. v-nigrum* sustained nearly 50% mortality and 81% of those dead beetles exhibited mycosis. This indicates that *H. axyridis* was likely exposed to *B. bassiana* but was not susceptible.

Although future introductions of polyphagous Coccinellidae similar to *H. axyridis* or *C. septempunctata* most likely will be avoided (due to negative impacts upon native species), it is likely that mono- or oligophagous species will be released. If potential biological control agents are highly susceptible to endemic entomopathogens, the potential for establishment may be diminished. Therefore, it will be important to know how potential biological control agents will fare against a new suite of entomopathogens.

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