Interactions between the fungal pathogen *Beauveria bassiana* and three species of coccinellid: *Harmonia axyridis*, *Coccinella septempunctata* and *Adalia bipunctata*

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Abstract *Harmonia axyridis* is a predatory coccinellid, native to central and eastern Asia. It has been available in many countries for use as a biological control agent of pest aphids and scale insects. In many of these countries, including the USA, *H. axyridis* has established. It is now considered an invasive alien species for a number of reasons, including its impact on functional biodiversity. *Beauveria bassiana* is known to be a natural mortality agent of overwintering coccinellids and is a potential candidate for the biological control of *H. axyridis*. In this paper we compare the susceptibility of three species of coccinellid, *H. axyridis* (cultures derived from Japan and UK), *Coccinella septempunctata* and *Adalia bipunctata* to infection by *B. bassiana* (commercial strain GHA) after exposure at three doses (10^5, 10^7, 10^9 conidia ml^{-1}). The two subpopulations of *H. axyridis* (Japan and UK) were more resistant to *B. bassiana* infection than either *A. bipunctata* or *C. septempunctata*. This is exemplified by the median lethal doses at 10 days post-inoculation (LD$_{50}$) of $10^{6.2}$, $10^{6.0}$, $10^{8.3}$, $10^{9.6}$ conidia ml^{-1} for *A. bipunctata*, *C. septempunctata*, *H. axyridis* (Japan) and *H. axyridis* (UK), respectively. Only doses of 10^9 conidia ml^{-1} resulted in mortality of *H. axyridis*, in contrast, 80% of *C. septempunctata* and 70% of *A. bipunctata* exposed to 10^7 conidia ml^{-1} of *B. bassiana* succumbed to infection. The fecundity (cumulative mean egg production over 10 days) of *A. bipunctata* and *H. axyridis* (UK) was also assessed. The fecundity of *C. septempunctata* could not be assessed because this species requires diapause prior to the onset of reproduction and these studies were on beetles that had recently eclosed (2–8 weeks). *Harmonia axyridis* (Japan) produced no eggs in most treatments including the control and so was excluded from analysis. High dose (10^9 conidia ml^{-1}) inoculation reduced the fecundity of *A. bipunctata* to zero but egg production was similar for individuals inoculated with doses

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of 10^5, 10^7 conidia ml^{-1} and control individuals. In contrast, all doses of *B. basssiana* reduced *H. axyridis* (UK) egg production dramatically. We discuss these results in relation to the potential for control of *H. axyridis* using *B. bassiana*.

Introduction

The fungus *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales) is a facultative pathogen infecting species from most insect orders (Keller and Zimmerman 1989). It is ubiquitous in the soil and recently propagules have been reported from the phylloplanes of hedgerow vegetation (Meyling and Eilenberg 2006), elm bark (Doberski and Tribe 1980), conifer plantations (Ormond 2007) and as an endophyte of corn (Lewis et al. 1996). As an insect pathogen it is found naturally infecting many insect species in the field (Riedel and Steenberg 1998; Ormond et al. 2006), particularly those which have a component of their life history associated with the soil, such as some species of over-wintering coccinellid. Indeed, *B. bassiana* is documented as a major mortality factor of seven-spot ladybirds *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) which, in temperate regions, spend the winter months in diapause under leaf litter where they are exposed to this fungal pathogen (Majerus and Kearns 1994; Ormond et al. 2006).

The interactions between *B. bassiana* and coccinellids have been studied from two main perspectives: mortality of overwintering populations exposed to naturally occurring *B. bassiana* (Iperti 1966; Barron and Wilson 1998; Ceryngier and Hodek 1996; Ormond et al. 2006) and mortality of non-target coccinellids exposed to *B. bassiana* based biorational pesticides (James et al. 1995; Pingel and Lewis 1996; Todorova et al. 1996; Roy and Pell 2000; Smith and Krischik 2000; Pell and Vandenberg 2002). There is no doubt that the latter has dominated. Intriguingly the arrival of the harlequin ladybird, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), as an invasive alien in many countries provides a new perspective on the interactions between coccinellids and *B. bassiana*. Whereas previously infection of coccinellids by *B. bassiana* has been seen as a negative non-target impact it could now be considered as an asset if the target is *H. axyridis*. However, the wide host range of *B. bassiana* should be considered in this context.

Harmonia axyridis is an exotic species in many countries (Brown et al. 2007) and is reported to be the dominant species of coccinellid in some habitats within its non-native range (Brown and Miller 1998; Colunga-Garcia and Gage 1998; Cottrell and Yeargan 1998; Wells and McPherson 1999; Michaud 2002; Cottrell and Shapiro-Ilan 2003). *Harmonia axyridis* is widely considered as an invasive alien species for a number of reasons, including: impacts on native fauna, overwintering aggregations in domestic dwellings and damage to orchard fruits (Majerus et al. 2006). Recent attention has been given to the control of *H. axyridis* (reviewed by Kenis et al. 2007) and *B. bassiana* is considered a potential candidate. However, endemic insect species are known to be susceptible to *B. bassiana* and so the differential susceptibility of these species to *B. bassiana* must be evaluated as a first stage of assessing the risks of this fungus as a possible control agent of *H. axyridis*.

In this paper we assess the susceptibility of *H. axyridis* and two native UK coccinellid species, *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae) and *C. septempunctata*, to

B. bassiana (Mycotrol GHA). In a previous study we demonstrated that adult *H. axyridis* reared in the laboratory from UK field collections differed behaviourally in comparison to adults derived from Japan field collections (Roy et al. 2007a). Therefore, we included UK and Japan subpopulations of *H. axyridis* in our studies. *Adalia bipunctata* has similar life history traits to *H. axyridis* in terms of sites for oviposition, larval development and pupation (diverse but often on deciduous trees). Furthermore both species overwinter in aggregations in buildings (Majerus 1994; Majerus et al. 2006). In contrast, *C. septempunctata* has different habitat preferences for oviposition, larval development and pupation (usually herbaceous layer) and often overwinters under leaf litter or in soil (Majerus 1994; Majerus et al. 2006). *Coccinella septempunctata* is commonly found infected with *B. bassiana* in the field, whereas mycosed *A. bipunctata* are rarely reported (Majerus 1994; Barron and Wilson 1998; Majerus et al. 2006). It is for these reasons that we decided to include these three coccinellid species in our studies.

Most studies that examine the potential of entomopathogens as biological control agents express efficacy as a median lethal dose (LD_{50}) and median lethal time to death (LT_{50}); therefore considering only the mortality of the study species (Inglis et al. 2001; Cottrell and Shapiro-Ilan 2003). More recent attention has focused on the importance of sublethal effects such as alterations to behaviour (Roy et al. 2006a; Roy et al. 2006b; Roy et al. 2007b). A number of studies have shown that fungal-infected hosts produce fewer progeny (Blanford and Thomas 2001; Baverstock et al. 2006). Reduction in fecundity could increase pathogen fitness by diverting host resources for the pathogen although there is, of course, a trade-off to the pathogen because fewer susceptible hosts will consequently be available for infection; although the extent of this is less for a facultative pathogen, such as *B. bassiana*, than a host-specific pathogen.

The aims of our studies were to assess the impact of a commercial isolate of *B. bassiana* (Mycotrol GHA) on the survival of three coccinellid species (*H. axyridis*, *A. bipunctata* and *C. septempunctata*) and the fecundity of two coccinellid species (*H. axyridis* and *A. bipunctata*).

Materials and methods

Insect and fungus cultures

Laboratory reared adult *H. axyridis*, *C. septempunctata* and *A. bipunctata* were used in all experiments. These cultures were obtained from the following field locations: *H. axyridis* from the UK (Battersea Park, London) and Japan (Fuchu, Honshu), *C. septempunctata* and *A. bipunctata* from the UK (Norfolk and Cambridgeshire, respectively). All adult coccinellids had undergone eclosion within 2–8 weeks of the bioassays. The coccinellids were maintained in batches of five individuals within 90 mm diameter single-vented Petri dishes at 22°C (14L:10D). All individuals were fed daily on an excess of adult pea aphids, *Acyrthosiphon pisum* Harris (Hemiptera: Aphididae) which were cultured on broad bean plants *Vicia faba* L. (cultivar: The Sutton).

The GHA strain of *B. bassiana* that is the active ingredient of the product Botanigard® (Laverlam) was used in all bioassays. The culture was stored in 10% glycerol (cryoprotectant) at –86°C until required. After retrieval from –86°C, the fungal plugs were macerated within the glycerol and spread onto Sabouraud Dextrose Agar (SDA). The fungus was then incubated at 20°C (16L:8D) for 2 weeks and subcultured again on to SDA. After a further 2 weeks, *B. bassiana* conidia could be harvested from the plates. The

fungal conidia from eight plates were dispersed in 20 ml of 0.03 % Tween 80 and held on ice. Spore concentration was calculated using a Neubauer haemocytometer and suspensions of 10^9, 10^7 and 10^5 conidia ml^{-1} were achieved through dilution in the surfactant Tween 80 (0.03%). All spore suspensions were again assessed with the Neubauer haemocytometer to ensure accuracy of concentration values. All bioassays used spore suspensions that were less than four hours old and had been stored on ice.

Bioassays

Batches of either 1 ml of fungal spore suspension or 1 ml of 0.03 % Tween 80 (control) were decanted into 1.5 ml Eppendorf tubes and stored on ice. Each individual coccinellid was dipped in new spore or control suspension (inverted five times) and transferred to 50 mm single-vented Petri dishes. Each dish contained a filter disc moistened with sterile distilled water and an excess of adult pea aphids. All dishes were placed in trays lined and covered with moistened paper to maintain high humidity for a period of 24 h. After 24 h the moistened filter paper discs were removed from each dish and the moistened paper was also removed from the tray. All individuals were fed daily on an excess of adult pea aphids. Replication of each treatment, and the number of male and female coccinellids within a bioassay varied depending on availability of laboratory cultured coccinellids (Table 1).

Mortality was recorded daily for each individual until 10 days post inoculation (days p.i.). Individual dead coccinellids were transferred to a 50 mm single-vented Petri dish containing a filter disc moistened with sterile distilled water. All dishes were placed in trays lined and covered with moistened paper to maintain high humidity. Dead coccinellids were assessed daily for external fungal growth (sporulation), which could be confirmed as *B. bassiana* on the basis of morphology.

Eggs were counted and removed on a daily basis for 10 days p.i.

Statistical analyses

Mortality

Mortality data were analysed using binary logistic regression in Minitab 14 (Minitab 2003). Control data were excluded from the analysis because of the exceptionally low mortality (only one individual (*A. bipunctata*) across all replicates and this individual showed no evidence of mycosis). Goodness-of-fit was used to compare the logit and the

Table 1 Number of replicates per treatment (Control or *B. bassiana* treatment: 10^5, 10^7 or 10^9 conidia ml^{-1}) for each species (or biotype) of coccinellid: *A. bipunctata*, *C. septempunctata*, *H. axyridis* (Japan) and *H. axyridis* (UK)

Coccinellid species	Control	10^5 *B. bassiana*	10^7 *B. bassiana*	10^9 *B. bassiana*
A. bipunctata	10	10 (6♀:4♂)	10	10
C. septempunctata	20	20	20	20
H. axyridis (Japan)	10 (6♀:4♂)	10 (6♀:4♂)	10 (7♀:3♂)	10 (4♀:6♂)
H. axyridis (UK)	20	20	20 (9♀:11♂)	20 (8♀:12♂)

An equal number of male and females were used for each treatment unless stated

Gompit (complementary log–log) model, and the latter was subsequently used for all analyses. Therefore, the relationship between mortality and $\log_{10}$ dose was described by a binary regression model with Gompit link function. If m_{ij} denotes the mortality rate for the *i*th species with $\log_{10}$ dose x_{ij} the model is:

$$\log_e[-\log_e(1 - m_{ij})] = a_i + bx_{ij}$$

where a_i denotes an intercept term for the *i*th species. The model assumes the same slope (b) across species. This assumption was tested but no differences in slope were detected. Significance tests were adjusted for over-dispersion using the Pearson method, by division of *t*-ratios by $\sqrt{(\chi^2/\mathrm{df})}$, equivalent to 1.21 for 10 day p.i.

The LD_{50} corresponds to 50% mortality and is obtained by setting $m_{ij} = 0.5$, i.e.

$$\log_{10} \mathrm{LD50}_i = -(0.367 + a_i)/b$$

A 95% confidence interval for the $\log_{10} LD_{50}$ is obtained by application of Fieller's theorem (Finney 1971). It should be noted that where an LD_{50} exceeds 10^9 conidia ml^{-1} the value is an extrapolation of the regression curve.

Fecundity

Fecundity (cumulative number of eggs laid per individual) was analysed using repeated measures ANOVA for *A. bipunctata* (Fig. 3) and *H. axyridis* UK (Fig. 4) in Genstat version 10 (Genstat 2007). Counts were transformed using log(count + 1) to approximate the assumptions of constant variance and normality. Significance tests for time effects and the interaction with treatment were adjusted to allow for a non-constant correlation between repeat observations on the same units using the Greenhouse–Geisser correction factor.

It should be remembered that at day 10 p.i. for some species-treatment combinations the replication was low because of high mortality, particularly for *A. bipunctata* inoculated with 10^9 conidia ml^{-1}, where mortality by day 10 was 100%. Therefore, analyses on fecundity of individuals at late stages of *B. bassiana* infection are presented tentatively.

Coccinella septempunctata fecundity was not analysed because this species does not reproduce prior to diapause and all experiments were conducted with 2–8 week old individuals (Majerus 1994). Data for *H. axyridis* (Japan) were also excluded because of a high number of zero values for all individuals (control and treatments).

Results

Mortality

By 5 days p.i., the proportion of coccinellids that had succumbed to *B. bassiana* infection was similar for *C. septempunctata* and *H. axyridis* (Japan) ($t = -1.93$, df = 7, $P = 0.095$; Fig. 1). There was a trend suggesting that a lower proportion of *H. axyridis* (UK) had died in comparison to *H. axyridis* (Japan) ($t = 1.66$, df = 7, $P = 0.07$; Fig. 1). The mortality of *A. bipunctata* was significantly higher compared to *H. axyridis* (Japan) ($t = -4.90$, df = 7, $P < 0.001$; Fig. 1).
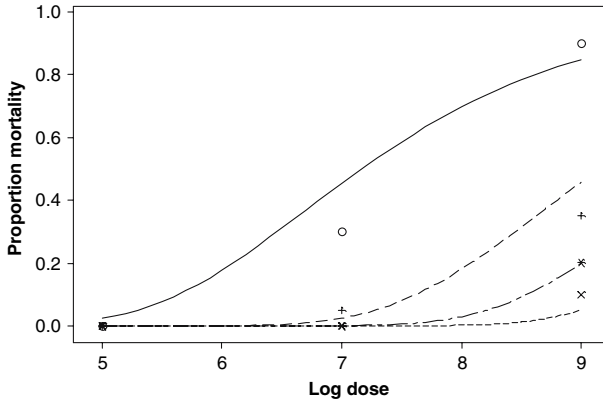
Fig. 1 Proportion mortality at 5 days for four species of coccinellid (*Coccinella septempunctata* (large hash line/+), *Adalia bipunctata* (solid black line/o), *Harmonia axyridis* (Japan) (broken hash line/*) and *Harmonia axyridis* (UK) (small hash line/x)) exposed to three doses of *B. bassiana* (10^5, 10^7 and 10^9 conidia ml^{-1}). Symbols represent experimental data and connected lines represent data as predicted by the Gompit (complementary log–log) model

By 10 days p.i., the proportion of *H. axyridis* (Japan) and *H. axyridis* (UK) that had died from *B. bassiana* infection was statistically similar ($t = 2.05$, df = 7, $P = 0.078$, Fig. 2), although there was a trend suggesting that *H. axyridis* (UK) were more resistant to infection than *H. axyridis* (Japan). Mortality of *C. septempunctata* ($t = -3.55$, df = 7, $P = 0.009$; Fig. 2) and *A. bipunctata* ($t = -2.85$, df = 7, $P = 0.024$; Fig. 2) was significantly greater than observed for *H. axyridis* (Japan and UK). So, in conclusion, the order of susceptibility from highest (greatest proportion dead) to lowest (smallest proportion dead) was *C. septempunctata*, *A. bipunctata*, *H. axyridis* (Japan) and *H. axyridis* (UK). This is apparent from the calculated LD$_{50}$ values (Table 2, Figs. 1 and 2).

Fecundity

There was a significant difference between treatments ($F_{3,8} = 6.6$, $P = 0.015$, Fig. 3), time ($F_{4,32} = 18.84$, $P = 0.001$; Fig. 3) and the treatment × time interaction ($F_{12,32} = 3.64$,

Fig. 2 Proportion mortality at 10 days for four species of coccinellid (*Coccinella septempunctata* (large hash line/+), *Adalia bipunctata* (solid black line/o), *Harmonia axyridis* (Japan) (broken hash line/*) and *Harmonia axyridis* (UK) (small hash line/x)) exposed to three doses of *B. bassiana* (10^5, 10^7 and 10^9 conidia ml^{-1}). Symbols represent experimental data and connected lines represent data as predicted by the Gompit (complementary log–log) model
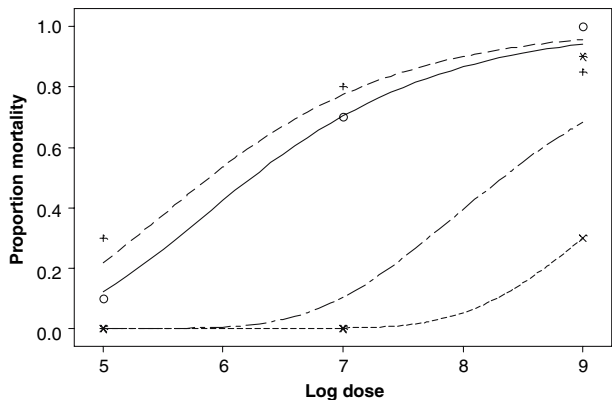
	Species	LD$_{50}$ (95% confidence interval)
Table 2 Estimated LD$_{50}$ (log$_{10}$ conidia ml^{-1}) at 10 days with 95% confidence interval for *A. bipunctata*, *C. septempunctata*, *H. axyridis* (Japan) and *H. axyridis* (UK)	*A. bipunctata*	$10^{6.2}$ ($10^{5.4}$, $10^{7.3}$)
	C. septempunctata	$10^{6.0}$ ($10^{5.0}$, $10^{6.7}$)
	H. axyridis (Japan)	$10^{8.3}$ ($10^{7.4}$, $10^{9.5}$)
	H. axyridis (UK)	$10^{9.6}$ ($10^{8.9}$, $10^{10.7}$)

$P = 0.031$; Fig. 3) for *A. bipunctata*. The cumulative egg count increased over time for all treatments except the 10^9 spores ml^{-1} (high dose) treatment. Indeed when the zero egg counts observed for the 10^9 spores ml^{-1} (high dose) treatment were excluded from the analysis there was no evidence of either a treatment × time interaction effect ($F_{8,24} = 1.74$, $P = 0.23$), or treatment effect ($F_{2,6} = 0.08$, $P = 0.93$).

There was no significant difference between treatments for *H. axyridis* ($F_{3,7} = 2.73$, $P = 0.124$; Fig. 4) but there was a significant difference between treatments in comparison to the control ($F_{1,7} = 7.46$, $P = 0.023$; Fig. 4). Cumulative egg count increased significantly over time for all treatments including the control ($F_{4,28} = 24.12$, $P = 0.001$; Fig. 4) but there was no significant treatment × time interaction ($F_{12,28} = 0.6$, $P = 0.75$; Fig. 4).

In summary *A. bipunctata* exposed to 10^9 conidia ml^{-1} of *B. bassiana* did not produce any eggs while the fecundity of individuals exposed to 10^5 and 10^7 conidia ml^{-1} of *B. bassiana* was similar to that of control individuals. In contrast the fecundity of *H. axyridis* inoculated with *B. bassiana* was lower, irrespective of dose, than control individuals (Figs. 3 and 4).

Discussion

Numerous studies have demonstrated the physiological susceptibility of coccinellids to *B. bassiana* (Iperti 1966; Todorova et al. 1996; Cottrell and Shapiro-Ilan 2003). In a recent paper, Cottrell and Shapiro-Ilan (2003) highlighted the differential susceptibility of a native coccinellid and an exotic coccinellid to an isolate of *B. bassiana* that had been derived from the native coccinellid. The exotic coccinellid, *H. axyridis*, was less susceptible to *B. bassiana* than the native coccinellid, *Olla v-nigrum* Mulsant (Coleoptera:
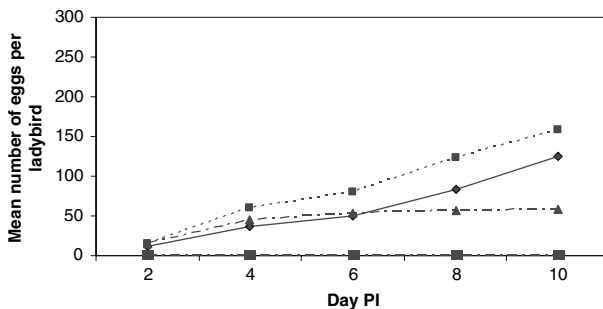

Fig. 3 Cumulative number (mean) of eggs laid per *Adalia bipunctata* over time (day p.i.) after inoculation at day 0 with *B. bassiana* at either a low dose (10^5 conidia ml^{-1}; small squares, dashed line), mid dose (10^7 conidia ml^{-1}; triangle, dashed line), high dose (10^9 conidia ml^{-1}; large square, dashed line) or control (0.03% tween 80; diamond, solid line)
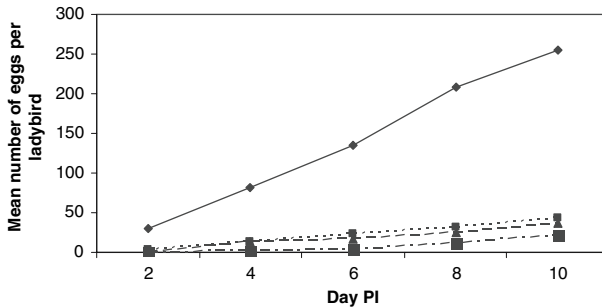
Fig. 4 Cumulative number (mean) of eggs laid per *Harmonia axyridis* (Japan and UK combined) over time (day p.i.) after inoculation at day 0 with *B. bassiana* at either a low dose (10^5 conidia ml^{-1}; small squares, dashed line), mid dose (10^7 conidia ml^{-1}; triangle, dashed line), high dose (10^9 conidia ml^{-1}; large square, dashed line) or control (0.03% tween 80; diamond, solid line)

Coccinellidae). In our study we compared the susceptibility of three species of coccinellid to a commercial isolate (geographic origin unknown) of *B. bassiana* (Mycotrol GHA) and conclude that *H. axyridis* is more resistant to infection than either *A. bipunctata* or *C. septempunctata* (which are both highly susceptible). Mortality of *H. axyridis* did not occur at doses less than 10^9 conidia ml^{-1}, whereas 70 and 80% mortality of *A. bipunctata* and *C. septempunctata* (respectively) occurred after inoculation at 10^7 conidia ml^{-1}. Inoculation at the highest dose (10^9 conidia ml^{-1}) resulted in 100% mortality of *A. bipunctata* and *C. septempunctata*. Interestingly 90% mortality of *H. axyridis* (Japan) was achieved at this high dose but only 30% mortality of *H. axyridis* (UK). Although the difference in mortality of the two *H. axyridis* subpopulations (derived from cultures initiated from UK and Japan collected adults) was not significant it is certainly worth further exploration. In previous work, Roy et al. (2007a) demonstrated a difference in the feeding behaviour of these two subpopulations and concluded that *H. axyridis* (UK) was a stronger intraguild predator than *H. axyridis* (Japan). The observed anomaly between the UK and Japan derived *H. axyridis* suggests that the individuals that have invaded the UK could have a different genetic profile to those in its native range.

We included *A. bipunctata* in our study for two reasons: first, there are no published studies reporting infection of *A. bipunctata* by *B. bassiana* and second, it has similar life history traits to *H. axyridis* and occupies similar habitats for both feeding and overwintering (Majerus 1994; Lanzoni et al. 2004; Majerus et al. 2006). Both *A. bipunctata* and *H. axyridis* are found in a range of habitats but favour deciduous trees (Majerus 1994; Majerus et al. 2006). *Beauveria bassiana* is generally considered to be a soil-borne pathogen (Keller and Zimmerman 1989) with limited distribution on foliage (Meyling and Eilenberg 2006). Therefore, it is widely assumed that coccinellids overwintering in leaf litter, such as *C. septempunctata*, are more likely to be exposed to *B. bassiana* than coccinellids, such as *H. axyridis* and *A. bipunctata*, which spend winter above ground. The lack of reports of interactions between *A. bipunctata* and *B. bassiana* could, perhaps, be linked to the spatial disparity of their habitats. From our studies it can be concluded that *A. bipunctata* is as physiologically susceptible as *C. septempunctata* to infection by *B. bassiana*. This supports the hypothesis that the lack of field observations of mycosed *A. bipunctata* is because they either do not contact sufficiently high doses of inoculum, or are not in abiotic environments suitable for infection. Furthermore, our study contributes a further example of the resilience of *H. axyridis*, compared to other coccinellids, to

B. bassiana. It should be a priority to extend these laboratory studies to the field through a combination of experimental manipulations and observational surveys of natural enemies, including insect pathogens. Indeed the UK on-line Harlequin Ladybird Survey (www.harlequin-survey.org) is now providing the opportunity for contributors to include this information.

Throughout our studies we used only one isolate of *B. bassiana*. A number of recent publications have shown that *B. bassiana* isolates are very genetically variable (Aquino de Muro et al. 2003, 2005; Estrada et al. 2007; Ormond 2007), and concomitantly highly phenotypically variable in parameters such as virulence (Bidochka et al. 2002; Devi and Rao 2006; Talaei-Hassanloui et al. 2006). The natural enemy escape hypothesis is often used as an explanation for the rapid establishment and proliferation of an invasive alien species; the geographical displacement of the species results in escape from predators and parasites and so gives the invading species an advantage over native species that are attacked by a suite of indigenous natural enemies (e.g. Colautti et al. 2004; Carroll et al. 2005). It is possible that an isolate derived from the native range of *H. axyridis* would be more virulent to *H. axyridis* than the isolates studied so far (Bazzocchi et al. 2004). However, it is striking that, despite different approaches to isolate choice in studies on *H. axyridis*, the conclusion that *H. axyridis* is resistant to infection is unanimous (Cottrell and Shapiro-Ilan 2003). It would be interesting to explore the mechanisms underlying the resistance of *H. axyridis* to *B. bassiana*.

Initial studies should focus on the infection process and mechanisms of defence by the host. At high humidity, *B. bassiana* conidia will germinate on the surface of a suitable host and differentiate into an appressorium, which penetrates directly through the cuticle. Insect hosts have a limited range of defences but can, as an initial response, produce melanin at the appressorium penetration site (James et al. 2003). Therefore, it could be hypothesised that melanic individuals, which are common in *H. axyridis*, would be more resistant to infection than non-melanic individuals. However, in preliminary bioassays comparing melanic and non-melanic individuals there was no difference in susceptibility (H. Roy personal observation). It is possible that the defensive chemistry of *H. axyridis* could be adversely affecting fungal development within the haemocoel and this could be investigated in vitro. *Beauveria bassiana* produces a number of secondary metabolites, such as bassianin, bassiacridin, beauvericin, bassianolide, beauverolides, tenellin and oosporein (Strasser et al. 2000; Vey et al. 2001; Quesada-Moraga and Vey 2004). Some of these have toxic effects (beauvericin, bassianin, bassianolide, beauverolides, tenellin) while others have an antibiotic or antifungal role (oosporein) (Strasser et al. 2000; Scholte et al. 2004). Perhaps the toxic alkaloids circulating in the haemocoel of *H. axyridis* are interacting with these secondary metabolites and negating their effects. Considerable further work is required to elucidate this.

Harmonia axyridis inoculated with *B. bassiana* produced fewer eggs than control individuals and this suggests that the fungus is invading the host. This impact on fecundity was not observed for *A. bipunctata* (and not tested for with *C. septempunctata* because a period of diapause is required prior to reproduction and our studies used recently eclosed adults). Most studies on insect pathogens consider only host mortality but recent attention has focussed on sublethal effects, such as reduced fecundity (e.g. Roy et al. 2006a; Roy et al. 2006b). Baverstock et al. (2006) demonstrated a reduction in fecundity of *B. bas-siana*-infected *A. pisum* (pea aphid) within 24 h of infection. *Acyrthosiphon pisum* were highly susceptible to the isolate of *B. bassiana* used in these studies (Mycotrol GHA) and it is possible that resources were directed away from host embryogenesis to benefit development of the pathogen. An alternative strategy involves a host maintaining or increasing

reproductive output in response to early stages of infection, to ensure that part of their reproductive potential is realised. Blanford and Thomas (2001) observed such a phenomenon in desert locusts, *Schistocerca gregaria* Forskal (Orthoptera: Acrididae), infected with *Metarhizium anisopliae* (Metsch.) Sorokin var *acridum* Milner & Driver (Ascomycota: Hypocreales). In both these studies the host was highly susceptible to the pathogen under investigation. Our studies suggest that the susceptible coccinellid, *A. bipunctata*, is employing the strategy of maintaining egg production throughout the infection process, whereas *H. axyridis*, which suffers much lower mortality as a consequence of *B. bassiana* inoculation, produces fewer eggs after exposure even to low doses (10^5 conidia ml^{-1}). We tentatively propose that *H. axyridis* may be diverting resources from egg production to invest in defence against the pathogen. This intriguing result requires further investigation to see, for example, whether this sublethal effect is consistent across a range of *B. bassiana* isolates and, indeed, for other pathogens. It would also be worth assessing whether the reduction in egg laying persists in the longer term.

The importance of assessing sublethal effects is highlighted through our study. It is clear that *H. axyridis* is more resistant, in terms of mortality, to *B. bassiana* than the other coccinellids included in these experiments and, therefore, it could be concluded that the potential for *B. bassiana* to control *H. axyridis* is negligible. However, including an assessment of fecundity within these experiments has provided further insight into the interactions between coccinellids and *B. bassiana*.

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