Avoidance of a generalist entomopathogenic fungus by the ladybird, Coccinella septempunctata

Emma L. Ormond¹, Alison P.M. Thomas¹, Judith K. Pell², Stephen N. Freeman³ & Helen E. Roy³

¹Department of Life Sciences, Anglia Ruskin University, Cambridge, UK; ²Department of Plant and Invertebrate Ecology, Rothamsted Research, Harpenden, Hertfordshire, UK; and ³NERC Centre for Ecology and Hydrology, Crowmarsh Gifford, Oxfordshire, UK

Correspondence: Helen E. Roy, NERC Centre for Ecology and Hydrology, Wallingford, Crowmarsh Gifford, Oxfordshire OX10 8BB, UK. Tel.: +1491 692 252; fax: +44 01491 692424; e-mail: hele@ceh.ac.uk

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Abstract
Fungal entomopathogens are ubiquitous within the environment and susceptible insects are predicted to be under strong selection pressure to detect and avoid virulent isolates. Beauveria bassiana is an entomopathogenic fungus with a wide host range including coccinellids. Seven-spot ladybirds, Coccinella septempunctata, overwinter predominantly in leaf litter and B. bassiana is one of their major mortality factors during winter in temperate regions. Behavioural assays were conducted to assess the ability of adult C. septempunctata to avoid lethal densities of B. bassiana conidia in soil or on leaves. Further assays considered avoidance by C. septempunctata of mycosed (B. bassiana) C. septempunctata cadavers compared with uninfected C. septempunctata cadavers or in vitro B. bassiana. Treatments in any bioassays entirely avoided by C. septempunctata were regarded as censored data, to overcome the difficulties associated with zeros in log-ratio analyses. Male and female C. septempunctata avoided contact with leaf surfaces and soil inoculated with B. bassiana and mycosed cadavers. The ability of C. septempunctata to detect and avoid B. bassiana conidia is an adaptation that undoubtedly increases survival and ultimately fitness. We predict that such behavioural responses are widespread and driven by the high cost of fungal infection to a host.

Introduction
Pathogens and their hosts are intrinsically linked, but their evolutionary interests diverge: selection on the pathogen is for greater exploitation of the host and selection on the host is for exclusion of the pathogen (Roy et al., 2006). Host–pathogen interactions can be considered within the context of a coevolutionary arms race whereby partners are evolving in an aggressive manner toward one another (Roy et al., 2006). An arms race manifests itself in a variety of adaptations, but host–pathogen relationships are often considered only in terms of the impact of host susceptibility and pathogen virulence on physiology and, more exactly, mortality. Host behaviour is often ignored, even though behavioural responses can affect the parameters essential to pathogen and host evolution such as transmission and longevity (Moore, 2002).

Recent evidence suggests that arthropods foraging for food and oviposition sites assess their environment for the risk of predation both to themselves and to their offspring (Blaustein et al., 2004; Offenberg et al., 2004). Previous studies have examined a range of behaviours adopted by animals to increase their survival in the presence of pathogenic fungi (Roy et al., 2006) and include behavioural fever (Covert & Reynolds, 1977; Watson et al., 1993; Elliot et al., 2002), hygienic behaviours (Jones et al., 1996; Spivak & Gilliam, 1998; Arathi et al., 2006; Stow & Beattie, 2008), grooming (Yanagawa & Shimizu, 2007), alarm behaviour (Myles, 2002a), necrophagy (Myles, 2002b) and avoidance (Milner et al., 1998). The detection and avoidance of pathogens is important for host survival, longevity and ultimately fitness; infection by an insect pathogen (entomopathogen) is often lethal to the host and reproductive success, and fitness, is usually reduced (Lima & Dill, 1990). However, very few studies have assessed the detection and avoidance of lethal pathogens even though pathogenic bacteria, viruses and fungi are ubiquitous worldwide and common natural enemies of many invertebrate species (Hajek, 2004; Meyling & Pell, 2006). Meyling & Pell (2006) demonstrated that the anthocorid bug, Anthocoris nemorum L. (Heteroptera: Anthocoridae) avoided leaf surfaces inoculated with the fungus Beauveria bassiana (Balsamo)
Vuillemin (Ascomycota: Cordycipitaceae). Termites (Isoptera: Rhinotermitidae) can detect the fungus Metarhizium anisopliae (Metschnikoff) (Ascomycota: Clavicipitaceae) in soil and in infected conspecifics (Staples & Milner, 2000; Myles, 2002a,b). Indeed, Mburu et al. (2009) demonstrated that the detection and avoidance of B. bassiana and M. anisopliae by termites (Macrotermes michaelseni) depends on the virulence of the isolate; infective isolates are recognized and avoided. Mole crickets (Orthoptera: Gryllotalpidae) respond to B. bassiana by altering their tunnelling behaviours (Thompson & Brandenburg, 2005). In contrast, the ectoparasitoid, Cephalonomia tarsalis (Ashmead) (Hymenoptera: Bethylidae) was not found to detect either the presence of B. bassiana conidia or infected conspecifics (Lord, 2001), nor were anopheline and culicine mosquitoes found to be repelled by mosquito nets treated with either M. anisopliae or B. bassiana (Mynone et al., 2010).

The anamorphic genus B. bassiana is globally ubiquitous and has a broad host range, although research into its fundamental ecology is limited (Roy & Cottrell, 2008; Vega et al., 2009; Roy et al., 2010) and its phylogeny and taxonomy are a source of considerable debate (Rehner & Buckley, 2005; Rehner et al., 2006). Indeed, B. bassiana comprises two distinct clades that cannot be separated morphologically (and it is, therefore, reasonable to refer to the genus only until further resolution of these clades is possible). Within these clades, genetic groups of B. bassiana appear to associate with particular host taxa (Couteaudier & Viaud, 1997; Berretta et al., 1998; Gaitan et al., 2002) and habitats (Bidochka et al., 2002; Ormond et al., 2010), although some isolates are generalist entomopathogens (Wang et al., 2003; Rehner & Buckley, 2005; Ormond et al., 2010). Recent studies suggest that isolates of B. bassiana can be placed along a nutritional continuum from commensal to saprobic to parasitic in environments both above and below the ground (Meyling & Eilenberg, 2006; Meyling & Pell, 2006; Meyling et al., 2006; Vega et al., 2009; Ormond et al., 2010). Parasitism by B. bassiana has been documented, by many, as a major mortality factor of overwintering coccinellids (Iperit, 1966; Ceryngier & Hodzic, 1996; Barron & Wilson, 1998; Ormond et al., 2006). We predict, therefore, that coccinellids should avoid virulent B. bassiana isolates within their environment to reduce transmission and infection. The ability to detect and avoid virulent isolates of B. bassiana, especially in the selection of an overwintering site, would increase fitness.

In this paper, we assess the ability of Coccinella septempunctata L. (Coleoptera: Coccinellidae) to detect and avoid B. bassiana on leaves, in soil and on mycosed conspecifics. To ensure that our experiments were ecologically relevant, we used densities of B. bassiana that represent those that would be expected in various field scenarios, such as on leaves after the application of a mycoinsecticide (Feng et al., 1994) and occurrence in natural soils (Ormond et al., 2010). We discuss the results in the context of the life history and current phylogenetic understanding of B. bassiana.

Materials and methods

Insects and plants

Adult C. septempunctata were collected from the field (Cambridgeshire, UK) during March and April 2006 and maintained in 9 cm Petri dishes (one male and one female per dish) in a controlled environment room (20 °C; 16:8 h L:D). Insects were provided with tissue paper as an egg-laying substrate and fed an excess of mixed instar pea aphids, Acyrthosiphon pisiun (Harris) (Homoptera: Aphididae), daily. The pea aphids were reared on broad bean plants (Vicia faba L. The Sutton) grown from seed in a glasshouse environment (20 °C; 16:8 h L:D). Coccinella septempunctata eggs were collected daily and maintained in the same controlled environment room until adult eclosion. Larvae were fed an excess of pea aphids and grain aphids, Sitobion avenae (F.) (Homoptera: Aphididae). Laboratory-reared adults were placed in cm Petri dishes (five ladybirds per dish) and fed an excess of pea aphids under the same environmental conditions. These adults were used in all experiments 1–3 weeks after eclosion.

For the experimental arenas, broad bean leaves were detached from plants (approximately 4 weeks old) and used immediately in experiments.

Fungus

Beauveria bassiana s.l. Anglia Ruskin University isolate B21 (originally isolated from adult C. septempunctata) was used in all the experiments. Stock cultures on Sabouraud dextrose agar (SDA) (Oxoid Ltd, Basingstoke, Hampshire, UK) were stored at 8 °C in 10% glycerol (Sigma-Aldrich, Gillingham, Dorset, UK) and subcultured onto SDA for experimental use. Subcultures (never more than two subcultures from isolation) were incubated in the dark at 22 °C. Conidal suspensions were prepared by scraping eight plates of fungal material (following 2–3 weeks’ growth on SDA) into 20 mL 0.03% Tween 80 (Sigma-Aldrich). The conidial suspension was vortexed for 2 min using a mini-vortexer (VWR, UK). Suspensions were filtered through four layers of sterilized muslin and the conidia concentration was determined using an improved Neubauer haemocytometer under a light microscope (x 400).

Production of cadavers

Adult C. septempunctata were dipped individually in B. bassiana suspensions (10⁶ conidia mL⁻¹) in 1.5 mL Eppendorf tubes and inverted five times (approximately 30-s
exposure). Inoculated adults were incubated individually in 3 cm Petri dishes under controlled conditions (22 °C; 16:8 L: D) for 5–10 days and fed an excess of pea aphids. On death, adult *C. septempunctata* were placed on damp tissue paper and maintained at high humidity (> 95% RH) to encourage sporulation. Uninfected adult *C. septempunctata* cadavers were produced by first dipping in 0.03% Tween 80 and then freezing to − 18 °C for 24 h.

**Effect of the presence of *B. bassiana* conidia on leaves, in soil or in mycosed conspecifics on the behaviour of *C. septempunctata***

The behavioural responses of *C. septempunctata* towards *B. bassiana* were assessed in a series of bioassays run in a controlled environment room (22 °C). Four *B. bassiana* treatments were used:

1. Broad bean leaves dipped completely or partially in a conidia suspension representing a field application of a mycoinsecticide (Feng *et al*., 1994).
2. Sterile soil inoculated with a conidia suspension representing the occurrence in natural soils (Ormond *et al*., 2010).
3. Cadavers of *C. septempunctata* sporulating with *B. bassiana* on soil.
4. *In vitro* cultured *B. bassiana* (fungal plug) on soil.

Before a bioassay, all insects were starved in individual 3 cm Petri dishes for 24–29 h (22 °C; 16:8 L: D) to increase foraging activity. The relative humidity in the arenas was high (> 75%) as a consequence of the water agar and soil substrates. There were 10 replicates for each sex in each experimental condition.

**Effect of the presence of *B. bassiana* conidia on broad bean leaves on the behaviour of *C. septempunctata* in choice and nonchoice bioassays**

Broad bean leaves were detached from the plant and either the right or the left side (mirrored by the mid-vein) at random was submerged in 0.03% Tween 80. The opposite half of the leaf was then submerged in a conidial suspension (decanted from a stock suspension to ensure that each leaf was dipped in a fresh conidial suspension and so received the same concentration of conidia) of *B. bassiana* (10⁶ conidia mL⁻¹). The leaves were left to air dry on tissue paper and then embedded in 2% tap water agar, abaxial side uppermost (Meyling & Pell, 2006), in a 9 cm triple vented Petri dish constituting a choice arena. For nonchoice arenas, the leaf was dipped entirely in *B. bassiana* conidial suspension or 0.03% Tween 80 with no inoculum (control).

A single *C. septempunctata* was introduced into the choice or the nonchoice experimental arena on the mid-vein of the leaf, just above the petiole. All treatments were performed within a single day and the sex of the chosen insect and the order in which the experimental treatments were observed were randomized. There were 10 replicates for each sex in each experimental condition. Observations were made for 10 min and the time spent by the insect on each side of the leaf or off the leaf was recorded to the nearest second. In addition, the following behaviours were recorded for the duration of the trial:

Active searching, which involved a combination of the following behaviours:

1. Intensive (slow directionless locomotion with frequent turning and examination of the substrate with antennae).
2. Extensive (rapid directional locomotion with very little turning behaviour or investigation of the substrate).

Or inactive behaviour, which involved a combination of the following behaviours:

1. Cleaning (grooming of limbs, body or antennae).
2. Resting (remaining still, often with legs retracted and head bent under thorax).

**Effect of the presence of *B. bassiana* conidia in soil on the behaviour of *C. septempunctata* in choice and nonchoice bioassay**

Soil from a commercial potting medium (58% loam, 25% peat, 17% sand + limestone) was sterilized in an autoclave (121 °C; 20 min). The water content of the soil was determined by weighing ten 10-g sample and then reweighing after 24-h drying in an oven at 120 °C. *Beauveria bassiana* conidial suspension (10⁸ conidia mL⁻¹) was added to 100 g volumes of soil to produce a soil concentration of 10⁷ conidia g⁻¹ of dry soil (representing occurrence in natural soil). Distilled water was subsequently added to ensure a final soil moisture content of 30% wet weight. Control soil was prepared as above, but sterile 0.03% Tween 80 was substituted for conidial suspension. Each arena consisted of a 9 cm triple vented Petri dish in which the inner surface of the side of the Petri dish was painted with Fluon® (Asahi Glass Fluoropolymers UK Ltd, Thornton Cleveleys, Lancashire, UK) to prevent *C. septempunctata* from climbing onto the lid of the dish. The arenas were divided by placing a 1 cm high strip of acetate across the centre of the Petri dish. Fungal inoculated soil was added to one side and control soil to the other (both to a depth of 0.5 cm). The acetate strip was removed immediately before each trial. In nonchoice arenas, either fungal inoculated soil or control soil filled the entire arena. A single *C. septempunctata* was introduced into the centre of the choice and nonchoice experimental arenas. All treatments were performed within a single day and the sex of the insect and the order in which the experimental conditions were presented was randomized. Observations were made for 10 min and the time each insect spent on either side of the experimental arena as well as the behavioural categories (as for leaf arenas) were recorded. There were 10 replicates for each sex in each experimental condition.
Comparison of the frequency of encounter with mycosed \((B. \text{ bassiana})\) \(C. \text{ septempunctata}\) or in vitro \(B. \text{ bassiana}\) or a dead \(C. \text{ septempunctata}\) by uninfected \(C. \text{ septempunctata}\)

Sterile (control) soil arenas were prepared as described above and two of the following objects (no choice) were placed, one each at opposite sides of the dish, 1 cm from the edge:

1. Adult uninfected \(C. \text{ septempunctata}\) killed by freezing (thawed for 30 min).
2. 5 mm\(^2\) \(B. \text{ bassiana}\) (isolate B21) fungal plug.
3. \(B. \text{ bassiana}\) mycosed cadavers of \(C. \text{ septempunctata}\).

A single live \(C. \text{ septempunctata}\) adult was introduced into the centre of each arena, mid-way between the two objects. All treatments were performed within a single day and the sex of the chosen insect and the order in which the experimental conditions were presented were randomized. Observations were made for 10 min and the number of encounters (investigation of the object with antennae or climbing on/over object) with each object was recorded. There were 10 replicates for each sex in each experimental condition.

Log-ratio analysis

Data describing temporal patterns in an animal’s utilization of a discrete number of geographical regions or behavioural options, either in a natural environment or in a laboratory assay, are described as ‘compositional data’. Compositional data analysis has a long history (Aitchison, 1986).

The key feature of such data is a lack of independence between the times an individual devotes to the options available; these are constrained to sum to the total time for which it is observed. In itself, this constraint causes little difficulty, conventionally overcome by directly modelling not the proportions of time allocated to the various options, but the ratios of pairs of these options, usually logarithmically transformed. Data transformed are unbounded; hence in a log-ratio analysis, the observed log-ratios are assumed to be normally distributed with constant, unknown variance and fitted with standard linear models (Aitchison, 1986).

Difficulty arises when, as in the present paper, one or more of the options are not chosen in some of the trials. A proportional use of zero leaves the log-ratio undefined, but excluding these trials risks bias, and nonparametric alternative analyses lack power. The problem is sometimes overcome by replacing the zero observations with an arbitrarily small positive number, but here, we prefer the more rigorous approach of Elston et al. (1996), in which observations for unutilized options are effectively regarded as censored observations bounded above by a small number. Thus, as ladybird behaviour was recorded to the nearest second, we regard unutilized options as censored observations within the range \((0–0.5)\). Retaining the assumption of normality, a log-likelihood for a set of observations is readily obtained (Elston et al., 1996) and easily maximized to obtain parameter estimates and, hence, expected times devoted to each option. Mathematically, the approach is most tractable when options are considered offered two at a time, avoiding the need for numerical integration otherwise required. Thus, the design in this paper allows all comparisons of interest to be made in this pairwise fashion. Closed-form estimates of relative preference do not exist; thus, likelihood maximization was carried out using an iterative procedure, and SEs were determined using an estimate of the Hessian matrix. Testing of hypotheses and model comparisons were carried out using standard likelihood-ratio tests.

The frequency with which uninfected \(C. \text{ septempunctata}\) encountered either mycosed \((B. \text{ bassiana})\) \(C. \text{ septempunctata}\) or in vitro \(B. \text{ bassiana}\) or a dead \(C. \text{ septempunctata}\) was analysed using a generalized linear model (GLM), assuming Poisson-distributed encounter frequencies and a log-link function. All log-ratio and GLM analyses were performed in \texttt{R} (Ihaka & Gentleman, 1996).

Results

Effect of the presence of \(B. \text{ bassiana}\) conidia on broad bean leaves on the behaviour of \(C. \text{ septempunctata}\) in choice and nonchoice bioassays

The time spent on the leaf by \(C. \text{ septempunctata}\) adults differed significantly between the three (control, choice, fungus) treatments \((\chi^2 = 163.97, \ P < 0.001; \text{Fig. 1})\). There was no difference in the time spent on the leaves by male and female \(C. \text{ septempunctata}\) in any of the three treatments (control: \(\chi^2 = 0.05, \ P > 0.05\); choice: \(\chi^2 = 0.29, \ P > 0.05\); fungus: \(\chi^2 = 0.95, \ P > 0.05\)). Ladybirds spent an estimated 33.4% of their total time on leaves that were only partially coated with fungal inoculums, significantly less than the 34.6% of time spent on the control leaves \((\chi^2 = 6.54, \ P < 0.05; \text{Fig. 1})\), but significantly more than the 0.7% of the time on leaves that had been completely exposed to a suspension of \(B. \text{ bassiana}\) conidia \((\chi^2 = 101.64, \ P < 0.001; \text{Fig. 1})\).

On leaves that had been partially dipped in \(B. \text{ bassiana}\) (choice), \(C. \text{ septempunctata}\) spent significantly more time on the control side than the fungus \((B. \text{ bassiana})\)-treated side \((\chi^2 = 57.51, \ P < 0.001; \text{Fig. 2})\).

Only active behaviours were observed on leaves that had been completely inoculated with \(B. \text{ bassiana}\) (Fig. 3), leading to failure in the models using these behavioural data to converge. Therefore, behavioural data from the completely inoculated leaf treatments were excluded from analyses and there was no significant difference in the time spent by...
C. septempunctata in active vs. inactive behaviours on the control and choice leaf treatments ($\chi^2 = 0.26, P > 0.05$).

**Effect of the presence of B. bassiana conidia in soil on the behaviour of C. septempunctata in choice**

*Coccinella septempunctata* adults spent almost three times longer on the control side compared with the *B. bassiana* side of the leaves assigned to the choice treatment. Bars = 95% CL.

*Coccinella septempunctata* adults rarely (< 1% of the time) engaged in inactive behaviour on either the *B. bassiana*-treated or control side of the soil arena, but significantly more time was spent in active behaviours by *C. septempunctata* on the *B. bassiana* (fungus)-treated side of the soil arena in comparison with the control side ($\chi^2 = 11.61, P < 0.001$; Fig. 4).

**Comparison of the frequency of encounter with mycosed (B. bassiana) C. septempunctata or in vitro B. bassiana or a dead C. septempunctata by uninfected C. septempunctata**

The Poisson model showed that *C. septempunctata* adults did not encounter the three objects (uninfected dead ladybirds, *in vitro B. bassiana* or sporulating conspecific ladybirds) with equal frequency ($\chi^2 = 9.72, P < 0.01$), and the fit of the model was good (residual deviance = 39.58, d.f. = 57). The estimated mean number of encounters in 10 min with a dead conspecific was 1.6.
overwinter in aggregations usually of up to 10 individuals (Majerus, 1994), but mycosed individuals were always found as isolated individuals at least 5 cm from the nearest aggregation (Iperli, 1966; Ormond, 2007).

Avoidance as a behavioural resistance strategy has been studied for a few insects. Lord (2001) demonstrated the inability of the ectoparasitoid C. tarsalis (Ashmead) (Hymenoptera: Bethylidae) to detect and avoid B. bassiana. In contrast, Meyling & Pell (2006) reported that A. nemorum exhibited avoidance of leaf surfaces contaminated with B. bassiana. Interestingly, the behaviour of A. nemorum was no different on soil inoculated with B. bassiana compared with control soil. In our study, we observed C. septempunctata adults avoiding B. bassiana in all test arenas; the sensitivity of C. septempunctata to B. bassiana in soil is not surprising, given that the soil is a major source of inoculum to overwintering C. septempunctata. In contrast, A. nemorum overwinters above ground and so the soil is a less relevant niche, in which to detect B. bassiana, for this true bug compared with C. septempunctata. It would be interesting to explore further the extent to which the context of the inoculum source, and the density, affects the interaction with susceptible host insects.

Genetic groups of B. bassiana associate with particular host taxa (Couteaudier & Viaud, 1997; Berretta et al., 1998; Gaitan et al., 2002; Wang et al., 2003; Rehner & Buckley, 2005), but can also vary across foliage types as well as between foliage and soil environments (Ormond et al., 2010). Thus, the situation is complex and generalist predators will encounter a diverse array of B. bassiana isolates. The isolate used in our studies was known to be pathogenic to C. septempunctata, but further research could assess whether such a predator responds uniformly to B. bassiana or whether the predators behaviour is modified according to the genotype of the pathogen. It would be particularly pertinent to examine the behavioural responses of ladybirds to isolates of commercial importance, such as Mycotrol (isolate GHA, Mycotech Corporation). Indeed, it could be predicted that the use of fungal pathogens augmentatively in the field will affect the foraging behaviour of generalist predators (Meyling & Pell, 2006). However, the interactions between nontarget insects, such as coccinellids, and the pathogen will be complex depending on abiotic and biotic factors. The interactions are likely to vary subtly, depending on the specific isolate; it is possible that a highly virulent isolate will elicit a more dramatic response than an avirulent isolate. Termites are able to distinguish between lethal and nonlethal isolates of M. anisopliae both on conspecifics and in their environment (Staples & Milner, 2000). The behavioural responses of hosts to fungal pathogens are also likely to be affected by the environmental context. Elliot et al. (2002) highlight the importance of extrinsic biotic and abiotic factors in determining the outcome of a

In contrast, only 0.65 and 0.8 encounters were made with the in vitro fungus and sporulating conspecific, respectively (Fig. 5), and these were not significantly different ($\chi^2 = 0.31, P > 0.05$).

**Discussion**

*Coccinella septempunctata* adults exhibited dramatic avoidance behaviours in response to the fungus *B. bassiana* on leaf surfaces, in soil and in mycosed *C. septempunctata*. High inoculum densities are required to cause mortality of *C. septempunctata* adults, but these could be achieved through extended contact with conidia during foraging on leaf surfaces, particularly after field application of a mycoinsecticide (Feng et al., 1994). Larvae are more susceptible to *B. bassiana* than adults (Roy et al., 2008) and, therefore, avoidance by adult beetles could also be related to reproductive strategies. It is interesting to note that both male and female *C. septempunctata* responded similarly in all experiments. *Beauveria bassiana* is a major overwintering mortality factor of *C. septempunctata*; 10–15% of adults succumbed to infection in a field study in England (Ormond et al., 2006). Avoidance of overwintering sites with high *B. bassiana* conidal densities would increase the survival of both sexes of *C. septempunctata* equally during dormancy. We demonstrated that *C. septempunctata* adults avoid soil inoculated with *B. bassiana* (at densities simulating field occurrence in natural soils) and also the frequency of encounter with a mycosed conspecific was less than with uninfected, dead conspecifics. Interestingly, field observations also highlighted the avoidance of mycosed conspecifics by seemingly uninfected conspecifics; *C. septempunctata*...
host–pathogen interaction. Fluctuating ambient temperatures increased the median survival time of the desert locust, Schistocerca gregaria, exposed to the pathogenic fungus, M. anisopliae.

Few studies assess the costs to host fitness of behavioural resistance. Harlequin ladybirds, Harmonia axyridis, have low susceptibility to an isolate of B. bassiana that infects other species of coccinellid, but the fecundity of inoculated harlequin ladybirds was reduced dramatically compared with control individuals (Roy et al., 2008). Although difficult to assess, understanding the fitness costs of avoidance behaviour is important when considering the evolutionary consequences of behavioural adaptations. Elliot et al. (2002) demonstrated that viable offspring were only produced by desert locusts able to fever; thus, the fitness benefit of this behavioural adaptation is apparent. Coccinellids contain a variety of bitter tasting and toxic substances that protect them from attack by predators and parasitoids (Ceryngier & Hodek, 1996; Karyistinou et al., 2004). There are very few detailed studies on coccinellid–pathogen interactions (Roy & Cottrell, 2008) and understanding is limited. However, like all insects, coccinellids have an innate immune system that they can use against immune assaults such as pathogen invasion (Rolff & Siva-Jothy, 2004). The insect immune system comprises various defence mechanisms including those modulated by haemocytes (Karyistinou et al., 2004) and humoral defence such as antibacterial peptides and the phenoloxidase enzyme cascade (Gillespie et al., 1997). It is very likely that behavioural avoidance will be less costly to fitness than utilizing valuable resources in an immune response. Fitness was not measured in our study, but should be a priority in future studies.

The mechanisms involved in the detection of B. bassiana by C. septempunctata were not examined in this study. It is likely that chemical cues are important. There are many studies that highlight the importance of chemical cues in triggering behavioural responses in insects (Roy et al., 1999, 2005; Dicke & Grostal, 2001; Nakashima et al., 2004; Baverstock et al., 2005; Meyling & Pell, 2006; Johne et al., 2008). Roy et al. (2005) have shown that entomopathogenic fungi produce a range of chemical volatiles. Coccinellids are known to use volatile chemicals produced by foraging larvae to assess patch quality (Doumbia et al., 1998). Johne et al. (2008) demonstrated the ability of the horse chestnut leaf miner (Cameraria ohridella) to distinguish between powdery mildew (Erysiphe flexuosa) and other phytopathogenic fungi primarily on the differing pattern of C8 compounds of these fungi. It would be interesting to assess whether particular chemicals are utilized, as signals of pathogenicity, by C. septempunctata and other predatory ladybirds. Some species of coccinellid are mycophagous and their behavioural responses to fungi are likely to differ from their predatory relations. Further studies could examine whether the detection and avoidance by C. septempunctata is specific to living B. bassiana conidia or whether heat-killed conidia also elicit a response. It would also be relevant to assess whether the behavioural response of C. septempunctata varies with the virulence of B. bassiana isolates (Mburu et al., 2009) or whether other entomopathogens (and, indeed, nonentomopathogenic fungi) are detected and avoided.

In conclusion, the response of C. septempunctata to B. bassiana was dramatic. This fungal pathogen poses a particular threat to C. septempunctata during the winter when this species of ladybird enters dormancy in leaf litter, a rich source of B. bassiana inoculum. The ability of C. septempunctata to avoid soil, in which the density of B. bassiana conidia is high, is an adaptation that will undoubtedly increase survival and ultimately fitness. We predict that such behavioural responses are widespread and driven by the high cost of fungal infection on a host.

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