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Horizontal transmission of a microsporidium from the convergent lady beetle, *Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae), to three coccinellid species of Nova Scotia

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Abstract

Convergent lady beetles, *Hippodamia convergens* Guérin-Méneville, are a popular choice for aphid control in North America. An unidentified microsporidium was found in *H. convergens* adults that were purchased from a commercial insectary in 2004. This study examined egg cannibalism and egg predation as a means of horizontal transmission of the unidentified microsporidium among *H. convergens* larvae and three coccinellid species found in Nova Scotia: *Coccinella septempunctata* (seven-spotted lady beetle), *C. trifasciata perplexa* (three-banded lady beetle), and *Harmonia axyridis* (multicolored Asian lady beetle). The microsporidium was transmitted with 100% efficiency when first instars fed on microsporidia-infected eggs. Mean spore count data from smear preparations of infected beetles suggest that the infection was as heavy in *C. trifasciata perplexa* (a native coccinellid) (11.2 ± 0.96 spores/100 μm^2) as it was in *H. convergens* (the natural host) (12.8 ± 1.16) but lighter in the introduced species *C. septempunctata* (7.5 ± 0.65) and *H. axyridis* (0.8 ± 0.11). For all of the beetle species examined, larval development was significantly longer for microsporidia-infected individuals than for their uninfected cohorts. The microsporidium had no effect on larval mortality. Based on the results of this study, field-collected *H. convergens* should be examined for microsporidia and uninfected individuals should be used to rear individuals for release in biological control programs. However, this is unlikely to happen because *H. convergens* are relatively easy and inexpensive to collect from their overwintering sites for redistribution.

Keywords: *Hippodamia*; *Coccinella*; *Harmonia*; Cannibalism; Host specificity; Microsporidia; Predation; Transmission

1. Introduction

Predaceous coccinellids are associated with biological control more often than other predatory organisms because they are important natural enemies of arthropod pests, including whiteflies, aphids, mealy bugs, scales, and mites (Obrycki and Kring, 1998). Convergent lady beetles, *Hippodamia convergens* Guérin-Méneville, are a popular choice for aphid control in North America because these beetles are easily collected and redistributed. Several billion *H. convergens* adults are collected annually from their overwintering sites in California. These beetles are then sold to commercial growers and home gardeners for augmentative biological control (Dreistadt and Flint, 1996).

An unidentified microsporidium was found in *H. convergens* that were purchased from a commercial insectary in 2004. Microsporidia appear to be common pathogens of predaceous coccinellids, having been reported from several field-collected species. The microsporidium *Nosema hippodamiae* Lipa and Steinhaus was described from *H. convergens* (Lipa and Steinhaus, 1959) and Sluss (1968) reported an unidentified microsporidium in both *H. convergens* and its endoparasitoid, *Dinocampus coccinellae* Shrank. It is not clear if this pathogen is *N. hippodamiae*. Two additional species of microsporidia have been reported from other field-collected coccinellids. *N. tracheophila* Cali and Briggs

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infects the seven-spotted lady beetle, *Coccinella septempunctata* L. (Cali and Briggs, 1967) and *N. coccinellae* Lipa is known to infect several coccinellid species, including *C. septempunctata* (Lipa, 1968; Lipa et al., 1975).

Although microsporidia have been reported from several coccinellid species (Lipa and Steinhaus, 1959; Cali and Briggs, 1967; Lipa, 1968; Lipa et al., 1975), little is known regarding the transmission routes and host specificity of these pathogens. Horizontal transmission often occurs when contaminated food is ingested (Andreadis, 1987; Tanada and Kaya, 1993) and many aphidophagous coccinellids are cannibalistic, feeding on their own eggs and larvae when aphids are in short supply (Agarwala, 1991, 1998; Agarwala and Dixon, 1992). Because the microsporidium in *H. convergens* is vertically transmitted with 100% efficiency (Joudrey, 2006), the cannibalistic tendencies of aphidophagous coccinellids may help facilitate the successful horizontal transmission of microsporidia among *H. convergens*, and from *H. convergens* to other coccinellid species.

In this study, egg cannibalism was examined as a means of horizontal transmission of an unidentified microsporidium among *H. convergens* larvae. Egg predation was used to determine if this microsporidium is transmitted horizontally to three aphidophagous coccinellids of Nova Scotia: *C. septempunctata* L. (seven-spotted lady beetle), *C. trifasciata perplexa* Mulsant (three-banded lady beetle), and *Harmonia axyridis* Pallas (multicolored Asian lady beetle).

2. Materials and methods

Uninfected and microsporidia-infected *H. convergens* were isolated from a shipment of beetles that was received from a commercial insectary in July 2004. Beetles were randomly selected for mating pairs. To confirm their infection status, we chose four to five eggs per cluster from egg-laying females (4–5 eggs per oviposition). These were smeared, stained with 5% buffered Giemsa (pH 6.9, Sigma Diagnostics), and examined for the presence of microsporidian spores by light microscopy.

Uninfected and microsporidia-infected individuals were maintained and reared in 120 mL clear, polyethylene cups (Canemco-Marivac Inc., QC). A 2.2-cm-diameter hole was made on the side of each cup, over which a fine mesh screen (80 μm, Bioquip, CA) was affixed. This provided air circulation and prevented the beetles from escaping. Cups were washed, soaked in a 10% bleach solution (10 min), rinsed, and then air-dried before each use. The inside of each lid was lined with a piece of filter paper (55 mm diameter) and beetles were supplied distilled water as needed through a moistened cotton roll (Crosstex International, NY).

Beetles were maintained on green peach aphids (*Myzus persicae* Sulzer) that were reared in environmental chambers under controlled conditions (16:8 L:D; 25 °C). Aphids were collected from rose bushes on the Saint Mary's University Campus and aphid colonies were reared on nasturtium (*Tropaeolum minus*, Dwarf Jewel Mix, Stokes Seeds Ltd., ON) that were grown from seed (16:8 L:D; 25 °C). When the aphid supply was low, beetles were fed an artificial diet that consisted of Lacewing and Ladybug Food (20 mL, Planet Natural, MT), pure honey (20 mL), and distilled water (2 mL). The diet was held in a sealed Petri dish under refrigeration. Prior to feeding, a small amount of the mixture was softened at room temperature and sterilized forceps were used to spread a small amount on the inside wall of an inverted scintillation vial lid. Lids with diet were placed inside each beetle rearing cup and new lids with diet were used to replace them as needed. *H. convergens* were reared in environmental chambers under controlled conditions (16:8 L:D; 25 °C).

Laboratory colonies of *C. septempunctata*, *C. trifasciata perplexa*, and *H. axyridis* were established from specimens collected on Saint Mary's University Campus. These beetles were maintained and reared in the same manner as were *H. convergens*. To ensure that these field-collected beetles were free of microsporidia, eggs and larvae were examined on a routine basis for microsporidian spores.

2.1. Horizontal transmission

The microsporidium in *H. convergens* is vertically transmitted with 100% efficiency (Joudrey, 2006); therefore, larvae and eggs that were used in the transmission trials were confirmed to be either microsporidia-free or microsporidia-infected by examining other eggs that were produced by the same parent female. Parent females were also examined for microsporidian spores at the conclusion of the trial. Smear preparations of eggs, larvae and females were stained in 5% buffered Giemsa (pH 6.9, Sigma Diagnostics) and examined by light microscopy.

The instruments used for transferring eggs and handling larvae (feather-weight forceps and a fine brush) were dipped in 70% ethanol (1 min), then rinsed in distilled water after each use. The entire procedure was carried out in a biological safety cabinet and control larvae were fed and observed before those of the treatment. Microsporidian spores are known to lose their viability when they are subjected to UV light or when they become desiccated (Maddox, 1977; Kelly and Anthony, 1979; Whitlock and Johnson, 1990); therefore, the interior surfaces of the cabinet were disinfected before and during the trial with 70% ethanol. This was followed by exposure to a germicidal UV light (Philips TUV36T5/SP (40W), 253.7 nm, 60 cm above working surface for 2 h) at the end of each daily observation period.

2.1.1. Horizontal transmission in Hippodamia convergens

Three eggs from uninfected *H. convergens* females were fed to one-day-old uninfected larvae ($n = 50$), which served as a reference (control) and microsporidia-infected eggs were fed to uninfected one-day-old *H. convergens* larvae ($n = 50$; treatment). All larvae used in this trial originated from five uninfected mating pairs.

Twenty polyethylene cups (10 control and 10 treatment) were prepared each day for five days. A sterile cotton roll was moistened with distilled water and placed in each cup. Three eggs (less than 24 h old) were transferred to the inside wall of each cup with a fine brush. A single larva was then transferred to each cup so that it was adjacent to the eggs. After 24 h had lapsed, the number of eggs eaten by each larva was recorded and uneaten eggs were removed. Larvae were fed an *ad libitum* diet of aphids and distilled water daily until the larvae completed their development and emerged as adults.

Data for individuals that did not eat any eggs during the first 24 h and those that died before their first molt were discarded. All other individuals (larvae, pupae, adults) were smeared, stained with 5% Giemsa, and examined for microsporidian spores by light microscopy. Presence or absence of spores was used to calculate percent horizontal transmission.

2.1.2. Horizontal transmission to three coccinellid species

H. convergens was used in this trial as a reference species to verify that the microsporidian spores within infected eggs were viable and able to infect the three other coccinellid species that were examined: *Coccinella septempunctata*, *C. trifasciata perplexa*, and *H. axyridis*.

The materials and methods used in this trial were similar to those outlined for *H. convergens* (Section 2.1.1). Six polyethylene cups (3 control and 3 treatment) were prepared for each species on each day. The procedure was repeated over 10 days until 60 uninfected larvae ($n = 30$ control and $n = 30$ treatment) of each species were set up. For each beetle species, uninfected one-day-old larvae that were used in this trial were obtained from five mating pairs. Larvae that failed to complete development, as well as those that completed development and emerged as adults, were smeared, stained and examined for microsporidian spores. Presence or absence of spores was used to calculate percent horizontal transmission. Larvae that did not eat any eggs during the first 24 h or had died before the first molt were not included in the analysis. Several individuals that died as pre-pupae or pupae were also excluded from the analysis because the cadavers of these individuals had become too dry to smear on microscope slides, making their examination difficult.

Spore counts were used to make inferences regarding the relative suitability of each beetle species as a host for the microsporidium. For each coccinellid species, microsporidian spores were quantified by counting the number of spores in smear preparations that were made from four adults from each treatment group. The dilution of spores during staining could have affected the number of spores observed. Prepared slides were randomly chosen; however, spores were counted only from individuals that had previously eaten three infected eggs as one-day-old larvae during the first 24 h of the trial. Spores were counted within 25 areas per smear (each area = 100 μm^2) that were randomly chosen on each slide ($n = 100$ counts/species; 1000× magnification). A photograph was taken at each location to ensure that spores were not counted twice from the same area.

The mean number of spores per area was calculated for each species. The raw data did not follow a normal distribution (D'Agostino and Pearson test, $P > 0.05$) and data was $\log_{10}$ transformed and then tested for equality of variances (Bartlett's test). Data from *H. convergens*, *C. septempunctata* and *C. trifasciata perplexa* had equal variances ($\chi^2 = 3.89$, $P = 0.14$) and were analyzed with a one-way ANOVA and Dunnett's multiple comparison test (Statistix 8, 2003) to determine significance ($P < 0.05$). Data from *H. axyridis* was excluded from the analysis because the variance of the transformed data was significant (Bartlett's test, $P < 0.05$).

2.2. Larval development

For each beetle species, mean developmental time (days) was calculated for individuals that completed development and emerged as adults. Only larvae that completed development and emerged as adults were used in the analyses. Data were checked for normality and a two-sample *t*-test was used to determine significance in larval development for *H. convergens* (first horizontal transmission trial; $P < 0.05$). A Mann-Whitney test was used to determine significance for *H. convergens*, *C. septempunctata*, *C. trifasciata perplexa* and *H. axyridis* (multi-species transmission trial; $P < 0.05$).

2.3. Larval mortality

Data on larval mortality were collected during both transmission trials and percentage larval mortality was calculated for each beetle species. Larvae that did not eat any eggs during the first 24 h of the study were not included in the analysis. A 2×2 χ^2 contingency table was used to determine significance between the controls and treatments for each beetle species.

3. Results

3.1. Horizontal transmission

Microsporidia were not detected in smear preparations made from the parent females that were used to produce one-day-old uninfected larvae for the transmission trials. These parent females had also produced the eggs that were fed to control larvae. Smear preparations of additional eggs and cohort larvae produced by these parent females were also free of microsporidian spores. Microsporidian spores were not detected during the routine examination of field-collected *C. septempunctata*, *C. trifasciata perplexa*, or *H. axyridis*. Spores were detected in all of the parent females that produced eggs as food for the one-day-old treatment larvae. All additional eggs and larvae that were examined from these microsporidia-infected females were also infected.

3.1.1. Horizontal transmission in Hippodamia convergens

Microsporidian spores were detected in all individuals (100% infection, $n = 43$) that fed on microsporidia-infected eggs during the first 24 h of the study (treatment, Table 1). Microsporidian spores were also detected in one individual from the control (2.4% infection, Table 1); however, this result was likely due to contamination because microsporidian spores were not detected in any of the eggs or cohort larvae produced by the same parent female, nor were microsporidia detected in the female herself. The source of contamination may have originated from the male parents because they were not examined for microsporidia. If one or more males were infected, they could have contaminated the rearing cups where the females oviposited.

3.1.2. Horizontal transmission to three coccinellid species

For all four beetle species examined, microsporidia were detected in all of the individuals that were fed microsporidia-infected eggs (treatment; 100% infection) (Table 1). In the case of *C. septempunctata*, microsporidian spores were also detected in one individual from the control (4.0% infection, $n = 25$).

The mean number of microsporidian spores observed in *H. convergens* was 12.8 ± 1.16 spores/100 μm^2. The mean number of spores observed from smear preparations of microsporidia-infected *C. septempunctata*, *C. trifasciata perplexa* and *H. axyridis* was 7.5 ± 0.65, 11.2 ± 0.96 and 0.8 ± 0.11 spores/100 μm^2, respectively ($n = 100$ for each species). With *H. convergens* as a reference (control), mean spore numbers were significantly different for *C. septempunctata* ($F = 15.4$; $df = 2$, 297; $P < 0.0001$); however, the mean number of spores observed in *C. trifasciata perplexa* did not differ significantly.

3.2. Larval development

During the first trial, *H. convergens* control larvae took significantly less time to develop than did the treatment larvae ($t = -2.68$, $df = 67$, $P = 0.005$) (Table 2). For all species examined in the multi-species trial, mean larval development (days) was significantly shorter for control larvae than for the treatment larvae (*H. convergens*, $W = 296.5$, $P = 0.001$; *C. septempunctata*, $W = 215.5$, $P = 0.001$; *C. trifasciata perplexa*, $W = 129.5$, $P = 0.002$; *H. axyridis*, $W = 449.5$, $P = 0.01$).

Table 1
Percent horizontal transmission of an unidentified microsporidium in *H. convergens* and three coccinellid species of Nova Scotia

	Control			Treatment		
	n	Number infected	Transmission (%)	*n*	Number infected	Transmission (%)
Hippodamia convergens[a]	42	1	2.4	43	43	100
H. convergens[b]	25	0	0	20	20	100
Coccinella septempunctata	25	1	4.0	24	24	100
C. trifasciata perplexa	26	0	0	26	26	100
Harmonia axyridis	23	0	0	23	23	100

[a] *Hippodamia convergens* in the first trial.
[b] *H. convergens* as a reference species in the multi-species trial.

Table 2
Total developmental time (days) and percent larval mortality for *H. convergens* and three coccinellid species of Nova Scotia

	Developmental time (days)			Larval mortality (%)		
	n	Mean ± SE	*P*-value	*n*	Mortality	*P*-value
Hippodamia convergens[a]						
Control	31	14.65 ± 0.12		43	27.9	
Treatment	38	15.11 ± 0.12	0.005	46	17.4	0.24
H. convergens[b]						
Control	22	13.60 ± 0.19		26	15.4	
Treatment	17	14.77 ± 0.18	0.001	26	34.6	0.11
Coccinella septempunctata						
Control	18	11.56 ± 0.15		25	28.0	
Treatment	17	12.53 ± 0.12	0.001	27	37.0	0.49
C. trifasciata perplexa						
Control	13	12.70 ± 0.17		28	53.6	
Treatment	15	13.80 ± 0.31	0.002	29	48.3	0.69
Harmonia axyridis						
Control	23	13.65 ± 0.19		23	0	
Treatment	23	14.09 ± 0.12	0.01	24	4.2	

[a] *Hippodamia convergens* in the first trial.
[b] *H. convergens* as a reference species in the multi-species trial. *Harmonia* mortality data could not be analyzed.

3.3. Larval mortality

There was no significant difference in larval mortality for *H. convergens* during the first trial ($\chi^2 = 0.89$, $df = 1$, $P = 0.24$) (Table 2). During the second trial, larval mortality did not differ between control and treatment larvae for *H. convergens* ($\chi^2 = 2.56$, $df = 1$, $P = 0.11$), *C. septempunctata* ($\chi^2 = 0.25$, $df = 1$, $P = 0.49$) or *C. trifasciata perplexa* ($\chi^2 = 0.16$, $df = 1$, $P = 0.69$). For *H. axyridis*, two cells had expected numbers less than five and in this case, a χ^2 approximation is probably invalid (Bailey, 1995).

4. Discussion

Studies that investigate the host specificity of microsporidia through the use of natural transmission methods are limited. Horizontal transmission in this study was investigated by allowing first instars to consume microsporidia-infected eggs. This is likely to occur under natural conditions because many predaceous coccinellids are cannibalistic (Agarwala, 1991, 1998; Agarwala and Dixon, 1992). Beetles consume eggs or larvae when prey availability is low and younger larvae are vulnerable to older ones. Results of this study suggest that if microsporidia-infected eggs or larvae are consumed by individuals of the four species of beetles examined, there is a high probability that the individual that ate them will become infected.

One previous study has investigated the horizontal transmission of a microsporidium in a coccinellid host under laboratory conditions. Cali and Briggs (1967) were able to infect adult *C. septempunctata* with *Nosema tracheophila* by directly feeding adult beetles a mixture of honey and spores. *C. septempunctata* is the natural host of *N. tracheophila* and the authors did not attempt to infect other coccinellids. In some cases, microsporidia may have a relatively broad host range when transmitted under natural conditions. For example, microsporidia isolated from European gypsy moth populations produced a variety of responses in nontarget Lepidoptera from North America (Solter et al., 1997) and the microsporidium *Brachiola algerae* (syn. *Nosema algerae*) is also known to have a wide host range (Vavra and Undeen, 1970; Undeen, 1976; van Essen and Anthony, 1976).

Studies have shown *H. convergens* to be ineffective for aphid suppression because beetles tend to disperse once they are released in the field (Kieckhefer and Olson, 1974; Dreistadt and Flint, 1996). However, *H. convergens* remains a popular choice for biological control because they are easy to collect and distribute. Even though the augmentative release of *H. convergens* may provide effective pest control in enclosed places such as greenhouses, it has been speculated that the distribution of *H. convergens* from California may result in the inadvertent importation and release of the microsporidian pathogen, *Nosema hippodamiae* (Obrycki and Kring, 1998). It is likely that this pathogen (or another microsporidium) has already been released in the field because the microsporidium examined in this study was detected in individuals from a shipment of beetles that was purchased from a commercial insectary for biological control. Furthermore, it is a common practice to release large quantities of *H. convergens* for aphid control in North America. Although microsporidia are known to cause chronic, debilitating disease that lowers host fitness (Tanada and Kaya, 1993), many species of microsporidia are thought to be host specific under natural conditions (Kluge and Caldwell, 1992), which may explain why *H. convergens* is still used for aphid control even though *N. hippodamiae* was discovered in 1959.

Microsporidia reported from field-collected coccinellids in previous studies are summarized in Table 3. Many of these descriptions were based on spore dimensions and light microscopic observations of pathogen development and tissue pathology. Based on their spore dimensions and the results of this study, one could speculate as to whether these microsporidia are distinct or if they represent one species that infects several different coccinellid hosts. To describe the microsporidium in this study, ultrastructural and molecular information are needed and tissue pathology must be described. Even with the acquisition of this information, it may be difficult to confirm if the microsporidium in this study represents a new species or one that has been described previously because of host overlap. For example, *C. septempunctata* is host to both *N. tracheophila* and *N. coccinellae* and was infected by the microsporidium in this study. Ultrastructural and molecular information are lacking for microsporidia reported in previous studies, which may make comparisons difficult. For the most part, microsporidia infect distinctive tissues in coccinellids (Table 3). Microsporidioses have been reported to progress along a similar course of development in both natural and non-target host individuals (Solter et al., 1997); therefore, tissue pathology studies may provide valuable information that can be used to identify the microsporidium in this study.

Although *C. trifasciata perplexa* is native to Canada, both *C. septempunctata* and *H. axyridis* are introduced species. It is not known if their introduction was intentional or accidental (Obrycki and Kring, 1998). Colonization of *C. septempunctata* and *H. axyridis* in North America has been reported since the 1970's and 1990's, respectively, and these two beetle species are known to suppress native coccinellid populations (Elliott et al., 1996; Snyder et al., 2004). Although all three species were found on the Saint Mary's University Campus, *H. convergens* are rarely found in Nova Scotia (Majka and McCorquodale, 2006). In the neighboring province of New Brunswick, *H. convergens*, *C. septempunctata*, *C. trifasciata perplexa*, and *H. axyridis* are known to occupy similar habitats and their seasonal occurrence is reported to overlap (Boiteau et al., 1999). Furthermore, the distribution of *H. convergens*, *C. septempunctata*, and *C. trifasciata perplexa* are reported to overlap in Manitoba (Turnock et al., 2003). These coccinellids may be exposed to (and feed upon) microsporidia-infected egg masses of *H. convergens* if infected beetles are released in the field.

Table 3
Microsporidia reported from field-collected coccinellids

Microsporidium and Host	Spore size (fixed and stained)	Tissues infected
Nosema hippodamiae (Lipa and Steinhaus, 1959) *Hippodamia convergens*	3.3–5.4 × 2.2–2.7 μm	Midgut, fat body
Nosema tracheophila (Cali and Briggs, 1967) *Coccinella septempunctata*	3.7 × 2.3 μm	Tracheal epithelium Blood cells Connective tissues
Nosema coccinellae (Lipa, 1968) *Adalia bipunctata* L.[a] *C. quinquepunctata* L.[a] *C. septempunctata* *Exochomus quadripustulatus* L.[a] *H. tredecimpunctata* L. *Myrrha octodecimguttata* L.	3.6–6.2 × 2.0–3.6 μm	Midgut epithelium Malpighian tubules Gonads, nerves Muscle tissues
Unidentified microsporidium (this study) *H. convergens* *C. septempunctata*[c] *C. trifasciata perplexa*[c] *Harmonia axyridis*[c]	3.9 × 2.5 μm[b]	

[a] Lipa et al., 1975.
[b] Joudrey (2006).
[c] Coccinellid hosts that have resulted from the consumption of infected *H. convergens* eggs.

Mean spore counts from infected larvae (Table 2) suggest that *C. trifasciata perplexa* (the only native coccinellid examined during this study) was as heavily infected as *H. convergens* (the natural host). Microsporidian spores were not as abundant in *C. septempunctata* or *H. axyridis*. This suggests that the latter two beetle hosts may not favor microsporidian development as much as *H. convergens* or *C. trifasciata perplexa*. *Harmonia axyridis* is a fairly robust and aggressive species (Koch, 2003), which may be explained, in part, by its ability to resist pathogens. *H. axyridis* has been shown to have low susceptibility to entomopathogenic nematodes and the fungus *Beauveria bassiana* (Cottrell and Shapiro-Ilan, 2003; Shapiro-Ilan and Cottrell, 2005).

In all cases, control larvae that were fed uninfected eggs developed significantly faster than did those that were fed microsporidia-infected eggs (treatment). These results are expected because microsporidia are known to cause chronic, sub-lethal effects that lower host fitness (Tanada and Kaya, 1993). In a previous study, this particular microsporidium reduced the fitness of *H. convergens* by lengthening the duration of larval development and by reducing the fecundity and survival of adults (Joudrey, 2006). Although no other studies have focused on the effects of microsporidia on the larval development of coccinellids, microsporidia are known to prolong larval development in other insects that are used for biological pest control (Zchori-Fein et al., 1992).

The microsporidium did not affect larval mortality in any of the beetle species examined during this study, including *H. convergens*, its natural host. However, other important life history parameters that are often used to measure host fitness (fecundity, adult survival, dispersal, prey consumption) were not examined. In relative terms, larval mortality was high for *C. trifasciata perplexa* larvae (both control and treatment) but was relatively low for *Harmonia axyridis* (Table 2). The surface of coccinellids eggs are known to be coated with species-specific alkaloids that may be toxic to other species (Cottrell, 2004, 2005; Omkar et al., 2004; Sato and Dixon, 2004); therefore, the relatively high larval mortality observed for *C. trifasciata perplexa* larvae may be caused by the consumption of the *H. convergens* eggs themselves, which may be toxic to *C. trifasciata perplexa*. The relatively low larval mortality observed for *H. axyridis* (control and treatment) suggests that *H. convergens* eggs are suitable as food for this particular beetle species. *H. axyridis* is a polyphagous species that is known to survive on the eggs of other coccinellids (Cottrell, 2004, 2005; Sato and Dixon, 2004). The polyphagous characteristic of this beetle, together with its apparent low susceptibility to pathogens (Cottrell and Shapiro-Ilan, 2003; Shapiro-Ilan and Cottrell, 2005), seem to favour *H. axyridis* with respect to interspecific competition.

The larvae in this study were given three eggs during the first 24 h of the trial (no-choice test) and it is possible that these larvae would not have eaten the eggs provided if suitable prey were also made available. The role of egg cannibalism and predation in the transmission of microsporidia among these beetle species in the field will not be fully understood until it is investigated.

The collection of *H. convergens* from overwintering sites may result in the inadvertent importation and release of

microsporidia in areas where these pathogens do not occur. Ideally, *H. convergens* that are used for biological control should be examined for microsporidia and uninfected individuals should be used to establish rearing colonies that can be used to produce uninfected beetles for distribution. This scenario is not likely to happen; however, because *H. convergens* are abundant in their overwintering sites and they are relatively easy and inexpensive to collect and distribute.

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