



Effects of a microsporidium from the convergent lady beetle, *Hippodamia convergens* Guérin–Méneville (Coleoptera: Coccinellidae), on three non-target coccinellids

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ABSTRACT

A microsporidium from *Hippodamia convergens* was transmitted horizontally to three non-target coccinellid hosts (*Adalia bipunctata* L., *Coccinella septempunctata* L. and *Harmonia axyridis* Pallas) under laboratory conditions. For all species examined, microsporidia-infected larvae took significantly longer to develop than did uninfected larvae but the microsporidium had no effect on larval mortality. Adult sex ratios of uninfected and microsporidia-infected adults were about 1:1 (♀:♂) and did not differ significantly. At the end of a 90-day trial, microsporidia-infected *H. convergens* produced significantly fewer eggs and did not live as long as uninfected individuals. Differences in fecundity and longevity were not observed for the three non-target coccinellids that were examined. Mean spore counts from smear preparations of microsporidia-infected *A. bipunctata* did not differ significantly from *H. convergens*, suggesting that *A. bipunctata* (a native coccinellid) is a suitable host for the microsporidium but infection was lighter in *C. septempunctata* and *H. axyridis* (introduced species). Vertical transmission of the pathogen was observed during the 90-day trial by examining eggs and larvae that were produced by microsporidia-infected adults. For all species examined, 100% vertical transmission of the pathogen was eventually observed. Three eugregarines were found in two adult *A. bipunctata*: Gregarine A trophozoites are similar in size to those of *Gregarina katherina* Watson (described earlier from *Coccinella* spp.), Gregarine B trophozoites are similar in size to those of Gregarine A but are morphology distinct, and Gregarine C trophozoites are similar in size to *G. barbarara* Watson (described earlier from *A. bipunctata*).

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1. Introduction

In North America, convergent lady beetles, *Hippodamia convergens* Guérin–Méneville, are collected each year from their overwintering sites in California. These beetles are packaged and sold to commercial growers and home gardeners for aphid control. Several natural enemies are associated with field-collected *H. convergens* (Bjørnson, 2008) and because beetles are not mass-reared or placed under quarantine prior to use, their natural enemies are inadvertently imported and released when beetles are used for biological control.

Three species of microsporidia have been described from coccinellids. In 1959, *Nosema hippodamiae* was described from field-collected *H. convergens* from California (Lipa and Steinhaus, 1959). Microsporidia are thought to be host specific under natural conditions (Kluge and Caldwell, 1992) and this may explain why *H. convergens* were collected and distributed for aphid control even after *N. hippodamiae* was discovered in 1959. *Nosema tracheophila* was later described from laboratory-infected *Coccinella septempunctata* L. (Cali and Briggs, 1967) and *N. coccinellae* Lipa was found

infesting several coccinellids that were examined from their overwintering sites (Lipa, 1968a; Lipa et al., 1975).

In a recent study, an unidentified microsporidium from *H. convergens* was transmitted to three non-target coccinellids (*C. septempunctata*, *C. trifasciata perplexa* Mulsant and *Harmonia axyridis* Pallas) when larvae were fed microsporidia-infected *H. convergens* eggs under laboratory conditions (Saito and Bjørnson, 2006). Successful pathogen transmission from *H. convergens* (the natural host) to other coccinellids raises questions regarding host specificity and the effects of this pathogen on non-target coccinellids. The objective of this study was to examine the effects of the microsporidium from *H. convergens* on life history characteristics (larval development and mortality, adult longevity and fecundity, sex ratio) of three non-target coccinellids: *Adalia bipunctata* L., *C. septempunctata*, and *H. axyridis*. Vertical transmission of the pathogen was also investigated.

Adalia bipunctata was chosen for this study because it is native to North America and is commercially available for biological control in both North America and Europe. Although *C. trifasciata perplexa* was the subject of a previous study (Saito and Bjørnson, 2006), this native coccinellid was difficult to rear and field-collected specimens were in short supply. Both *C. septempunctata* and *H. axyridis* are introduced species: the former was introduced

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into North America from Europe, whereas the latter is from Asia. Colonization of these two beetle species has been reported since the 1970's and 1990's, respectively (Obrycki et al., 2000) and they appear to be responsible for a recent and serious decline of indigenous coccinellid species (Cormier et al., 2000; Elliot et al., 1996; Koch, 2003; Obrycki et al., 2000; Turnock et al., 2003).

2. Materials and methods

Uninfected and microsporidia-infected *H. convergens* were obtained from a shipment of beetles that were purchased from a commercial insectary in July 2004. Beetles were reared in 120 mL clear, polyethylene cups (Canemco-Marivac Inc., QC) in environmental chambers (16L:8D; $25 \pm 1^\circ\text{C}$: $20 \pm 1^\circ\text{C}$). Each rearing cup had a 2.2-cm diameter hole in its side that was covered with a fine mesh screen (80 μm , Bioquip, CA). The screen allowed air to circulate but prevented beetles from escaping. Cups were washed, soaked in a 10% bleach solution (10 min), rinsed, and then air-dried before use. A piece of filter paper (55 mm diameter) was used to line the inside of each lid. 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 under controlled conditions (16L:8D; $25 \pm 1^\circ\text{C}$: $20 \pm 1^\circ\text{C}$). Aphid colonies were reared on nasturtium (*Tropaeolum minus* L., Dwarf Jewel Mix, Stokes Seeds Ltd., ON) that were grown from seed (16L:8D; $25 \pm 1^\circ\text{C}$: $20 \pm 1^\circ\text{C}$). When beetles were being maintained (when they were not used in trials or used to rear offspring), they were fed an artificial diet that consisted of Lacewing and Ladybug Food (20 mL, Planet Natural, MT), honey (20 mL), and distilled water (2 mL). This diet was nutritionally sufficient to keep beetles alive; however, it proved insufficient as a sole source of food for oviposition. Fresh diet was kept under refrigeration and was supplied to beetles as needed. Prior to feeding, a small amount of diet was softened at room temperature and sterilized spatulas were used to spread the diet on the inside wall of each rearing cup at the edge of the screen-covered hole.

Laboratory colonies of *A. bipunctata*, *C. septempunctata*, and *H. axyridis* were established from specimens collected on Saint Mary's University Campus. The progeny of these individuals were used to establish microsporidia-free beetle colonies that were maintained and reared in the same manner as was *H. convergens*. Eggs and larvae from these colonies were examined on a routine basis to ensure that individuals were free of microsporidia.

2.1. Horizontal transmission

During the experimental trials, all larval and adult coccinellids were fed an *ad libitum* diet of green peach aphids that was augmented with bird cherry-oat aphids (*Rhopalosiphum padi* L.; purchased from a commercial insectary) and rose aphids (*Macrosiphum rosae* L.; collected from shrub roses on the Saint Mary's University campus). Instruments used for transferring eggs, handling larvae, and feeding (feather-weight forceps, spatulas and fine brushes) were dipped in 70% ethanol (1 min), then rinsed in distilled water after each use to prevent contamination. Procedures (beetle feeding and observation) were carried out in a biological safety cabinet (Baker Company, SteriGARD III Advanced, SG403A). Control (uninfected) groups were fed before treatment (microsporidia-infected) groups. 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 with 70% ethanol, followed by exposure to a germicidal UV light (Philips TUV36T5/SP 40W, 253.7 nm,

60 cm above working surface) for 15 min before and after each daily observation period.

Larvae and eggs used to set up the trial were confirmed as either microsporidia-free or microsporidia-infected by examining other eggs and larvae that were produced by the same parent female. After enough eggs were collected for setting up the trial, parent females were also examined. Smear preparations of eggs, larvae and parent beetles were fixed in methanol, stained in 10% buffered Giemsa and examined for microsporidian spores by light microscopy. Throughout the trial, this staining methodology was used to prepare and examine individuals for microsporidian spores.

Hippodamia convergens was used as a reference species to verify that the microsporidian spores within infected eggs were viable and able to infect the non-target coccinellids species. For *H. convergens*, 14 uninfected mating pairs were used for rearing larvae that were used in the trial and 12 microsporidia-infected mating pairs were used to produce infected eggs that were fed to larvae from all treatment groups. Twelve uninfected mating pairs were used to rear *A. bipunctata*, *C. septempunctata*, and *H. axyridis* larvae.

For each species, one uninfected *H. convergens* egg was fed to each 1-day-old uninfected larva ($n = 50$ larvae), which served as a reference (control). Larval age was an important consideration because larvae finish consuming their egg shells and start searching for food when they are about 1-day-old. Conversely, one microsporidia-infected *H. convergens* egg was fed to each uninfected 1-day-old larva ($n = 50$ larvae; treatment).

Twenty polyethylene cups (10 control and 10 treatment) were prepared for each species on alternate days. This procedure was repeated five times until 100 uninfected larvae ($n = 50$ control and $n = 50$ treatment) of each species were set up. Larvae of all species were reared individually. A sterile cotton roll was moistened with distilled water and placed in each cup. One *H. convergens* egg (less than 24 h old) was transferred onto a piece of sterilized filter paper (6 mm diameter) that was pre-moistened with distilled water. The disk was placed carefully at the bottom of a rearing cup and one 1-day-old larva was transferred onto the paper so that it was adjacent to the egg. Larvae that did not eat the egg after 24 h had lapsed and those that died prior to their first molt were discarded. Larvae were then reared on an *ad libitum* diet of aphids and distilled water that were provided daily until the larvae completed development and emerged as adults. Larvae that failed to complete development were smeared and examined for microsporidian spores. Presence and absence of spores in all individuals (larvae that failed development, emerged males, emerged females that were employed in the following observations) were used to produce percent horizontal transmission.

2.2. Effects on larval development and mortality

For each species, a one-tailed *t*-test (control > treatment, $\alpha = 0.05$) was used to determine significance in larval development time (from first instar to adult emergence) between individuals from control and treatment groups. Adults were sexed following eclosion and this allowed male and female larvae to be differentiated. Larval development data were reanalyzed to determine the effect of the microsporidium on the development time of male and female larvae (uninfected male vs. uninfected female larvae; infected male vs. infected female larvae). Data were tested for normality (Shapiro–Wilk *W* test) and only individuals that completed their development and emerged as adults were included in the analysis. A χ^2 test ($\alpha = 0.05$) was used to analyze differences in larval mortality between control and treatment groups. Larvae that did not eat the egg after 24 h and those that died prior to their first molt were excluded from the analysis.

2.3. Sex ratio

Adults were sexed upon emergence and a χ^2 test was used to test significance ($\alpha = 0.05$) in sex ratios between control and treatment groups for each beetle species. After all beetles were sexed, newly emerged female beetles were mated and used in 90-day fecundity and longevity trials. Male beetles that emerged were smeared and examined for microsporidian spores.

2.4. Effects on adult fecundity and longevity

Newly emerged, virgin females were placed individually in cups and provided artificial diet and distilled water for four days. Females were fed aphids on the fifth day following emergence and they were mated on the sixth day. In the case of *H. convergens*, females were mated with uninfected males that were obtained from a shipment of beetles. For all other beetle species, virgin females were mated with uninfected and genetically distant males that originated from uninfected parents. Mating pairs were provided distilled water and an *ad libitum* diet of aphids. After mating, male beetles were smeared and examined for microsporidian spores. Mating periods were longer for some beetle species (24 h for *A. bipunctata*, 48 h for *H. convergens* and *H. axyridis*, and 72 h for *C. septempunctata*) to ensure that viable eggs were produced.

Mated females were provided distilled water and aphids daily for the duration of the trial and artificial diet was also provided as needed. Eggs were removed and counted daily. All of the females were smeared upon death or at the end of the trial and examined for microsporidian spores. Age-specific oviposition curves were constructed for each species and a one-tailed *t*-test (control > treatment, $\alpha = 0.05$) was used to determine significance in fecundity (grand means) between individuals from control and treatment groups. Data were tested for normality (Shapiro–Wilk *W* test) and non-normal data (*H. convergens* and *C. septempunctata*) were power-transformed. A χ^2 test ($\alpha = 0.05$) was used to test for significance in adult longevity by comparing the ratios of live and dead beetles between control and treatment groups at the end of the trial (90 days post-emergence).

2.5. Spore counts

Spore counts were used to determine the relative suitability of each beetle species as host for the microsporidium. For each species, smear preparations were made from randomly chosen females from the treatment groups ($n = 4$ per species). Heads and pronota were removed before the remainder of the body was smeared and examined for microsporidian spores. The mean ages of the examined females were 77.0, 72.0, 73.5, and 90.0 days (post-eclosion) for *H. convergens*, *A. bipunctata*, *C. septempunctata* and *H. axyridis*, respectively. Twenty-five areas (each area: $100 \mu\text{m}^2$) were examined from random sites on each slide (total $n = 100$ counts/species, $1000\times$ magnification). The mean number of spores per area was calculated for each species and spore counts from non-target species were compared to those of *H. convergens*. Raw spore count data were log transformed and re-assessed for normality (Shapiro–Wilk *W* test). Transformed data were analyzed for significance with a one-way ANOVA and Dunnett's multiple comparison test ($\alpha = 0.05$) (Statistix 8, 2003).

2.6. Vertical transmission

For each beetle species, eggs and larvae produced by females of the treatment groups ($n = 4$ for each species) were examined for microsporidian spores over the lifetime of each female. Microsporidian spores were easier to detect in first-instar beetle larvae than in eggs; therefore, eggs were isolated daily and allowed to hatch

(3–4 days). A maximum of 10 randomly selected larvae (or 10 eggs if they did not hatch) from each female were smeared on each day of the trial, and examined for microsporidian spores. Presence or absence of spores was used to determine daily percent vertical transmission (number of larvae that contained spores/total number of larvae examined) and to construct vertical transmission curves for each beetle species.

3. Results

3.1. Horizontal transmission

Microsporidian spores were not detected in smear preparations made from any of the parent males that were mated with female beetles during the trial. Female *H. convergens* that were used to produce uninfected eggs (that were fed to 1-day-old control larvae or used as first-instar larvae for the trial) were also free of microsporidian spores. To ensure that a low infection would not be overlooked, additional eggs and cohort larvae were also examined but spores were not observed in any of the smear preparations. Conversely, spores were detected in all *H. convergens* females that were used to produce eggs that served as food for 1-day-old treatment larvae. All additional eggs and larvae that were examined from these microsporidia-infected females were infected with spores.

At the end of the 90-day trial, microsporidian spores were not detected in *H. convergens*, *A. bipunctata*, *C. septempunctata*, or *H. axyridis* from the control groups including males, females, and larvae that failed to complete development ($n = 47, 42, 45$, and 44 , respectively). Microsporidian spores were detected in the majority of smear preparations of individuals from the treatment groups (*H. convergens*, 40 infected/46 total = 87% transmission; *A. bipunctata*, 35/38 = 92.1%; *C. septempunctata*, 40/46 = 87%; *H. axyridis*, 38/42 = 90.5%).

3.2. Effects on larval development and mortality

For all species examined, treatment larvae took significantly longer to develop than did control larvae (*H. convergens*, $t = -4.55$, $df = 81$, $P = 0.001$; *A. bipunctata*, $t = -3.78$, $df = 58$, $P = 0.001$; *C. septempunctata*, $t = -3.8$, $df = 61$, $P = 0.001$; *H. axyridis*, $t = -5.54$, $df = 71$, $P = 0.001$) (Table 1). There was no significant difference in larval development based on sex (uninfected male vs. uninfected female larvae; infected male vs. infected female larvae; $P > 0.05$).

Table 1

Developmental time (days) and percent larval mortality for *H. convergens* and three non-target coccinellids infected with a microsporidium under laboratory conditions

	Developmental time (days) ^a			Larval mortality (%)		
	<i>n</i>	Mean \pm SE	<i>P</i> -value	<i>n</i>	Mortality (%)	<i>P</i> -value
<i>Hippodamia convergens</i>						
Control	47	18.02 \pm 0.01		48	2.1	
Treatment	37	18.65 \pm 0.01	0.001	40	7.5	0.45 ^a
<i>Adalia bipunctata</i>						
Control	42	15.79 \pm 0.08		42	0	
Treatment	32	16.31 \pm 0.11	0.001	35	8.6	0.05 ^a
<i>Coccinella septempunctata</i>						
Control	34	17.74 \pm 0.16		45	24.4	
Treatment	32	18.69 \pm 0.19	0.001	40	20.0	0.62
<i>Harmonia axyridis</i>						
Control	43	18.12 \pm 0.11		44	2.3	
Treatment	36	19.08 \pm 0.13	0.001	38	5.3	0.46 ^a

^a Development time measured between first instar and adult eclosion.

^a Two cells (both control and treatment) had expected values less than five (results were likely invalid).

Significant differences in larval mortality were not observed. Results from *C. septempunctata* were valid ($\chi^2 = 0.24$, $df = 1$, $P = 0.62$); however, χ^2 results for the other beetle species were probably invalid since two cells (both control and treatment) had expected counts less than five (*H. convergens*, $\chi^2 = 0.56$, $df = 1$, $P = 0.45$; *A. bipunctata*, $\chi^2 = 3.75$, $df = 1$, $P = 0.05$; *H. axyridis*, $\chi^2 = 0.55$, $df = 1$, $P = 0.46$) (Table 1).

3.3. Sex ratio

Sex ratios of emerged adults were about 1:1 ♀:♂ (Table 2). Significant differences were not observed among individuals of the control and treatment groups (*H. convergens*, $\chi^2 = 0.82$, $df = 1$, $P = 0.37$; *A. bipunctata*, $\chi^2 = 0.07$, $df = 1$, $P = 0.79$; *C. septempunctata*, $\chi^2 = 0.001$, $df = 1$, $P = 0.99$; *H. axyridis*, $\chi^2 = 0.15$, $df = 1$, $P = 0.7$).

3.4. Effects on adult fecundity and longevity

Females from the treatment groups that were not infected with microsporidia at the end of the 90-day trial were excluded from the analysis (3 *H. convergens*, 3 *C. septempunctata* and 1 *H. axyridis*). Unidentified eugregarines were observed in a single *A. bipunctata* female (control) and one male (treatment). Although eugregarines are considered to be weak pathogens that cause little damage to host tissues (Tanada and Kaya, 1993), the infected female was also excluded from the analysis. Eugregarine trophozoites were described according to Clopton (2004).

Gregarine A ($n = 22$ trophozoites; Table 3) was observed in the *A. bipunctata* male. No associations (syzygy) were observed. Trophozoites stained lightly and were granular in appearance. The protomerite appeared broadly ovoid, being wider than long. The deutomerite was dolioform to elliptoid, and a single spherical nucleus was visible in more than half of the specimens (mean diameter, $8.4 \mu\text{m}$, $n = 12$). Gregarine B ($n = 34$) was observed in the *A. bipunctata* female. No associations were observed. Trophozoites of Gregarine B were similar in size to those of Gregarine A but the former had a distinctly smaller and more darkly stained deutomerite. The protomerite was ovoid and the deutomerite was panduriform. About one third of the deutomerites contained a single spherical nucleus (mean diameter, $7.8 \mu\text{m}$, $n = 11$). Gregarine C ($n = 13$) was also observed in the *A. bipunctata* female. Trophozoites of Gregarine C were much larger than those of Gregarines A and B and were nearly translucent and granular in appearance (Table 3). Syzygy was observed as bi-associations of primitives with satellite trophozoites of similar dimensions. The pro-

tomterite was shallowly ovoid, being wider than long. The majority of deutomerites were dolioform but some were pyriform or obpyriform, containing a single spherical nucleus (mean diameter, $11.9 \mu\text{m}$, $n = 9$).

Age-specific oviposition curves (mean eggs/day) were constructed for each beetle species (Fig. 1). During the 90-day trial, *H. convergens* females from the treatment group produced significantly fewer eggs than did those of the control group ($t = -2.0$, $df = 34$, $P = 0.03$). However, no significant differences in fecundity were observed between control and treatment *A. bipunctata*, *C. septempunctata*, and *H. axyridis* females ($t = -0.42$, $df = 33$, $P = 0.66$; $t = -0.63$, $df = 30$, $P = 0.27$; $t = -1.27$, $df = 35$, $P = 0.89$, respectively) (Table 2).

Percent mortality for all beetle species (Table 2) was calculated at the end of the trial. *H. convergens* females of the control group lived significantly longer than did females from the treatment group ($\chi^2 = 4.53$, $df = 1$, $P = 0.03$). Significant differences were not observed for *A. bipunctata* ($\chi^2 = 0.54$, $df = 1$, $P = 0.46$) and *C. septempunctata* ($\chi^2 = 0.05$, $df = 1$, $P = 0.83$). In the case of *H. axyridis* ($\chi^2 = 0.26$, $df = 1$, $P = 0.61$), two cells (for both the control and treatment) had expected numbers that were less than five and this would likely render the χ^2 approximation invalid.

3.5. Spore counts

Mean spore counts were used to determine the overall suitability of each beetle species as a host for the microsporidium (Table 2). Mean spore counts for *A. bipunctata* (134.4 ± 8.8 spores/ $100 \mu\text{m}^2$) did not differ significantly from *H. convergens* (101.5 ± 5.6), the natural host of the microsporidium; however, spore counts for *C. septempunctata* (32.4 ± 3.1) and *H. axyridis* (24.8 ± 3.3) differed significantly ($F = 176$, $df = 3$, $P < 0.0001$).

3.6. Vertical transmission

Percent vertical transmission data were acquired by examining eggs that were produced by randomly chosen females ($n = 4$ for each species) (Fig. 2). In the case of *H. convergens*, vertical transmission of the microsporidium was detected in 70% of the eggs that were produced on the first day of oviposition and almost 100% transmission was observed about 9 days after oviposition had begun. Microsporidian spores were also detected in about 37% of the eggs produced by *A. bipunctata* on the first day of oviposition. The number of eggs that contained spores increased until 26 days later when all were infected. For *C. septempunctata*, microsporidian

Table 2

Sex ratio, fecundity, percent adult mortality, and spore count data for *H. convergens* and three non-target coccinellids infected with a microsporidium under laboratory conditions

	Sex ratio (♀:♂)	Fecundity			Mortality (%)			Spore counts ($100 \mu\text{m}^2$)
		<i>n</i>	Mean \pm SE	<i>P</i> -value	<i>n</i>	Mortality (%)	<i>P</i> -value	Mean \pm SE
<i>Hippodamia convergens</i>								
Control	(22:25)	22	1458 \pm 86		22	50.0		
Treatment	(21:16)	21	1199 \pm 104	0.03	21	81.0	0.03	101.5 \pm 5.6
<i>Adalia bipunctata</i>								
Control	(21:21)	21	1327 \pm 105		21	47.6		
Treatment	(15:17)	15	1385 \pm 95	0.66	15	60.0	0.46	134.4 \pm 8.8 ^a
<i>Coccinella septempunctata</i>								
Control	(18:16)	17	2111 \pm 319		18	38.9		
Treatment	(17:15)	16	1900 \pm 270	0.27	17	35.3	0.83	32.4 \pm 3.1 ^b
<i>Harmonia axyridis</i>								
Control	(22:21)	22	2139 \pm 160		22	9.1		
Treatment	(20:16)	20	2381 \pm 104	0.89	20	5.0	0.61 ^a	24.8 \pm 3.3 ^b

^a Two cells (both control and treatment) had expected values less than five (results were likely invalid). ANOVA and Dunnett's multiple comparison with mean spore count of *H. convergens* as a reference.

^a No significant difference.

^b Significant difference ($P < 0.0001$).

Table 3
Mean dimensions ($\mu\text{m} \pm \text{SE}$) and measurement ratios of eugregarine trophozoites found in *Adalia bipunctata*

Gregarine	LP	LD	WP	WD	TL	Range (TL \times WD)	TLA	LP:TL	WP:WD
A (n = 22)	12.7 \pm 0.6	42.3 \pm 1.6	15.8 \pm 0.7	26.3 \pm 1.6	55.0 \pm 1.9	34.1–64.9 \times 16.5–46.0		4.3	1.7
B (n = 34)	9.2 \pm 0.3	25.2 \pm 0.7	9.5 \pm 0.3	14.8 \pm 0.6	34.4 \pm 0.9	24.4–44.9 \times 9.3–24.5		3.7	1.6
C Primate (n = 13)	14.3 \pm 1.4	86.3 \pm 6.6	29.2 \pm 3.3	51.2 \pm 5.6	100.7 \pm 7.3	62.2–137.2 \times 20.9–77.6	232.5	7.0	1.8
Satellite (n = 2)	18.0 \pm 8.6	113.7 \pm 2.1	46.0 \pm 7.8	70.1 \pm 0.3	131.8 \pm 10.6	121.2–142.4 \times 69.8–70.5		7.3	1.5

LP, protomerite length; LD, deuteromerite length; WP, primite width; WD, deuteromerite width; TL, total trophozoite length; TLA, total length of association.

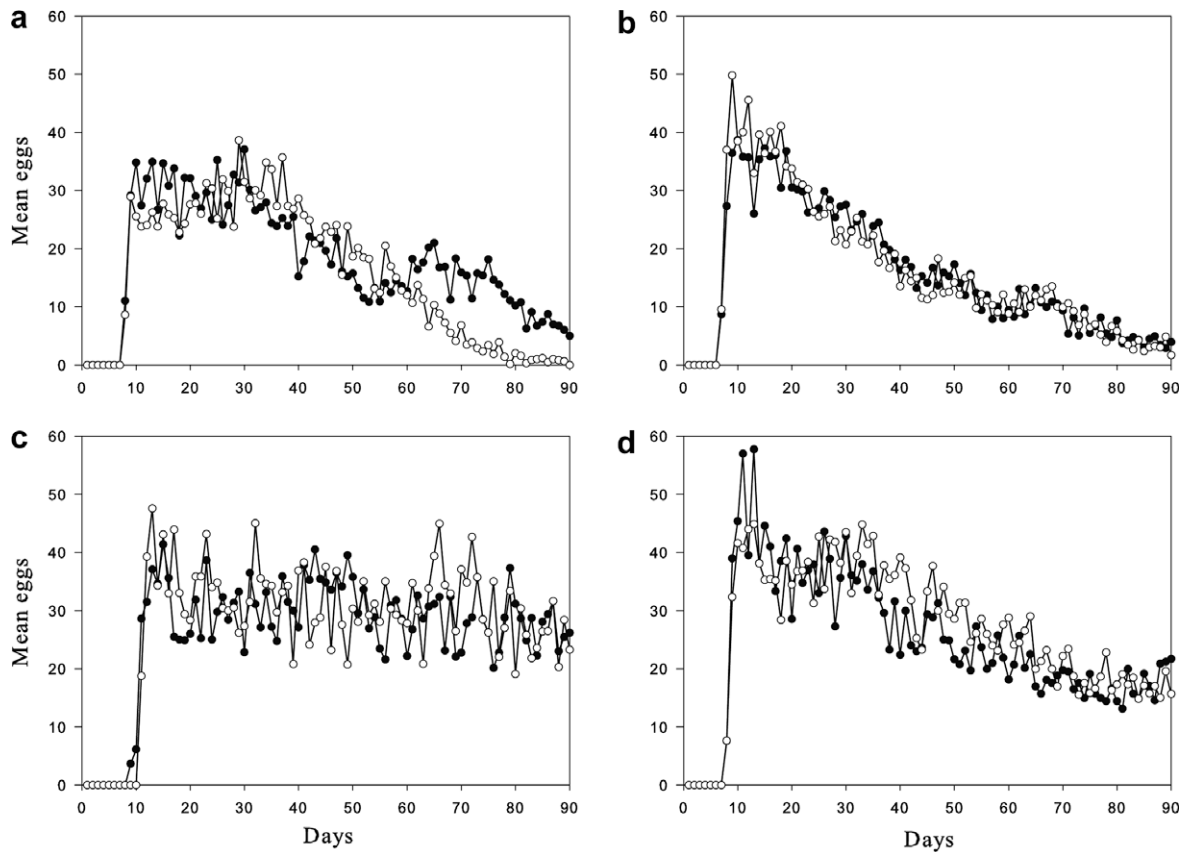


Fig. 1. Age-specific oviposition curves (mean eggs produced per day) of uninfected and microsporidia-infected females during a 90-day trial. Solid circles, uninfected; clear circles, microsporidia-infected. (a) *H. convergens* (n = 22 uninfected, 21 infected), (b) *A. bipunctata* (n = 21, 15), (c) *C. septempunctata* (n = 17, 16), (d) *H. axyridis* (n = 22, 20).

spores were detected in 12.5% of the eggs that were produced on the first day and in more than 80% of the eggs that were produced seven days later. In the case of *H. axyridis*, microsporidian spores were initially detected in only a few eggs (2.5%) but eventually spores were detected in all of the eggs that were produced (100%).

4. Discussion

Despite rigorous efforts to ensure that the non-target beetles used in the trials were free of microsporidia and other pathogens, three unidentified eugregarines were found in two adult *A. bipunctata*. Based on trophozoite dimensions, Gregarine A (34.1–64.9 \times 16.5–46.0 μm , TL \times WD) were similar in size to *Gregarina katherina* Watson (45–70 \times 20–34 μm ; Watson, 1915), described from *Coccinella novemnotata* Herbst, *C. septempunctata bruckii* Mulsant, *C. californica* Mann and *C. trifasciata* L. (Hoshide, 1951; Lipa, 1968b). Trophozoites of Gregarine B (24.4–44.9 \times 9.3–24.5 μm) have similar dimensions to those of Gregarine A but their morphol-

ogy was distinct. Although *G. ruszkowskii* is reported from *A. bipunctata* (Lipa et al., 1975), both Gregarine A and B were too small to be *G. ruszkowskii* (65–92 \times 30–83 μm , Lipa, 1967). Gregarine C (primate, 62.2–137.2 \times 20.9–77.6 μm) was similar in size to *G. barbarara* Watson, previously described from *A. bipunctata* (60–100 \times 40–60 μm ; Watson, 1916).

Horizontal transmission was lower during this study than was reported previously by Saito and Bjørnson (2006) and this may be due to a difference in spore dose. One microsporidia-infected *H. convergens* egg was fed to each treatment larva during this study but as many as three infected eggs (and presumably a larger spore dose) were eaten by beetle larvae in the previous study.

Larval development for microsporidia-infected larvae (treatment) was delayed by about one day when compared to uninfected larvae (control). Larval mortality, however, was not affected. These findings are consistent with those reported in a previous study (Saito and Bjørnson, 2006); however, *H. convergens*, *C. septempunctata* and *H. axyridis* larvae took longer to develop during this study

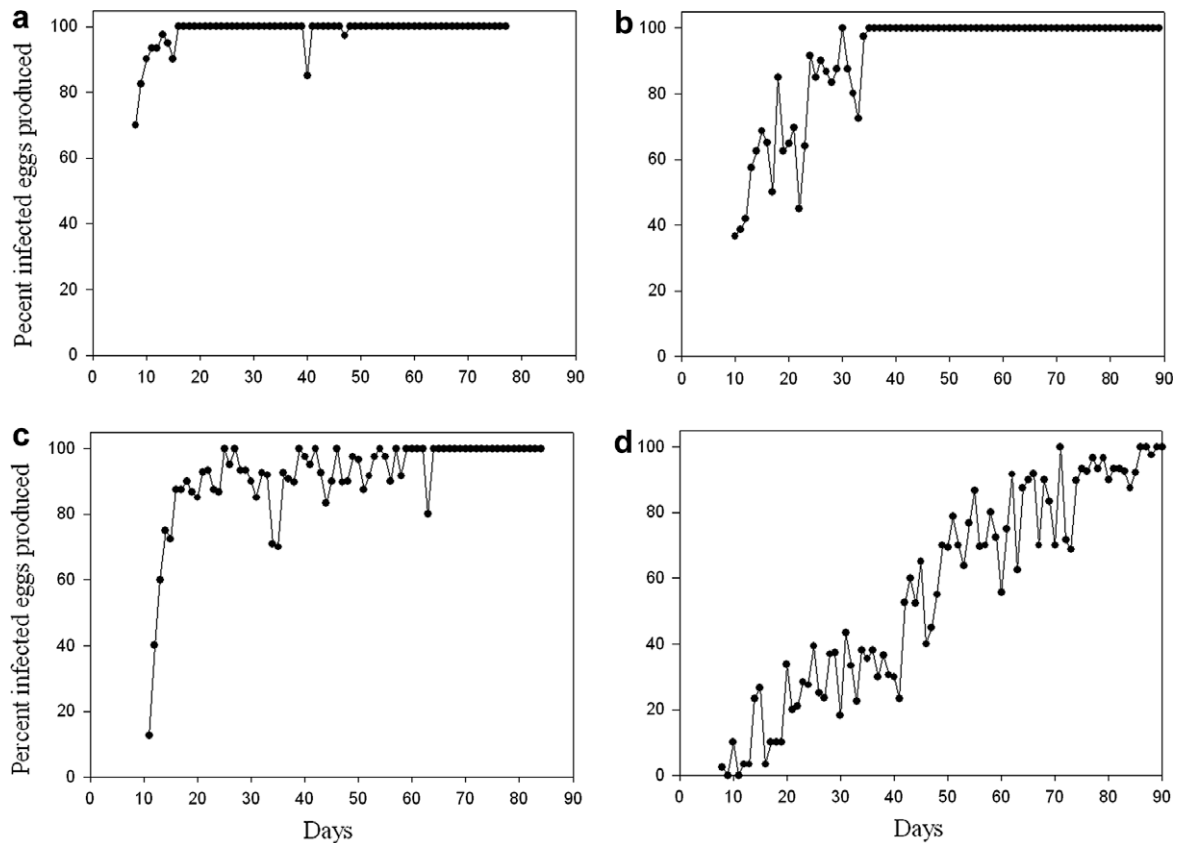


Fig. 2. Vertical transmission of microsporidia (percent microsporidia-infected eggs produced) by coccinellids that were infected with microsporidia under laboratory conditions (a) *H. convergens*, (b) *A. bipunctata*, (c) *C. septempunctata*, (d) *H. axyridis*. Beetles were mated six days following eclosion (Day 0).

than they did previously. Differences in development times may be attributed to different rearing temperatures that were used in the two studies. Larval mortality was observed for most species (both control and treatment) but larval mortality was relatively high for *C. septempunctata* larvae from both the control and treatment groups. High larval mortality for *C. septempunctata* larvae was also observed previously (Saito and Bjørnson, 2006). Coccinellid eggs are coated with species-specific alkaloids that may be toxic to other species (Cottrell, 2004, 2005; Omkar et al., 2004; Sato and Dixon, 2004) and this may explain the high mortality observed for *C. septempunctata* larvae. Larval mortality may also have been caused, in part, by the cherry-oat aphids (*Rhopalosiphum padi*) that were used for food in this study because this aphid species is considered to provide relatively low quality food for *C. septempunctata* (Hauge et al., 1998).

Results from this study suggest that *A. bipunctata* (a native species) was a suitable host for the microsporidium but low spore counts in *C. septempunctata* and *H. axyridis* (both introduced, highly invasive species) suggest that these two host species were less favorable for the reproductive success of the pathogen. Nevertheless, the microsporidium appeared to reproduce successfully during the adult life span in three host species. For *H. convergens*, newly emerged adults had 12.8 ± 1.16 spores/ $100 \mu\text{m}^2$ (Saito and Bjørnson, 2006), whereas 101.5 ± 5.6 spores/ $100 \mu\text{m}^2$ were observed in older adults from this study (mean age: 77 days post emergence). Similar comparisons may be made for *C. septempunctata* and *H. axyridis*. Newly emerged adults had 7.5 ± 0.65 and 0.8 ± 0.11 spores/ $100 \mu\text{m}^2$, respectively (Saito and Bjørnson, 2006) but older adults of the same species had 32.4 ± 3.1 (mean age: 74 days post emergence) and 24.8 ± 3.3 spores/ $100 \mu\text{m}^2$ (mean age: 90 days post emergence), respectively.

Although spore count data demonstrated that successful reproduction of the pathogen took place within the non-target hosts, it is difficult to explain why the non-target hosts were not adversely affected. Prolonged larval development was observed for all host species examined and this is typical of microsporidiosis. Furthermore, the consumption of one microsporidia-infected egg by first-instar larvae was sufficient to cause adverse effects in adult fecundity and longevity in the natural host (*H. convergens*).

The pathogen did not affect adult fecundity and longevity of the three non-target coccinellids but this may be explained, in part, by the conditions that beetles were subjected to during the trial. Adult beetles had constant access to clean water and were provided an *ad libitum* diet of aphids and artificial diet (pollen, yeast and honey). When microsporidia-infected beetles are fed artificial diet, the energy gained from the diet may compensate for the loss of energy caused by the microsporidium because honey and sugar provide energy (Hagen, 1962; Thompson, 1999) and pollen and fungi provide beetles with alternative sources of protein (Hagen, 1962). For example, *Coleomegilla maculata* (DeGeer) larvae are able to complete development when fed only pollen (Hodek, 1996) and larval survival, adult fecundity and percent egg hatch of *A. bipunctata* are improved when beetles are fed frozen pollen and *Ephestia kuehniella* Zeller eggs rather than when they are fed moth eggs alone (De Clercq et al., 2005). It has also been suggested that many polyphagous coccinellids, *A. bipunctata* and *H. axyridis* for example, employ a mixed feeding habit to select for a favorable balance of nutrients from various food sources, including plant materials (Hodek, 1996). Food quality appeared to increase beetle fecundity, whether or not individuals were ultimately infected with the microsporidium. Both uninfected and microsporidia-infected

H. convergens fed aphids and artificial diet during this study laid more eggs (grand mean of 1458 and 1199 eggs, respectively) than ones fed aphids only (*M. persicae*) (928 and 545 eggs, respectively; Joudrey and Bjørnson, 2007). These results suggest that the artificial diet was responsible for the increase in fecundity that was observed for *H. convergens*.

Although differences in prey species and environment must be considered, other coccinellid species examined in this study also performed better than they did during previous studies. Uninfected and microsporidia-infected *A. bipunctata* from this study, which were fed both aphids and artificial diet, produced an average of 1327 and 1385 eggs, respectively and 52.4% of the control and 40% of the treatment beetles survived until the end of the 90-day trial (Table 2). When fed only pea aphids, *Acyrtosiphon pisum* (Harris), *A. bipunctata* produce an average of 809.9 eggs and lived 70.5 days (16L:8D, 22 °C; Ueno et al., 2004). Similarly, both uninfected and microsporidia-infected *C. septempunctata* from the present study laid more eggs (mean: 2111 and 1900 eggs, respectively) and lived longer than they did during previous studies. In a study conducted by Kawauchi (1985), *C. septempunctata* produce 1660.5 eggs when fed cotton aphids, *Aphis gossypii* Glover (14L:10D, 25 °C). When fed cowpea aphids, *Aphis craccivora* Koch, *C. septempunctata* laid 1060.7 eggs and lived about 70 days (14L:10D, 25 °C; Omkar et al., 2005).

For all species examined, 100% vertical transmission was eventually observed (Fig. 2) and the transmission patterns appeared to reflect the susceptibility of a particular beetle species to the microsporidium. *H. convergens* (natural host, 101 spores/100 μm^2 , 70% initial vertical transmission that increased to 100% transmission 9 days after the first eggs were produced) was the best suited host for the pathogen but *A. bipunctata* (a native species) was the most susceptible non-target host (134 spores/100 μm^2 , 37% initial transmission that increased to 100% transmission 26 days later). The two introduced coccinellid species seemed to be less susceptible to the microsporidium: *C. septempunctata* (32 spores/100 μm^2 , 12.5% initial transmission that increased to 100% transmission 54 days later) and *H. axyridis* (25 spores/100 μm^2 , 2.5% initial transmission that increased to 100% transmission 79 days later). Long-term studies on vertical transmission of microsporidia are needed to assess the fate of these pathogens once they are released into new local environments.

Based on the results of this study, the host range of the microsporidium from *H. convergens* overlaps with the host ranges of *Nosema hippodamiae*, *N. coccinellae* and *N. tracheophila* reported in previous studies (Cali and Briggs, 1967; Lipa, 1968a; Lipa and Steinhaus, 1959; Lipa et al., 1975). Spore sizes of these microsporidia are similar but the geographical locality of these pathogens is distinct: *Nosema hippodamiae* and *N. tracheophila* are reported from the USA, whereas *N. coccinellae* is reported from Europe. *A. bipunctata* (a known host of *N. coccinellae*) are native to North America (Gordon, 1985) but this species may be purchased from Europe and imported to North America for aphid control. Therefore, it is possible for *N. coccinellae* to be transported from Europe to North America if microsporidia-infected *A. bipunctata* are imported for biological control programs. Although *N. coccinellae* is reported only from European populations of *A. bipunctata*, it is possible that the undescribed microsporidium in *H. convergens* originated from native *A. bipunctata* populations.

Microsporidiosis of non-target hosts have been observed in Lepidoptera (Solter et al., 1997; Solter and Maddox, 1998) and one could argue that host range expansion to non-target host species is unlikely to occur in a natural environment. In our study, however, the pathogen was readily transmitted to non-target coccinellids when they ate one microsporidia-infected *H. convergens* egg. Although effects on fecundity and adult longevity were not observed, larval development was delayed about one day, and per-

cent vertical transmission increased as the hosts aged, especially in the case of *C. septempunctata* and *H. axyridis* (Fig. 2).

Intraguild predation (IGP) and cannibalism are common among predaceous coccinellids (Agarwala, 1991, 1998; Agarwala and Dixon, 1992), not only when prey availability is low but also when prey are abundant (Gupta et al., 2006). Eggs and young larvae are especially vulnerable to older, larger ones. Microsporidia-infected larvae take longer to develop and cannibalism of infected larvae by uninfected ones may facilitate horizontal transmission of the microsporidium. The coccinellid species that were studied here overlap in their distributions and habitats in North America (Boiteau et al., 1999; Cormier et al., 2000; Gordon, 1985; LaMana and Miller, 1996; Majka and McCorquodale, 2006; Yasuda et al., 2004), and IGP among these species have been observed and studied (Kajita et al., 2000, 2006; Yasuda et al., 2004). Distribution overlap provides an opportunity for the microsporidium from *H. convergens* to expand its distribution and host range once infected *H. convergens* are released.

This study was designed to reduce environmental stresses so that the effects of microsporidiosis on beetle life history characteristics could be observed. However, such stresses may accentuate differences in fitness between uninfected and infected beetles. Adult beetles use energy searching for prey and for reproduction (Dixon, 2000). In their natural environment, beetles must fly and search for patchily distributed prey and reproduce with limited resources. Climactic conditions may be unfavorable for optimal growth and reproduction. Physical exercise, flight and heat may provide enough stress to decrease disease resistance (Adamo and Parsons, 2006); therefore, microsporidiosis may have a greater impact on the fitness of non-target coccinellids in their natural environment than was observed during this study.

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