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Short communication

Effects of an unidentified microsporidium on the convergent lady beetle, *Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae), used for biological control

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Abstract

Convergent lady beetles, *Hippodamia convergens* Guérin-Méneville, are collected from overwintering sites in California and redistributed for aphid control in home gardens and agroecosystems. The effects of an unidentified microsporidium on the life history characteristics of commercially available *H. convergens* were examined. Mean development for microsporidia-infected and uninfected *H. convergens* was 15.40 ± 0.14 and 14.76 ± 0.16 days, respectively ($P = 0.01$). Larval mortality did not differ significantly. Cumulative mean egg production for microsporidia-infected and uninfected females was 545.8 ± 92.6 and 928.3 ± 86.4 eggs, respectively ($P = 0.004$) and mean survival was 64.5 ± 5.6 and 77.1 ± 4.5 days, respectively ($P = 0.04$). Microsporidian spores (3.6×2.4 μm) are similar in size to those of *Nosema hippodamiae*.

Keywords: *Hippodamia convergens*; Microsporidia; Biological control; Development; Fecundity; Survival

1. Introduction

In North America, convergent lady beetles, *Hippodamia convergens* Guérin-Méneville, are frequently used for aphid control in home gardens and agroecosystems. Billions of *H. convergens* adults are collected annually from their overwintering sites in the Sierra Nevada Mountains of southern California. Insectaries then sell these beetles to commercial growers and home gardeners throughout the United States and Canada where they are released in large numbers (Obrycki and Kring, 1998).

Although the microsporidium *Nosema hippodamiae* was identified from *H. convergens* in 1959 (Lipa and Steinhaus, 1959), the effects of microsporidia on the life history characteristics of *H. convergens* have not been studied. In 2004, an unidentified microsporidium was found in *H. convergens* that were obtained from a commercial insectary for biological control. The objectives of this study were to investigate the effects of this unidentified microsporidium on life history characteristics of *H. convergens*. Larval development, adult fecundity and survival of microsporidia-infected *H. convergens* were compared to those of uninfected beetles.

2. Materials and methods

Microsporidia-infected and uninfected *H. convergens* were isolated from a shipment of beetles that was purchased from a commercial insectary (July 2004). Larvae and adults used in the trials were reared individually from eggs. To confirm infection, parent females were smeared and examined for microsporidian spores at the end of the trials. Because vertical transmission of this microsporidium is 100% efficient (Joudrey, 2006), additional eggs and larvae from these parent females were also examined to confirm infection.

Hippodamia convergens were reared individually in 120 mL clear, polyethylene cups (Marivac Ltd., QC).

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A 2.2-cm diameter hole was cut in the side of each cup and a fine mesh screen (80 μm; Bioquip, CA) was affixed to the perimeter. This allowed for air circulation and prevented the beetles from escaping. Cups were soaked in a 10% bleach solution (10 min) before use, then rinsed with distilled water and allowed to dry. A piece of filter paper (5.5-cm diameter) was used to line the lid of each cup. Instruments were surface sterilized before each use by dipping them in 70% ethanol.

Nasturtiums (Dwarf Jewel mixed; Stokes Seed Ltd., ON) were grown from seed (16L:8D, 25 ± 1 °C). Three-week old plants were used to rear green peach aphids (*Myzus persicae* Sulzer) which were then fed to *H. convergens* throughout the trials.

2.1. Larval development

Eggs were collected randomly from both uninfected and microsporidia-infected *H. convergens* females ($n = 5$ females, respectively). Individual eggs were isolated in polyethylene cups and newly hatched larvae were fed an *ad libitum* diet of *M. persicae* daily (16L:8D; 25 ± 1 °C). Distilled water was provided through a moistened cotton wick (Crosstex International, NY). Eggs and larvae were examined every 6 h and larval instar was recorded. Larvae that died before completing development were smeared, stained with 10% buffered Giemsa (pH 6.9, Sigma Diagnostics), and examined for microsporidian spores by light microscopy. Larvae that completed development and emerged as adults were sexed before being stained and examined. Spore measurements ($n = 50$) were taken from prepared slides.

For all analyses, data were checked for normality when relevant. A Mann–Whitney test was used to determine significance among development times for eggs, combined larval instars (1–4), combined prepupal and pupal stages, and total larval development. Only larvae and pupae that completed development were included in the analyses. A conservative testing procedure ($\alpha = 0.0125$) was used to protect against Type I errors. A $2 \times 2\chi^2$ contingency table was used to determine significance for sex ratios and larval mortality.

2.2. Adult fecundity and survival

Eggs clutches were isolated in polyethylene cups and newly hatched larvae were reared to adult on an *ad libitum* diet of *M. persicae* (16L:8D; 25 ± 1 °C). Following eclosion, adult females were isolated in cups for 5 days on an artificial diet that consisted of Lacewing and Ladybug Food (10 mL, Planet Natural, MT), pure honey (10 mL) and distilled water (1 mL). Water was supplied daily through a moistened cotton wick. From the sixth day on, females were supplied an *ad libitum* diet of green peach aphids. Females were then mated by placing a male of unknown age with a virgin female for 24 h. Males were smeared, stained and examined for microsporidian spores by light microscopy. Females were examined daily and the number of eggs that were produced was recorded. Upon death or at the end of the 90-day trial, each female was stained and examined.

A two sample *t*-test was used to determine significance for cumulative mean eggs produced by uninfected and microsporidia-infected females. A Mann–Whitney test was used to determine significance for mean survival, whereas a $2 \times 2\chi^2$ contingency table was used to analyze survival.

3. Results

3.1. Larval development

No significant difference was detected for development times between microsporidia-infected and uninfected eggs ($U = 1590$, $P = 0.013$; Table 1). Development times were significantly longer for microsporidia-infected *H. convergens* larvae and pupae than for uninfected individuals ($U = 372.5$ and $U = 331$, respectively; $P = 0.001$). Total development times were significantly longer for microsporidia-infected larvae ($U = 277$, $P < 0.001$); however, larval mortality did not differ significantly ($\chi^2 = 0.40$, $df = 1$, $P > 0.05$; Table 1).

Sex ratios were 1.06:1 (17♀:16♂) and 1.25:1 (20♀:16♂) for microsporidia-infected and uninfected *H. convergens*, respectively ($\chi^2 = 0.11$, $df = 1$, $P > 0.05$). Microsporidian spores from prepared slides measured 3.6 × 2.4 μm ($n = 50$).

Table 1
Larval development and mortality for microsporidia-infected and uninfected *H. convergens*

	Mean development ± SE (days)				Mortality (%)	
	Uninfected	*n*	Infected	*n*	Uninfected	Infected
Egg	3.32 ± 0.02	51	3.27 ± 0.02a	51		
1st instar	2.49 ± 0.04	44	2.50 ± 0.05	39	13.7	23.5
2nd instar	1.82 ± 0.11	41	1.84 ± 0.04	38	6.8	2.6
3rd instar	1.98 ± 0.03	38	2.05 ± 0.05	37	7.3	2.6
4th instar	3.27 ± 0.08	38	3.69 ± 0.14b	37	0.0	0.0
Prepupa	0.91 ± 0.02	37	0.97 ± 0.03	37	2.6	0.0
Pupa	4.46 ± 0.03	36	4.54 ± 0.03b	33	2.7	10.8
Total	14.76 ± 0.16	36	15.40 ± 0.14b	33	29.4	35.3a

a, Not significantly different (development $P > 0.0125$; larval mortality, $\chi^2 = 0.40$, $P > 0.05$). b, Significantly different (combined larval instars 1–4, combined prepupal and pupal stages, and total larval development, $P < 0.0125$).

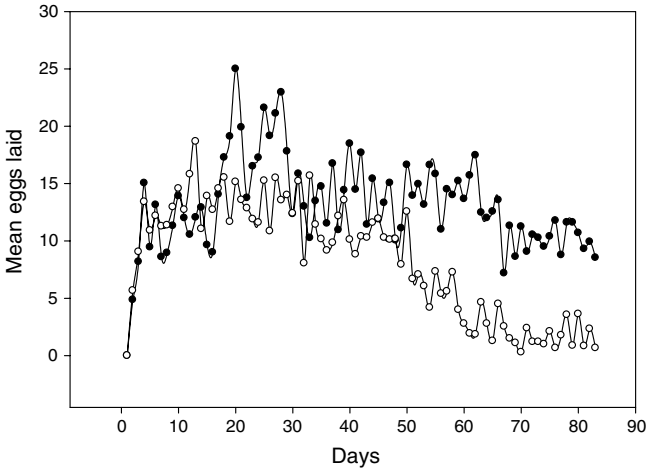

Fig. 1. Age-specific oviposition curves showing mean eggs laid per day by uninfected and microsporidia-infected *H. convergens*. Solid circles, uninfected; clear circles, microsporidia-infected.

3.2. Adult fecundity and survival

Cumulative mean egg production was 545.8 ± 92.6 and 928.3 ± 86.4 eggs for microsporidia-infected ($n = 24$) and uninfected ($n = 22$) females, respectively ($t = 3.006$, $df = 44$, $P = 0.004$). Age-specific oviposition curves are shown in Fig. 1.

Mean survival for microsporidia-infected and uninfected beetles was 64.5 ± 5.6 ($n = 24$), and 77.1 ± 4.5 days ($n = 22$), respectively ($U = 354.5$, $P = 0.04$). At the end of the 90-day trial, 59% (13/22) of the uninfected females were still alive, whereas only 25% (6/24) of the infected females had survived ($\chi^2 = 5.50$, $df = 1$, $P < 0.05$).

4. Discussion

Although microsporidia are known to infect aphidophagous coccinellids (Cali and Briggs, 1967; Lipa, 1968; Lipa et al., 1975), the effects of microsporidia on life history characteristics have not been studied. In this study, microsporidioses delayed the development of *H. convergens* larvae. Lifetime fecundity data for both microsporidia-infected and uninfected *H. convergens* are within the range previously reported by Hagen and Sluss (1966). However, microsporidia-infected *H. convergens* produced fewer eggs per day and fewer eggs overall than did uninfected females. Mean survival was significantly lower for microsporidia-infected females than for uninfected females. Sex ratios for both microsporidia-infected and uninfected *H. convergens* were about 1:1, which is consistent with those reported for *H. convergens* in earlier studies (Smith, 1966; Heimpel and Lundgren, 2000).

When microsporidia-infected individuals are contrasted with uninfected ones, the effects caused by the microsporidium become more apparent. Based on the mean data collected during this study, uninfected adult females live about 77 days and produce about 930 eggs. Microsporidia-infected females live 64 days and produce approximately 545 eggs, 41% fewer eggs than are produced by uninfected females. Uninfected larvae develop to adults within 14.76 days, whereas infected larvae take 15.40 days to develop (Table 1). Prolonged development, observed for microsporidia-infected larvae, may increase their susceptibility to predation.

The release of microsporidia-infected *H. convergens* for biological control may result in the dissemination of microsporidia into new areas and potentially to new coccinellid hosts. For example, *H. convergens* is rarely found in Nova Scotia, with the first and only specimen collected in Halifax in 2001 (Majka and McCorquodale, 2006). However, *H. convergens* are commercially available for aphid control, and under laboratory conditions, the microsporidium in *H. convergens* has been transmitted successfully to three coccinellids of Nova Scotia: *Coccinella septempunctata* (seven-spotted lady beetle), *C. trifasciata perplexa* (three-banded lady beetle), and *Harmonia axyridis* (multicolored Asian lady beetle) (Saito and Bjørnson, in press).

Two of four species of microsporidia identified from predacious coccinellids have been reported from *H. convergens*. In 1959, *N. hippodamiae* Lipa and Steinhaus was observed in midgut and fat body (Lipa and Steinhaus, 1959), whereas Sluss (1968) reported an unidentified microsporidium in both *H. convergens* and its endoparasitoid, *Dinocampus coccinellae* (Schrank). In the latter case, spore-like bodies were observed in several tissues, including fat body, muscle, gut, Malpighian tubules, and testes (Sluss, 1968) but it is unclear if the pathogen is *N. hippodamiae* or another microsporidium. *N. tracheophila* Cali and Briggs has been observed in the tracheal epithelium and connective tissues of the seven-spotted lady beetle, *C. septempunctata* L. (Cali and Briggs, 1967) and *N. coccinellae* Lipa is known to infect the midgut epithelium, Malpighian tubules, gonads, nerves, and muscle tissues of *C. septempunctata*, *Hippodamia tredecimpunctata* L., and *Myrrha octodecimguttata* L. (Lipa, 1968).

Nosema hippodamiae spores measure 3.3–5.4 × 2.2–2.7 μm (fixed and stained; Lipa and Steinhaus, 1959). Microsporidian spores from *H. convergens* in this study measured 3.6 × 2.4 μm and although these spores are within the range reported for *N. hippodamiae*, they are also similar in size to those of *N. tracheophila* (3.7 × 2.3 μm; Cali and Briggs, 1967). *N. tracheophila*, however, is not known to infect *H. convergens*. Molecular characterization, pathogen ultrastructure and tissue pathology studies are needed to identify the microsporidium in this study as *N. hippodamiae*, *N. tracheophila* or another microsporidium.

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