Journal of Invertebrate Pathology 99 (2008) 294–301

Contents lists available at ScienceDirect

Journal of Invertebrate Pathology

journal homepage: www.elsevier.com/locate/yjipa

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

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ARTICLE INFO

Article history:
Received 7 May 2008
Accepted 27 August 2008
Available online 6 September 2008

Keywords:
Adalia bipunctata
Coccinella septempunctata
Harmonia axyridis
Hippodamia convergens
Coccinellids
Microsporidia

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*).

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 infecting 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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doi:10.1016/j.jip.2008.08.005

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 ± 1 °C:20 ± 1 °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 ± 1 °C:20 ± 1 °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 ± 1 °C:20 ± 1 °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, SterilGARD 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.