Available online at www.sciencedirect.com
ScienceDirect
Biological Control 44 (2008) 305–311
Biological Control
www.elsevier.com/locate/ybcon

Natural enemies of the convergent lady beetle, *Hippodamia convergens* Guérin-Méneville: Their inadvertent importation and potential significance for augmentative biological control

S. Bjørnson *

Department of Biology, Saint Mary's University, 923 Robie Street, Halifax, NS, Canada B3H 3C3

Received 1 July 2007; accepted 2 October 2007
Available online 11 October 2007

Abstract

Each year, *Hippodamia convergens* adults are collected from their overwintering sites in California and made commercially available for aphid control in agriculture and in home gardens. This study examines the prevalence of natural enemies encountered in commercially available *H. convergens* from three commercial suppliers. Microsporidia were detected in individuals from 13 of 22 shipments (0.9% prevalence, range 0–3%). Spore dimensions ($3.9 \pm 0.1 \times 2.5 \pm 0.1$ μm; $n = 50$) were similar to those of *Nosema hippodamiae* (reported previously from *H. convergens*), *Nosema tracheophila* and *Nosema coccinellae* (reported from other coccinellids). Because *H. convergens* are sold in large quantities, thousands of microsporidia-infected beetles could be released each time *H. convergens* are used. Three distinct eugregarines were observed in *H. convergens* (0.2% prevalence) but none were similar in size to *Gregarina barbarara*, the only eugregarine reported from *H. convergens.* Although eugregarines are usually considered as mutualist symbionts or weak pathogens, the relationship between eugregarines and coccinellids is not fully understood. The hymenopteran parasitoid, *Dinocampus coccinellae*, was found in beetles from all shipments (8% mean parasitism, range: 3–15%). Significantly more females were parasitized than males. The importation and release of *D. coccinellae* undermine the success of biological control programs and may artificially increase the number of parasitoids in an area where *H. convergens* are released. *Verticillium* sp. (2% prevalence; range: 0–9%) was also observed but its role is unknown. Beetle quantities and sex ratios were also assessed.

Keywords: *Hippodamia convergens*; *Dinocampus coccinellae*; Eugregarines; Microsporidia; Natural enemies; Biological control

1. Introduction

Convergent lady beetles, *Hippodamia convergens* Guérin-Méneville, were first used for augmentative biological control in the early 1900s when beetles were collected from their overwintering sites in the Sierra Nevada Mountains of California for controlling outbreaks of melon aphid (*Aphis gossypii* Glover) on cantaloupe (Carnes, 1912). The use of *H. convergens* for biological control has since become a traditional practice and beetles are readily available for aphid control in agriculture and in home gardens.

Like many other lady beetles, *H. convergens* are known to host a variety of invertebrate parasites, including a braconid parasitoid (*Dinocampus coccinellae* Schrank), two encyrtid parasitoids (*Homalotylus terminalis terminalis* (Say) and *H. terminalis californicus* Girault) (see Richerson, 1970; Ceryngier and Hodek, 1996) and a parasitic mite (*Tetrapolipus hippodamiae* McDaniel and Morrill) (McDaniel and Morrill, 1969). A variety of microorganisms have also been observed in *H. convergens*, including the microsporidium *Nosema hippodamiae* Lipa and Steinhaus (Lipa and Steinhaus, 1959; Lipa and Semyanov, 1967), two unidentified microsporidia (Saito and Bjørnson, 2006; Sluss, 1968), the eugregarine *Gregarina barbarara* Watson (Lipa, 1968b) and an unidentified bacterium (Miller and Thompson, 1927).

* Fax: +1 902 420 5261.
E-mail address: susan.bjornson@smu.ca

1049-9644/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.biocontrol.2007.10.001

Reports of endoparasitoids and pathogens in *H. convergens* have not hampered the widespread practice of collecting, shipping and releasing beetles for augmentative biological control. However, the types of natural enemies that are inadvertently imported and the proportion of beetles that harbor them are unknown. This study examines the prevalence of natural enemies encountered in commercially available *H. convergens* from three commercial suppliers of natural enemies. Beetle quantities and sex ratios were evaluated for *H. convergens* immediately following shipment.

2. Materials and methods

Hippodamia convergens were obtained from three commercial sources on a biweekly basis from mid-July to mid-December 2004. Beetles were ordered according to the smallest quantity that was available from each source. Each shipment from Source A was expected to contain 500 beetles, whereas shipments from Source B and C were expected to contain 1000 and 1500 beetles, respectively. Adult beetles from Source A were purchased online and shipped by Priority Post (Canada Post Corporation), whereas those from Source B were shipped directly to a horticultural retail outlet where they were kept under refrigeration until they were picked up in person. Beetles from Source C were shipped by overnight courier. A total of 22 shipments were received from all sources: 10 shipments from Source A, three from Source B (beetles from this source were unavailable after August 2004), and nine from Source C. Suppliers were aware of the destination of their products but were unaware that beetles were involved in a study regarding quality.

Upon arrival, beetles from each shipment were removed from their packaging materials (a small burlap sack containing paper strips or wood shavings) and released into a 30.5 cm^3 cage fitted with 70 μm mesh netting and a clear plastic side panel (Bioquip, Rancho Dominguez, CA). Water was provided through saturated cotton wicks ($1.5 \times 3/8''$, Crosstex International, Hauppauge, NY) and beetles were left undisturbed for 1 h. Live beetles were then removed at random from the cage using an insect vacuum (Bioquip). Beetles were placed into 120-ml clear, polyethylene cups (Marivac Canada Inc., Montréal, QC) so that each cup contained about 125–200 beetles. Each cup was equipped with a 2.5-cm diameter hole that was fitted with a fine-mesh screen (80 μm mesh, Bioquip) to allow for air movement and prevent beetles from escaping. The underside of each lid was lined with filter paper (5.5-cm diameter) and a chalk line was drawn about 1 cm under the rim of each cup. Beetles were unable to cross the chalk line and tended to remain within the cup when lids were removed for brief periods during sampling. Cups that contained live beetles were stored in a Sanyo MLR-350H environmental test chamber (24 h; 10 °C; dark) until they were removed for examination. Both live and dead beetles were counted from each shipment.

Cups that contained live beetles were chosen at random to determine which beetles would be examined from each shipment. Sub-samples of live beetles ($n = 100$) were dissected and examined for endoparasitoids. These individuals were also used to determine sex ratio. In all cases, two characteristics were used to determine sex: the shape of the terminal abdominal sternite, which is a common diagnostic feature in coccinellids (Heimpel and Lundgren, 2000), and the reproductive organs of dissected individuals. The abdomens of other individuals ($n = 100$) were smeared directly onto glass microscope slides. These were fixed in methanol, stained in 10% buffered Giemsa (Sigma Diagnostics, pH 6.9) and examined by light microscopy for microorganisms. Smear preparations were made from both live and dead beetles.

A Shapiro–Wilk test was used to assess normality of percent survival data. A one-way ANOVA and Tukey's post hoc comparison were used to determine significance. A power analysis was used to determine if there was a large enough sample size to reject the null hypothesis. A χ^2 goodness of fit test was used to determine significance between observed and expected sex ratios. A *t*-test was used to determine significance between percent parasitism of male and female *H. convergens* by *D. coccinellae* and to determine if parasitism was seasonal.

3. Results

In most cases, shipments were free of extraneous materials. However, one shipment contained dried twigs and foliage and another contained a tenebrionid beetle that was identified as *Eulabis bicarinata* Eschscholtz.

Shipments from source A took 3 or more days to arrive by Canada Post. Beetles arrived in small cardboard boxes without other packing materials or cold packs. Each box had several holes punched in its side to provide ventilation. All of the shipments from Source A exceeded the total number of beetles that was specified by the supplier. The number of live beetles also exceeded the expected number; however, the percentage of live beetles from this source ranged from 57% to 95% (mean, 72%). The three shipments from Source B contained more beetles than were expected and the number of live beetles also exceeded the number that was specified by the supplier. The percentage of live beetles from Source B ranged from 85% to 93% (mean, 89%). Beetles from Source C were shipped in cardboard boxes with cold packs. Total beetle counts from Source C were variable and both total counts and live numbers were below what was expected for 6 of 9 shipments. The remaining shipments exceeded the expected number of beetles by about 3500–4500 individuals. Percent live beetles from this source ranged from 70% to 98% (mean, 87%). Percent survival of beetles from Source C was significantly different from Source A ($F = 5.31$, $df = 2, 19$, $P = 0.015$) but no other differences were found.

Total numbers of male and female beetles from each source were used to determine sex ratio. Sex ratios for

H. convergens from Sources A and C were significantly different ($P < 0.05$) from the expected sex ratio of 1♀:1♂ (Heimpel and Lundgren, 2000). Sex ratios for beetles from these sources were 1:0.75 (571 ♀, 429 ♂; $\chi^2 = 20.16$) and 1:0.51 (597 ♀, 303 ♂; $\chi^2 = 96.04$), respectively. The sex ratio for beetles from Source C was 1:0.81 (166 ♀, 134 ♂; $\chi^2 = 3.41$) and did not differ significantly from the expected sex ratio.

Microsporidia were detected in individuals from 13 of 22 shipments that were received. The specific tissues that were infected with microsporidia are not known because smear preparations of whole beetle abdomens were examined. Mean prevalence for individuals from all samples was 0.9% (range: 0–3%). Microsporidian spores were detected in individuals from four of the 10 shipments from Source A, all three shipments from Source B and six of the nine shipments from Source C. With the exception of two late season shipments (November and December), most of the infected beetles were received between mid-July to the beginning of August. Microsporidian spores measured $3.9 \pm 0.1 \times 2.5 \pm 0.1$ μm ($n = 50$).

Although eugregarines were observed infrequently in *H. convergens* (five individuals from four shipments, overall mean: 0.2%), they were found in beetles from all three sources. No seasonality was observed in association with the eugregarine infections. Based on morphology and dimensions, three distinct trophozoites were observed in *H. convergens*, and although life cycle stages were not observed, trophozoites were described according to the criteria outlined by Clopton (2004).

Trophozoites of Gregarine A were observed in three individuals from Sources B and C ($n = 33$, 2, 26, respectively). Trophozoites from these individuals had similar dimensions (Table 1) but no associations (syzygy) were observed. The epimerite was not evident. The protomerite appeared panduriform, being longer than wide and having a visible constriction in the mid region. In some individuals, the region anterior of the constriction stained more intensely that the rest of the protomerite. Presumably the darkened region anterior of the constriction was the epimerite, which was evident in only a few individuals. The deuteromerite was deeply ovoid, bearing a single nucleus.

Trophozoites of Gregarine B ($n = 12$) were observed in one individual from Source B. Most trophozoites were solitary but two pairs were observed in bi-association. Trophozoites of Gregarine B were similar in size to those of Gregarine A (Table 1) but they were distinct in appearance. The epimerite was not evident. The protomerite was broadly ovoid, being wider than long and lacking a visible constriction. It stained lightly and appeared less dense than the deuteromerite, which was deeply ovoid and contained a single nucleus. The nucleus of Gregarine B was less conspicuous than that of Gregarine A. The primary differences between trophozoites of this gregarine and those of Gregarine A are the overall appearance of the protomerite and differences in length/width ratios.

Trophozoites of Gregarine C ($n = 5$) were observed in one individual from Source A. Three trophozoites were much larger than those of Gregarines A and B (Table 1). Two of these were observed as primites in association with two smaller satellite trophozoites. The epimerite was not observed. The protomerite was shallowly ovoid, being wider than long and lacking a visible constriction. Like Gregarine B, the protomerite stained less intensely than the deuteromerite, which was deeply ovoid and bore a single nucleus.

The endoparasitoid *D. coccinellae* was found in beetles from all shipments. Higher numbers of endoparasitoids were observed in beetles that were received from mid-July to the end of August (mean: 9.7; $n = 11$ shipments) when compared to those that were received from September to mid-December (mean: 6.3; $n = 11$ shipments) ($t = 2.64$, $df = 20$, $P < 0.05$). Each parasitized beetle contained one to several *D. coccinellae* larvae. Larval morphology was similar to that reported by Balduf (1926) but only two of the three reported instars were observed. First-instars had light-brown, sclerotized heads with prominent, fang-like mandibles and soft whitish bodies. Mature larvae had whitish bodies that were large and grub-like. Mean parasitism by *D. coccinellae* was 8.0% (range: 3–15%) and mean

Table 1
Mean dimensions (μm ± SE) and measurement ratios of eugregarine trophozoites found in *Hippodamia convergens*

	LP	LD	WP	WD	TL	Range (TL × WD)	TLA	LP:TL	WP:WD	*n*
Gregarine A										
Source B	13.6 ± 0.5	31.2 ± 0.8	8.3 ± 0.4	14.2 ± 0.8	44.8 ± 1.1	37.3–52.4 × 8.1–21.0		1:3.3	1:1.7	20
Source C	13.9 ± 1.6	30.1 ± 4.0	9.8 ± 1.0	15.7 ± 2.0	44.0 ± 5.6	38.5–49.6 × 13.7–17.6		1:3.2	1:1.6	2
Source C	16.2 ± 0.6	41.6 ± 1.1	15.1 ± 0.7	24.7 ± 1.4	57.7 ± 1.6	45.0–69.6 × 15.8–35.3		1:3.6	1:1.6	21
Gregarine B										
Source B	14.8 ± 0.8	46.1 ± 2.9	20.3 ± 1.8	31.3 ± 3.1	61.0 ± 3.5	47.1–85.3 × 18.7–55.4	132.7	1:4.1	1:1.5	12
Gregarine C										
Source A[a]	24.9 ± 1.0	90.9 ± 3.9	35.7 ± 4.2	57.3 ± 6.2	115.8 ± 3.0	110.1–120.0 × 46.6–67.9	161.0	1:4.7	1:1.6	3
Source A[b]	11.2 ± 0.8	34.8 ± 0.6	18.5 ± 1.4	19.9 ± 4.6	46.0 ± 0.2	45.8–46.2 × 15.3–24.6		1:4.1	1:1.1	2

[a] Primite.
[b] Satellite; LP, protomerite length; LD, deuteromerite length; WP, primite width; WD, deuteromerite width; TL, total trophozoite length; TLA, total length of association.

parasitism (±SE) was significantly higher for females than for males (5.4 ± 0.5 and 2.6 ± 0.5, respectively) ($F = 1.29$, $t = 3.97$, $P < 0.05$).

A fungus was observed in beetles that had been received between mid-July and mid-September. It was identified as *Verticillium* sp. and was observed in up to 2% (range: 0–9%) of the beetles examined. The bodies of infected beetles were covered in mycelia and were hard, spongy or cheese-like when dissected.

4. Discussion

Although *H. convergens* is one of the most commonly used natural enemies in North America (van Lenteren, 2003), quality control guidelines for this predator have not been developed, presumably because *H. convergens* are field-collected and not mass-produced. The quality and efficacy of *H. convergens* cannot be properly assessed or guaranteed without clearly defined expectations regarding performance.

The harvesting of *H. convergens* from overwintering sites presents an opportunity for the inadvertent importation of a variety of natural enemies as well as other arthropods and plant materials. This may seem to be an inevitable outcome from field-collected biological control agents (i.e., parasitoids and predators) but the inclusion of their natural enemies and foreign plants or arthropods may pose future ecological problems, depending on what is imported. Larger and more obvious materials may be easy to detect but endoparasitoids, pathogens and other natural enemies are often cryptic and easily overlooked unless individuals are examined carefully. Foreign plant material and a tenebrionid were found in shipments during this study as well as less obvious endoparasitoids, a pathogen and other natural enemies.

Although the time of collection cannot be verified, it is likely that beetles collected from fall or spring aggregations are stored under cool conditions until they are sold. The large fat bodies that were observed in dissected beetles throughout the study period support this assumption. Relatively long shipping periods and lack of cold packs in boxes during shipment could contribute to higher beetle mortality (Source A), whereas lower mortality was achieved when beetles were shipped expediently and kept under refrigeration (Source B).

Hippodamia convergens has an approximate sex ratio of 1:1 (Heimpel and Lundgren, 2000). Beetles from Sources A and C had sex ratios with significantly greater numbers of females than males. Although *Wolbachia* are known to alter the sex ratios of some coccinellids including *Adalia bipunctata* L. and *Harmonia axyridis* Pallas (Majerus et al., 1999; Sokolova et al., 2002), these endosymbionts have not been reported from *H. convergens*. Beetles in this study were not examined for *Wolbachia*, *Rickettsia* or other microorganisms that are known to cause sex ratio distortions in coccinellids because they are not readily detected by light microscopy.

Microsporidia were uncommon pathogens in *H. convergens*, being present in less than 1% of beetles examined (0.9%, range 0–3%). The prevalence of microsporidia in *H. convergens* is relatively low when compared to the prevalence of *Nosema coccinellae* Lipa in other lady beetles (Table 2); however, the release of even a few microsporidia-infected individuals into the local environment could cause unforeseen problems that are irreversible (Kluge and Caldwell, 1992).

Microsporidia prevalence may increase if beetles remain confined for prolonged periods in cloth storage bags, particularly if ambient temperatures increase. Under such conditions, beetles may become more active and physical confinement may help facilitate pathogen transmission under conditions that are presumably stressful.

The basis of augmentative biological control with *H. convergens* is to release large numbers of individuals on a routine basis throughout the growing season when aphids are problematic. Depending on the season and the severity of aphid outbreaks, several packages of beetles may be released at one time. These are often sold in quantities that range from hundreds of beetles per package to more than 100,000. If 1% of these beetles are infected with microsporidia, as was observed during this study, thousands of infected beetles could be released each time *H. convergens* are used for biological control.

Both unfed and fed convergent lady beetles are known to disperse within days following their release (Davis and Kirkland, 1982), and this tendency helps facilitate pathogen dispersal, presenting a risk to local coccinellids that provide pest control in nature. The microsporidium in *H. convergens* is capable of infecting other coccinellids (*Coccinella septempunctata* L., *C. trifasciata perplexa* Mulsant, *H. axyridis*) when infected eggs are consumed under laboratory conditions (Saito and Bjornson, 2006) but the effects of microsporidia on native or exotic coccinellids has not been fully investigated. *H. convergens* is distributed throughout the continental United States and the southern regions of most Canadian provinces (Gordon, 1985; Majka and McCorquodale, 2006). In most, but not all cases, the natural distribution of *H. convergens* in North America overlaps with the areas where beetles are released for biological control. Although released beetles are likely to mix with native beetles, this is difficult to verify because released beetles cannot be readily distinguished from those that are native. The collection of a single *H. convergens* specimen in Nova Scotia (Majka and McCorquodale, 2006), where *H. convergens* is not native may be evidence that these beetles are capable of overwintering in release areas. This provides an opportunity for microsporidia and other natural enemies to become established where they may not have existed previously.

Host specificity for *N. coccinellae* appears to be relatively broad when compared to *N. tracheophila* Cali and Briggs and *N. hippodamiae* (Table 2), and it is possible that the latter two microsporidia infect other coccinellids that have not yet been identified as hosts. Spore dimensions of

Table 2
Microsporidia and their prevalence in field-collected and laboratory-reared coccinellids

Microsporidium	Spore size (μm)	Hosts	% Infected	Author
Nosema coccinellae[a]	4.4–6.7 × 2.3–3.4 (fresh)	*Coccinella septempunctata*	24.1[c]	Lipa (1968a)
	3.6–6.2 × 2.0–3.6 (fixed)	*Hippodamia tredecimpunctata*	2.5[c]	
		Myrrha octodecimpunctata	8.7[c]	
		Adalia bipunctata	0.6	Lipa et al. (1975)
		C. septempunctata	2.3	
		C. quinquepunctata	13.7	
		Exochromus quadripustulatus	0.8	
	3.5–6.3 × 1.9–2.6 μm	*C. septempunctata*	24.1	Lipa and Semjanov (1967)
		Myrrha octodecimguttata	8.7	
N. hippodamiae[a]	3.3–5.4 × 2.2–2.7 (fixed)	*Hippodamia convergens*	18.2[d]	Lipa and Steinhaus (1959)
N. tracheophila[b]	4.0–5.3 × 2.2–3.1 (fresh)	*C. septempunctata*	n/a	Cali and Briggs (1967)
	3.1–4.4 × 1.9–3.2 (fixed)			
Unidentified[a]	7.4 × 3.1 (fresh)	*H. convergens*	n/a	Sluss (1968)

[a] Microsporidia from field-collected coccinellids.
[b] Infection induced under laboratory conditions.
[c] Maximum percent infection according to a particular region.
[d] Prevalence among adult specimens submitted for examination.

the microsporidium observed in this study are similar to those of *N. coccinellae*, *N. tracheophila* and *N. hippodamiae* (Lipa and Steinhaus, 1959; Cali and Briggs, 1967; Lipa, 1968a). Some differences in tissue pathology have been observed for these microsporidia but these may be attributed to the different hosts that are infected. For example, the main argument for distinction between *N. tracheophila* and *N. hippodamiae* is that the former appears in a different (but related) host and has different sites of infection (Cali and Briggs, 1967). In the case of *N. coccinellae*, however, tissue pathology appears to change depending on the host that is infected (Lipa, 1968a). The identity or origin of microsporidia from field-collected coccinellids cannot be confirmed without additional information from molecular and ultrastructural studies.

Eugregarines were rarely observed in *H. convergens* in this study. Prevalence was based solely on the observation of trophozoites and prevalence may have been underestimated because other eugregarine life stages were not recognized. Other researchers use more sophisticated histological protocols for preserving eugregarines (Lipa, 1967) to help ensure that trophozoites and other life stages remain intact. These same protocols were not used in the current study due to the large quantity of beetles that were examined.

Based on trophozoite dimensions, it is unlikely that the eugregarines observed in *H. convergens* in this study are *G. barbarara*, the only eugregarine reported from *H. convergens* (141 × 78 μm; Lipa, 1968b). Total trophozoite dimensions (TL × WD) for Gregarine A are closest to those of *Gregarina katherina* Watson (45–70 × 20–34 μm; Watson, 1915) which was originally described from *Coccinella novemnotata* Herbst. and later reported from *C. bruckii* Mulsant, *C. californica* Mann and *C. trifasciata* L. (Hoshide, 1951; Lipa, 1968b). Trophozoites of Gregarines A and B have similar dimensions but their morphology is distinct. The larger trophozoites of Gregarine C are most similar in size to *G. fragilis* Watson, which was first reported from *Coccinella* sp. (110 × 60 μm, Watson, 1915) and later from *C. trifasciata* (Lipa, 1968b). Although the primites of *G. fragilis* are somewhat larger than its satellites (Watson, 1916), these differences are not as striking as those observed for Gregarine C, and Lipa (1968b) reports shorter primites and longer satellites for *G. fragilis* from *C. trifasciata*. The observed primite and satellite size differences for Gregarine C may be academic because trophozoite association (syzygy) is not a definite indication of maturity (Watson, 1916).

Although eugregarines are considered as either mutualist symbionts or weak pathogens (Lipa, 1967), pathogenicity has not been demonstrated in most cases. Large numbers of eugregarine trophozoites in the midgut are suspected to interfere with nutrient absorption and food movement but it is generally thought that any cellular damage caused by trophozoite attachment to the midgut epithelia is compensated by cellular regeneration (see Lipa, 1967).

The relationship between eugregarines and their coccinellid hosts is not fully understood. Lipa et al. (1975) observed hundreds of *Gregarina coccinellae* Lipa trophozoites in the midgut of *Exochromus quadripustulatus* L., *Harmonia quadripunctata* Pont. and *Myrrha octodecimguttata* L., which had an apparent but unspecified affect on host fitness. Others report that beetles with high numbers of eugregarine trophozoites die as a result of intestinal blockage (see Ceryngier and Hodek, 1996) and the longevity and fecundity of some infected beetles is reduced (Laudého et al., 1969). High numbers of trophozoites in the midgut may add stress to individuals when they are infected with other organisms that are pathogenic.

Dinocampus coccinellae is a common endoparasitoid of adult coccinellids and is reported from many hosts, including *H. convergens* (see Richerson, 1970). This parasitoid is distributed throughout North America and Europe where it tends to favor larger hosts (Balduf, 1926; Ceryngier

and Hodek, 1996). *Hippodamia convergens* females are often larger than the males and this may explain why they were parasitized more frequently.

Dinocampus coccinellae eggs were not used to determine percent parasitism in *H. convergens* because they are small, difficult to identify and are easily overlooked (Balduf, 1926). Mean parasitism of *H. convergens* by *D. coccinellae* in this study was likely underestimated at 8.0%. This result is similar to the 9.6% overall mean parasitism rate that was observed previously by Balduf (1926).

The importation and release of *D. coccinellae* as a result of biological control efforts could be argued as unimportant because this parasitoid attacks many coccinellid hosts and it is circumpolar in its distribution (Balduf, 1926; Ceryngier and Hodek, 1996). However, the importation of parasitoids undermines the success of a biological control program and may artificially increase the number of parasitoids in a particular area where *H. convergens* are released. Although *D. coccinellae* tends to favor larger hosts, it has been rather unsuccessful at parasitizing *H. axyridis* (Firlej et al., 2005), a relatively aggressive coccinellid that is known to displace native species.

Fungi are known to affect the overwintering success of some lady beetles. *Beauveria bassiana* (Bals.), *Metarhizium anisopliae* (Metsch.) Sorok., *Paecilomyces farinosus* (Holm ex S.F. Gray), and *P. fumosoroseus* (Wise), are reported to reduce survivability of overwintering coccinellids (Lipa and Semjanov, 1967; Lipa et al., 1975; Ceryngier and Hodek, 1996) but these fungi have not been reported from *H. convergens*. In my study, *Verticillium* sp. was observed in beetles primarily between mid-August and mid-September but the role that this fungus plays in the overwintering success of *H. convergens* is not known and *Verticillium* sp. are reported to be non virulent in other coccinellids (Ceryngier and Hodek, 1996).

The release of field-collected beetles is destined to result in the inadvertent release of pathogens, endoparasitoids and other natural enemies. Their role and identity are in need of further study to determine host specificity and their effects on *H. convergens* as a commercially available means of controlling aphid pests. Until then, the current practice of using *H. convergens* for biological control should be re-evaluated. Microsporidia and endoparasitoids are known to negatively impact the performance of beneficials; therefore, it is important to ensure that beneficial arthropods used for biological control are free of these natural enemies. Routine screening for pathogens is important to ensure that only disease and parasitoid-free arthropods are used in biological control programs. This helps ensure the success of the program and provides a measure of quality to growers that use them for pest control. Release of healthy individuals is also important to prevent the establishment of unforeseen natural enemies in areas where they did not exist previously. Mass-reared beetles and other beneficial arthropods are preferred for biological control because an acceptable level of quality can be assured.

Acknowledgments

My thanks to Phil Joudrey for his technical support, Mark Goettel and Christine Norohna (Agriculture and Agrifood Canada) for fungal identification and Chris Majka (Nova Scotia Museum of Natural History) for tenebrionid identification. Research funding was provided by Saint Mary's University (FGSR) and NSERC (Discovery Grant). Laboratory equipment was acquired through the Canadian Foundation for Innovation (New Opportunities Fund).

References

Balduf, V.W., 1926. The bionomics of *Dinocampus coccinellae* Schrank. Annals of the Entomological Society of America 19, 465–498.

Cali, A., Briggs, J.D., 1967. The biology and life history of *Nosema tracheophila* sp. n. (Protozoa: Cnidospora: Microsporidea) found in *Coccinella septempunctata* Linnaeus (Coleoptera: Coccinellidae). Journal of Invertebrate Pathology 9, 515–522.

Carnes, E.K., 1912. Collecting ladybirds (Coccinellidae) by the ton. California State Commission of Horticulture. Monthly Bulletin 1, 71–81.

Ceryngier, P., Hodek, I., 1996. Enemies of Coccinellidae. In: Hodek, I., Honěk, A. (Eds.), Ecology of Coccinellidae. Kluwer Academic Publishers, Boston, pp. 319–350.

Clopton, R.E., 2004. Standard nomenclature and metrics of plane shapes for use in gregarine taxonomy. Comparative Parasitology 71, 130–140.

Davis, J.R., Kirkland, R.L., 1982. Physiological and environmental factors related to the dispersal flight of the convergent lady beetle, *Hippodamia convergens* (Guerin-Meneville). Journal of the Kansas Entomological Society 55, 187–196.

Firlej, A., Boivin, G., Lucas, É., Coderre, D., 2005. First report of *Harmonia axyridis* Pallas being attacked by *Dinocampus coccinellae* Schrank in Canada. Biological Invasions 7, 553–556.

Gordon, R.D., 1985. The Coccinellidae (Coleoptera) of America north of Mexico. Journal of the New York Entomological Society 93, 1–912.

Heimpel, G.E., Lundgren, J.G., 2000. Sex ratios of commercially reared biological control agents. Biological Control 19, 77–93.

Hoshide, H., 1951. Studies on the gregarines from the Coleoptera of Japan. Yamaguchi Journal of Science 2, 93–106.

Kluge, R.L., Caldwell, P.M., 1992. Microsporidian diseases and biological weed control agents: To release or not to release? Biocontrol News and Information 13, 43N–47N.

Laudého, Y., Ormières, R., Iperti, G., 1969. Les entomophages de *Parlatoria blandchardi* Targ. dans les palmeraies de l'Adrar Mauritanien. II. Étude d'un parasite de Coccinellidae, *Gregarine katherina* Watson. Annales De Zoologie-écologie Animale 1, 395–406.

Lipa, J.J., 1967. Studies on gregarines (Gregarinomorpha) of arthropods in Poland. Acta Protozoologica 5, 97–179.

Lipa, J.J., 1968a. *Nosema coccinellae* sp. n., a new microsporidian parasite of *Coccinella septempunctata*, *Hippodamia tredecimpunctata*, and *Myrrha octodecimguttata*. Acta Protozoologica 5, 369–374.

Lipa, J.J., 1968b. On gregarine parasites of Coccinellidae in California, USA. Acta Protozoologica 6, 263–272.

Lipa, J.J., Pruszynski, S., Bartkowski, J., 1975. The parasites and survival of the lady bird beetles (Coccinellidae) during winter. Acta Parasitologica Polonica 23, 453–461.

Lipa, J.J., Semjanov, V.P., 1967. The parasites of the ladybirds (Coleoptera, Coccinellidae) in the Leningrad region. Entomologicheskoe Obozrenie 46, 75–80.

Lipa, J.J., Steinhaus, E.A., 1959. *Nosema hippodamiae* n. sp., a microsporidian parasite of *Hippodamia convergens* Guérin (Coleoptera, Coccinellidae). Journal of Insect Pathology 1, 304–308.

Majerus, T.M., Graf von der Schulenburg, J.H., Majerus, M.E., Hurst, G.D., 1999. Molecular identification of a male-killing agent in the

ladybird *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae). Insect Molecular Biology 8, 551–555.

Majka, C.G., McCorquodale, D.B., 2006. The Coccinellidae (Coleoptera) of the Maritime Provinces of Canada: new records, biogeographic notes, and conservation concerns. Zootaxa 1154, 49–68.

McDaniel, B., Morrill, W., 1969. A new species of *Tetrapolipus* from *Hippodamia convergens* from South Dakota (Acarina: Podopolipidae). Annals of the Entomological Society of America 62, 1465–1468.

Miller, R.L., Thompson, W.L., 1927. Life histories of lady-beetle predators of the citrus aphid. The Florida Entomologist 11, 1–8.

Richerson, J.V., 1970. A world list of parasites of Coccinellidae. Journal of the Entomological Society of British Columbia 67, 33–48.

Saito, T., Bjørnson, S., 2006. Horizontal transmission of a microsporidium from the convergent lady beetle, *Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae), to three coccinellid species of Nova Scotia. Biological Control 39, 427–433.

Sluss, R., 1968. Behavioral and anatomical responses of the convergent lady beetle to parasitism by *Perilitus coccinellae* (Shrank) (Hymenoptera: Braconidae). Journal of Invertebrate Pathology 10, 9–27.

Sokolova, M.I., Zinkevich, N.S., Zakharov, I.A., 2002. Bacteria in ovarioles of females from maleless families of ladybird beetles *Adalia bipunctata* L. (Coleoptera: Coccinellidae) naturally infected with *Rickettsia*, *Wolbachia*, and *Spiroplasma*. Journal of Invertebrate Pathology 79, 72–79.

van Lenteren, J.C., 2003. Commercial availability of biological control agents. In: van Lenteren, J.C. (Ed.), Quality Control and Production of Biological Control Agents: Theories and Testing Procedures. CABI Publishing, Wallingford, UK, pp. 167–179.

Watson, M.E., 1915. Some new gregarine parasites from Arthropoda. The Journal of Parasitology 2, 27–36.

Watson, M.E., 1916. Studies on gregarines including descriptions of twenty-one new species and a synopsis of the eugregarine records from the Myriapoda, Coleoptera and Orthoptera of the world. Illinois Biological Monographs 2, 1–258.