Biological Control 47 (2008) 82–88

Contents lists available at ScienceDirect

Biological Control

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

Preference and suitability of greenbug, *Schizaphis graminum* (Hemiptera: Aphididae) mummies parasitized by *Lysiphlebus testaceipes* (Hymenoptera: Aphidiidae) as food for *Coccinella septempunctata* and *Hippodamia convergens* (Coleoptera: Coccinellidae)

Tom A. Royer [a,*], Kristopher L. Giles [a], Makuena M. Lebusa [b], Mark E. Payton [c]

[a] *Department of Entomology and Plant Pathology, 127 NRC, Oklahoma State University, Stillwater, OK 74078, USA*
[b] *University of South Africa, Department of Agriculture, Animal Health and Human Ecology, P.O. Box 392, UNISA 0003, South Africa*
[c] *Department of Statistics, Oklahoma State University, USA*

ARTICLE INFO

Article history:
Received 10 April 2008
Accepted 11 July 2008
Available online 18 July 2008

Keywords:
Schizaphis graminum
Coccinella septempunctata
Hippodamia convergens
Lysiphlebus testaceipes
Biological control
Prey suitability
Intraguild predation

ABSTRACT

We conducted studies to (1) examine the stage-specific ability of *Coccinella septempunctata* L. and *Hippodamia convergens* Guérin-Méneville to prey on mummies of *Schizaphis graminum* (Rondani) parasitized by *Lysiphlebus testaceipes* Cresson, (2) evaluate whether fourth instars of both species are able to discriminate between mummies and unparasitized *S. graminum*, and (3) quantitatively describe the suitability of mummies parasitized by *L. testaceipes* as a food source for larvae of both species. Results revealed that second–fourth instar *H. convergens* and *C. septempunctata* could prey upon fully formed mummies, but consumption of mummies by fourth instars required considerably more time compared with greenbugs. Fourth instars of both coccinellid species readily accepted and consumed mummies. However, while *C. septempunctata* did not discriminate between mummies and live greenbugs, *H. convergens* was observed to occasionally reject mummies. A pure diet of mummies was unsuitable for larvae of *C. septempunctata* or *H. convergens* to complete development to adults, whereas a mixture of mummies and greenbugs allowed larvae to complete their lifecycle but delayed development and reduced the size of adult ladybeetles. Implications of these findings on *C. septempunctata* and *H. convergens*, and the potential effects of intraguild predation by these Coccinellidae on *L. testaceipes* are discussed.

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1. Introduction

Polis et al. (1989) categorized and described several interactions between species, including competition, predation/parasitism, mutualism, commensalism, and amensalism. One interaction commonly referred to as intraguild predation (IGP) is defined as the killing and eating of species that compete for a common resource. Thus, members of the guild interact through predation and competition. IGP can occur between predators, parasitoids or between predators and parasitoids, and can produce various effects within populations, communities and ecosystems (Polis et al., 1989; Rosenheim 1998).

Winter wheat and sorghum are grown in large areas of the Southern Great Plains of the US and both crops are regularly infested with greenbugs, *Schizaphis graminum* Rondani (Homoptera: Aphididae). The aphidophagous predators *Coccinella septempunctata* L. and *Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae) are found in abundance in both crops along with the solitary parasitoid *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Aphidiidae) and each are frequently observed attacking greenbugs (French et al., 2001; Giles et al., 2003; Jones, 2001, 2005; Kring and Kring, 1988; Michels et al., 1997). When abundant, Coccinellidae and/or *L. testaceipes* quickly suppress populations of greenbugs in wheat or sorghum fields (Fernandes et al., 1998; Giles et al., 2003; Jones, 2001; Rice and Wilde, 1988). Indeed, suppression of greenbugs by *L. testaceipes* is so predictable that parasitoid mummy ratios have been incorporated into pest management sampling decision rules in winter wheat (Elliott et al., 2004; Royer et al., 2004). Similar to observations in other systems (Colfer and Rosenheim, 2001; Wheeler et al., 1968), Coccinellidae in the Southern Plains are regularly observed eating unparasitized and parasitized greenbugs that infest winter wheat and sorghum (K.L.G. unpublished data; Lebusa, 2004), however, the consequences of this IGP have not been evaluated.

Müller and Brodeur (2002) argued that two competitors could persistently coexist only when the resource or ecological niche is partitioned between them. The observed coexistence of Coccinellidae and *L. testaceipes* in winter wheat and sorghum implies that predation on mummies has a limited or equivalent impact on the

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[*] Corresponding author. Fax: +1 405 744 6039.
E-mail address: tom.royer@okstate.edu (T.A. Royer).

1049-9644/$ - see front matter Published by Elsevier Inc.
doi:10.1016/j.biocontrol.2008.07.004

coccinellid and parasitoid populations. Several factors may contribute to their coexistence, including immigration, discrimination by the predators (Raymond et al., 2000), or other asymmetric interactions (Polis et al., 1989). Additionally, the low probability of widespread greenbug outbreaks occurring in sorghum and wheat fields that harbor Coccinellidae and *L. testaceipes* (Giles et al., 2003; Jones, 2001; Rice and Wilde, 1988) suggests that this form of IGP (Coccinellidae feeding on mummies) has little effect on the ability of *L. testaceipes* to suppress greenbug populations in the Southern Plains (Giles et al., 2003; Jones, 2001).

Investigations that quantitatively address feeding preference for and suitability of mummies by Coccinellidae and the consequences of such predation on coccinellids would provide insights on prey discrimination and the potential asymmetric interactions among these natural enemies. Takizawa et al. (2000) evaluated the suitability of parasitized pea aphids on the development of fourth instar Coccinellidae, but we are not aware of other studies that have evaluated this type of IGP and its effects on ladybeetle survival and development. We report on laboratory investigations that addressed three questions regarding potential interactions between *L. testaceipes* and the predators *C. septempunctata* and *H. convergens*: (1) which larval stages of *C. septempunctata* and *H. convergens* are able to attack and consume mummified greenbugs, (2) do *C. septempunctata* and *H. convergens* discriminately select prey when confronted with a mixed population of mummified and unparasitized greenbugs, and (3) are mummified greenbugs a suitable food source for *C. septempunctata* and *H. convergens*?

2. Materials and methods

2.1. Insect colonies

Greenbug ('Biotype E') colonies were maintained in a greenhouse (22 ± 2 °C) on sorghum plants (var. SG-925, Asgrow®, Monsanto Co., St. Louis, MO) grown in 15-cm diameter plastic pots using a fritted clay and sphagnum moss medium. Aphid-free plants were grown under greenhouse conditions (22 ± 2 °C) in 15 cm-diameter pots covered with plexi-glass or acetate cylinders (33 cm tall) that were vented with nylon mesh fabric on the top end and in two locations on the side to prevent contamination by other aphids and parasitoids. When plant stems reached ~8 mm diameter, they were placed into double-walled fine nylon-mesh cages (40 × 34 × 50 cm) and greenbugs were released and allowed to settle and multiply. Plants that became severely necrotic from greenbug feeding were replaced with fresh, greenbug-infested plants as needed.

A colony of *L. testaceipes* collected from Apache, OK was established and maintained in the laboratory at 22 ± 2 °C and 12:12 (L:D) on 'Biotype E' greenbugs in separate double-walled fine nylon-mesh cages. Our rearing method consisted of three steps. (1) Sorghum plants were grown under greenhouse conditions (22 ± 2 °C) in pots in the greenhouse and covered with acetate cylinders (33 cm h × 15 cm dia) that were vented with nylon mesh fabric (in two locations on the cylinder). (2) When the sorghum stems reached ~8 mm in diameter, they were placed into larger cages (3 plants per cage) and infested with greenbugs which were allowed to settle and multiply for 1–2 days. (3) Cylindrical vented cages were removed and the infested sorghum plants were transferred to double-walled fine nylon-mesh cages (3 plants per cage) that housed a laboratory colony of *L. testaceipes*. Plants that became necrotic from greenbug feeding were replaced with fresh, greenbug-infested plants ca. 5–8 days. Cages were inspected daily, and all newly formed mummies (aphids that became visibly mummified within the 24 h interval) were removed from the sorghum plants with a fine pair of forceps, placed in a 5 ml micro-centrifuge plastic vial that was labeled with the date of collection and held in a growth chamber at 6 °C and a photoperiod 16:8 (L:D). This temporary storage method allowed us to accumulate and hold viable mummies under arrested development for up to 2 weeks at a temperature set at their lower development threshold (Elliott et al., 1994; Jones, 2005).

Coccinella septempunctata and *H. convergens* were collected from alfalfa fields in Stillwater OK, separated into mating pairs and maintained in a growth chamber at 22 ± 0.5 °C and a photoperiod of 16:8 (L:D) h in 0.236 L cardboard ice cream containers with a fine mesh cover. The beetle colonies were maintained by feeding beetles *ad libitum* supply of pea aphids (*Acyrthosiphon pisum* Harris) from a stock laboratory colony that was maintained on *Vicia faba* L. and an artificial supplementary diet of 1:1:1 wheat flour-honey-yeast mixture. Males were removed when the first eggs were deposited to avoid egg cannibalism. Eggs were allowed to hatch under the same conditions, and upon eclosion, the larvae were separated into individual 5 ml glass vials stopped with nylon screened caps prior to initiating the feeding studies. For all studies, *C. septempunctata* and *H. convergens* larvae originated from a minimum of three mated pairs for each treatment.

2.2. Prey stages used in the experiments

The two prey items that we evaluated were (1) unparasitized greenbugs (late instars or adults) or (2) newly formed mummified greenbugs parasitized by *L. testaceipes* (mummies).

2.3. Feeding capability

This study was conducted to document whether first, second, third, and fourth instars and newly emerged adults of *C. septempunctata* and *H. convergens* could consume greenbug mummies parasitized by *L. testaceipes*. With the exception of the first instar evaluation, all larvae were maintained on 4 mg of greenbugs/day (approximately 30) and starved for 24 h before feeding trials were initiated to assure that they were not undergoing a period of inactivity due to satiation before the experiment began (Simelane et al. 2008). The feeding capability study was conducted for 24 h in 5 ml glass vials stopped with nylon screened caps. Five newly-emerged individuals of each developmental stage were evaluated for each coccinellid species. First instars were provided four mummies; second instars were provided eight mummies; third instars were provided sixteen mummies; and fourth instars and adult beetles were provided thirty mummies (30 mummies ≈ 4 mg). After 24 h, all remaining mummies were inspected with a dissecting microscope for evidence of feeding and recorded as either fed upon or intact.

2.4. Handling time and preference studies

Based on results of the feeding capability studies, fourth instars were used to evaluate handling time and diet preference because they could easily consume both diet items. For both handling time and preference evaluations, individual larvae were placed in a feeding arena (9 cm diameter Petri dish). Prey items were placed at the center of the Petri dish before an individual larva was released in the center.

Using no-choice trials, newly-emerged fourth instars (*C. septempunctata* and *H. convergens*) were starved for 24 h and provided with either 10 large (late instar or adult) greenbugs or 10 mummies. Larvae were observed for 30 min following their placement in the arena. We recorded the time (seconds) required to attack and completely consume (>50% of the aphid/mummy was consumed) each individual prey item ("handling time"). To ensure times were recorded accurately, only two individuals of the same species, each with different prey items, were observed during each 30 min evaluation. Fifteen and twenty pairs of *C. septempunctata* and *H. convergens*, respectively, were observed during this portion of the study. The nature of our paired experimental procedure required that (1) handling times between treatments be calculated as a difference (greenbug handling time—mummy handling time) for each pair, and (2) these differences be statistically compared among pairs, but separately for each Coccinellidae species.

For the choice preference trials, twenty newly-emerged fourth instar *C. septempunctata* and *H. convergens* were starved for 24 h and presented with 10 large (late instar or adult) greenbugs and 10 mummies per individual in the arena. The number of encounters (number of individuals that were physically contacted), number of attacks (prey was physically seized) and number of prey consumed completely (consumption of mummy > 50%) was recorded for 30 min following the presentation of the prey items. Because individual larvae were exposed to both prey items simultaneously, preference was evaluated by comparing differences in attack and consumption ratios (greenbugs-mummies) among individuals, but separately for each Coccinellidae species. Attack ratio was calculated using the formula: # attacked/# encountered, and consumption ratio was calculated using the formula: # consumed/# encountered. Individual differences in attack and consumption ratios (greenbug attack ratio—mummy attack ratio; greenbug consumption ratio—mummy consumption ratio) were used to analyze preference between prey types.

2.5. Suitability study

This study was conducted to evaluate the suitability of greenbug mummies parasitized by *L. testaceipes* as a food source for the survival and development of *C. septempunctata* and *H. convergens*. Prior to providing the diet treatments initiated in the 3rd instar (see below), each coccinellid larva used in the experiment was provided a daily supply of 4 mg/day of greenbugs. All diet quantities were measured using a digital Sartorium M3P microbalance (resolution: 0.0001 mg). All larvae were reared individually in 5 ml glass vials stopped with nylon screened caps.

We evaluated the effects of three diets on third and/or fourth instar larvae based on results of the feeding capability experiment which showed that third and fourth instars were capable of completely eating mummies. The diet treatments were defined by two factors: their composition, (4 mg/day of greenbugs, 4 mg/day of a 50/50 ratio of greenbugs/mummies or 4 mg/day of 100% mummies) and the larva stage that the diet was initiated (third instar or fourth instar larva). Suitability was evaluated by comparing survival and development of larvae assigned to these diet treatments, so the experiment contained a total of five treatments (MIX3, MUM3, MIX4, MUM4 and CONTROL) As with previous studies that quantitatively described suitability of prey items for Coccinellidae (Giles et al., 2002, 2005; Phoofolo et al., 2007); the sub-optimal daily diet level of 4 mg was chosen to eliminate the confounding effects of satiation, and attempt to reflect average daily consumption levels of naturally occurring ladybeetles (Obrycki et al., 1998). Additionally, water (saturated cotton wick) was provided daily to all larvae to eliminate potential confounding effects of dehydration (Michaud and Grant, 2005).

Each treatment (MIX3, MUM3, MIX4, MUM4 and CONTROL) was replicated 30 times for each species. All larvae were held in a growth chamber at 22 °C (16L:8D). Individual larvae were visually examined every 12 h for molting, death, pupation, and adult emergence. The ventral body area (mm^2) of each emerged adult was calculated by measuring the body length and width using the formulae: [½(body length)] × [½(body width)] (Obrycki et al., 1998; Giles et al., 2002; Phoofolo et al. 2007).

2.6. Statistical analyses

For all experiments, data were analyzed separately for each Coccinellidae species. For the no-choice evaluation of handling time difference between greenbugs and mummies, the average difference (greenbug handling time—mean mummy handling time) among paired fourth instars was compared using paired *t*-tests (PROC TTEST; SAS Institute, 2004). Also, for the choice test evaluation of preference between greenbugs and mummies, paired *t*-tests (PROC TTEST) were used to compare the average differences in attack ratio (H_O: attack ratio difference = 0) and consumption ratio (H_O: consumption ratio difference = 0) among fourth instars.

For prey suitability studies, differences in developmental time and ventral body size among diet treatments were evaluated using ANOVA (PROC MIXED) with a Satterthwaite adjustment for degrees of freedom, followed by protected pairwise *t*-test comparisons (LSMEANS). During six separate time periods, five individuals of each species were supplied with each of the diet treatments; therefore, time period was included as a random factor during analysis. χ^2 tests applied to contingency tables (PROC FREQ) were used to compare differences in survival and adult sex ratios among diet treatments.

3. Results

3.1. Feeding capability

First instar *C. septempunctata* and *H. convergens* larvae did not consume mummies (Table 1). Second instars attacked but only partially consumed individual mummies, whereas third and fourth instars and adults completely consumed (consumed > 50% of the body) mummies.

3.2. Handling time and preference studies

H. convergens larvae spent an average of 71.7 ± 6.0 more seconds ($df = 19$; $t = -11.9$; $P < 0.001$) and *C. septempunctata* larvae spent an average of 40.4 ± 9.6 more seconds ($df = 14$; $t = -4.2$; $P < 0.001$) handling a mummy compared to a greenbug. *H. convergens* larvae did not always attack a prey item even when it was encountered (Table 2), but *C. septempunctata* larvae attacked all prey that they encountered. In choice tests, attack ratios (# attacked/# encountered) were not significantly different (greenbug attack ratio—mummy attack ratio) for *H. convergens* (0.116 ± 0.061; $df = 19$; $t = 1.9$; $P = 0.072$) or *C. septempunctata* ($df = 19$; $t = 0$; $P = 1.0$). However, consumption ratios (# consumed/# encountered) were statistically different (greenbug consumption ratio—mummy consumption ratio) for *H. convergens* (0.177 ± 0.067; $df = 19$; $t = 2.6$; $P = 0.016$) but not for *C. septempunctata*, ($df = 19$; $t = 0$; $P = 1.0$). Based on this analysis, *H. convergens* larvae occasionally rejected mummies as a food source, whereas

Table 1
Consumption of greenbug mummies parasitized by *Lysiphlebus testaceipes* by larvae of *Coccinella septempunctata* (C-7) and *Hippodamia convergens* (HCON) in a 24-hr no-choice test

Larva Stage	Total # of mummies provided	Total # of mummies consumed	
		C-7	*HCON*
1st instars ($n = 5$)	10 (2 per larva)	0	0
2nd instars ($n = 5$)	20 (4 per larva)	19*	17*
3rd instars ($n = 5$)	80 (16 per larva)	80	80
4th instars ($n = 5$)	150 (30 per larva)	150	150
Adults ($n = 5$)	150 (30 per adult)	150	150

Feeding was attempted but mummies were only partially <50% consumed.

C. septempunctata completely consumed all mummies that were encountered and did not discriminate between food sources.

3.3. Suitability study

We compared survival through all larval stages, and survival through larval + pupal stages, among individual larvae that consumed greenbugs only, a mixed diet of greenbugs and mummies, or an exclusive diet of mummies. Three *C. septempunctata* that were assigned to the control cohort and five that were assigned to the MIX3 cohort died before reaching the fourth instar. All surviving fourth instar *C. septempunctata* larvae pupated but did not necessarily emerge as adult beetles. For *H. convergens* no significant differences in cumulative larval survival were observed among the diet treatments initiated at either the third or fourth instar (Table 3). For *H. convergens* and *C. septempunctata*, there were significant differences in cumulative survival through the larval + pupal stages among treatments (Table 3); no pupae survived to adult emergence when supplied with an exclusive diet of mummies initiated at either the third and fourth instar. There were no differences in male: female ratios of surviving adults that were fed greenbugs only or mixed diets.

There were significant differences in larval developmental times for both *C. septempunctata* and *H. convergens* among the diet treatments (Table 4). Larval development was significantly delayed for *H. convergens* and *C. septempunctata* supplied with an exclusive diet of mummies at the third and fourth instar. Additionally, the mean larval developmental times for individuals supplied with a mixture of greenbugs and mummies initiated at the third instar were significantly longer for both *C. septempunctata* and *H. convergens* than larvae in the control cohort. Interestingly, a mixed diet initiated at the beginning of the fourth instar did not significantly delay overall larval developmental time for *C. septempunctata* or *H. convergens*.

There were significant differences in the pupal developmental times among diet treatments for *C. septempunctata* and *H. convergens* (Table 4). For *C. septempunctata*, a mixed diet initiated at the beginning of the third instar caused a significant delay in pupal development, but did not affect pupal development when initiated at the fourth instar. However, for *H. convergens*, pupal development times increased when larvae were supplied with a mixed diet was initiated at both the third and fourth instar.

There were significant differences in the size of adult beetles for both *C. septempunctata* and *H. convergens* due to diet treatments (Table 4). Compared to the control cohort, the average ventral area (mm^2) of *H. convergens* adults was smaller when supplied with a mixed diet of greenbugs and mummies at the beginning of the third or fourth instar. However, only *C. septempunctata* beetles that received a mixed diet of greenbugs and mummies as third instar larvae were smaller than the control cohort; we saw no differences in ventral area between individuals supplied with a mixed diet as fourth instar larvae versus the controls. We also saw no differences in male/female ratio among surviving adult beetles (Table 4).

4. Discussion

Asymmetric IGP occurs when one predator of the same prey is also susceptible to predation by another (Meyhöfer and Klug, 2002). Coccinellid and aphidiid species often have common aphid prey (Hagen, 1986; Majerus, 1994) and because of their polyphagous behavior, Coccinellidae will consume parasitized aphids as they feed. Most Coccinellidae feed on a wide range of prey types (Hodek and Honek, 1996); however, that range of diet items may be restricted by the feeding capabilities of different growth stages. Our study demonstrated that several life stages (second instars through adults) of *C. septempunctata* and *H. convergens* can kill *L. testaceipes* within mummies as they forage, but second instars have a limited capacity to eat mummies.

Table 2
Mean (±SEM) number of encounters, attacks, prey consumed for 4th instar *C. septempunctata* or *H. convergens* provided with unparasitized greenbugs (GB) versus greenbug mummies parasitized by *L. testaceipes* (MUM)

Species	n	Prey	Encounters	Attacks	Consumed
C. septempuncatata	20	GB	5.25 ± 0.39	5.25 ± 0.39	5.25 ± 0.39
		MUM	5.60 ± 0.37	5.60 ± 0.37	5.60 ± 0.37
H. convergens	20	GB	4.10 ± 0.51	3.60 ± 0.58	3.50 ± 0.60
		MUM	4.40 ± 0.35	3.60 ± 0.53	3.20 ± 0.56

Table 3
Effects of diet treatments[a] on larval and larval + pupal survivorship and male:female ratios of emerged adults for *C. septempunctata* and *H. convergens* larvae reared at 22 °C

Species/treatment	n	Proportion[b] Surviving Past Larval or Pupal Stage		
		Larval	Larval + Pupal	M:F ratio
C. septempunctata				
Control	30	0.90ab	0.90ab	0.56
MIX3	30	0.83b	0.83b	0.60
MUM3	30	1a	0c	–
MIX4	30	1a	1a	0.60
MUM4	30	1a	0c	–
	χ^2	13.9	122.3	0.15
	df	4	4	2
	P	0.007	<0.001	0.929
H. convergens				
Control	30	1	1a	0.53
MIX3	30	0.93	0.93a	0.61
MUM3	30	1	0 b	–
MIX4	30	0.93	0.93a	0.46
MUM4	30	1	0b	–
	χ^2	6.2	134.7	1.15
	df	4	4	2
	P	0.187	<0.001	0.563

[a] Treatments: Control = cohort received 4 mg/day of GB; MIX3 = cohort received a mixture of 4 mg/day of GB + MUM beginning at 3rd instar; MUM3 = cohort received a diet of 4 mg/day MUM beginning at 3rd instar; MIX4 = cohort received a mixture of 4 mg/day of GB + MUM beginning at 4th instar; MUM4 = cohort received a mixture of 4 mg/day of MUM beginning at 4th instar

[b] Proportions within a column followed by the same letter are not significantly different using 2 × 2 χ^2 test, $P = 0.05$, PROC FREQ.

Table 4
The effects of diet treatments[a] on developmental times (±SE) and adult ventral area (±SE) for *C. septempunctata* and *H. convergens* larvae reared at 22 °C

Species/treatment	*n*	Developmental Time (days)				
		3rd Instar	4th Instar	Larval (1st–4th Instar)	Pupal	Ventral area (mm^2)
C. septempunctata						
Control	27	3.0 ± 0.2bc	4.8 ± 0.3d	11.8 ± 0.4d	5.6 ± 0.3b	23.4 ± 0.2a
MIX3	25	3.3 ± 0.2b	6.9 ± 0.3b	14.3 ± 0.4c	7.9 ± 0.3a	16.4 ± 0.2b
MUM3	30	6.9 ± 0.2a	8.3 ± 0.3a	19.2 ± 0.4a	–	–
MIX4	30	2.9 ± 0.2bc	5.8 ± 0.3c	12.6 ± 0.4d	5.9 ± 0.3b	22.9 ± 0.3a
MUM4	30	2.8 ± 0.2c	8.5 ± 0.3a	15.3 ± 0.4b	–	–
H. convergens						
Control	30	3.0 ± 0.1b	3.5 ± 0.3c	10.5 ± 0.3c	3.9 ± 0.1b	12.1 ± 0.2a
MIX3	30	3.1 ± 0.1b	5.7 ± 0.3b	12.8 ± 0.3b	4.9 ± 0.1a	9.1 ± 0.1c
MUM3	30	3.8 ± 0.1a	6.1 ± 0.3ab	14.0 ± 0.3a	–	–
MIX4	30	3.0 ± 0.1b	4.0 ± 0.3c	11.0 ± 0.3c	4.7 ± 0.1a	10.2 ± 0.1b
MUM4	30	3.0 ± 0.1b	6.8 ± 0.3a	13.8 ± 0.3a	–	–

Development times (LSMEANS) of a species within a column followed by the same letter are not significantly different using protected pair-wise *t*-tests, $\alpha = 0.05$, PROC MIXED.

[a] Treatments: Control = cohort received 4 mg/day of GB; MIX3 = cohort received a mixture of 4 mg/day of GB + MUM beginning at 3rd instar MUM3 = cohort received a diet of 4 mg/day MUM beginning at 3rd instar; MIX4 = cohort received a mixture of 4 mg/day of GB + MUM beginning at 4th instar; MUM4 = cohort received a mixture of 4 mg/day of MUM beginning at 4th instar.

Roy et al. (2003) stated that simple Petri dish arenas provide environments in which preliminary observations on predator behavior can be made, but such studies are insufficient for making conclusions on their potential behavior in more complex, multidimensional arenas. They observed in a feeding study involving *C. septempunctata* and *A. pisum* aphids that were infected with *Pandora neoaphidis*, *C. septempunctata* larvae fed for a longer time and consumed entire infected aphids in a complex arena in contrast to a Petri dish arena where larvae fed, but did not entirely consume infected aphids. Laboratory studies of other species indicate that parasitized aphids face the same risk of predation as unparasitized aphids (i.e. no preference) regardless of the stage of development and the foraging behavior of the predator (Colfer and Rosenheim, 2001; Meyling et al., 2004; Roger et al., 2001). However, several researchers, while acknowledging that mummies are highly susceptible to predation, showed that mummies are less likely to be eaten than their unparasitized herbivore host in larger field studies, especially when both prey items are mixed (Meyhöfer and Hindayana, 2000; Snyder and Ives, 2001; Snyder et al., 2004). Based on the results of our Petri dish lab study, *H. convergens* larvae occasionally rejected (did not fully consume) *L. testaceipes* mummies following initial attack while *C. septempunctata* larvae demonstrated no discrimination (i.e. a null switching response, Murdoch and Marks (1973) between unparasitized greenbugs and *L. testaceipes* mummies.

In our study, an exclusive diet of *L. testaceipes* mummies was unsuitable for *C. septempunctata* and *H. convergens* to complete development to adulthood. Larvae that consumed a mixture of mummies and greenbugs could, however, complete their lifecycle albeit with a delay in developmental time and a reduction in adult body size. These differences were most pronounced when the mixed diet was initiated at the beginning of the third instar. These results are similar to findings by Takizawa et al. (2000) who showed that survival of *C. septempunctata* was lower, development was longer, and adult size was smaller when fed an exclusive diet of *Aphidius colemani* Viereck mummies.

One difference between our study and Takizawa et al. (2000) was that they compared development of larvae that received *ad libitum* levels of food and measured differences in consumption by the test animals. They found that the coccinellid larvae consumed different amounts of each diet (mg aphids > mg parasitized aphids > mg mummies). Because they did not restrict the daily level of food, it is difficult to directly evaluate suitability of each prey item because of the confounding effects of differing levels of food consumption among diets (Giles et al., 2002). In our study, the amount of food that was consumed daily by each predator was equivalent for each prey diet (4 g/day) so we quantitatively compared suitability of the prey items.

These results reinforce the importance of supplying water when directly comparing the suitability of diets (e.g. mummies versus unparasitized greenbugs) for coccinellids or for other species in feeding studies (Michaud and Grant, 2005). Larval developmental times at 22 °C for *C. septempunctata* and *H. convergens* supplied 4 mg of greenbugs per day in our study were ~24% faster than those reported in previous studies with identical experimental conditions (Giles et al., 2005; Phoofolo et al., 2007).

IGP can theoretically interfere with biological control of a pest through diminution of the top-down cascade effect imparted by secondary consumers on primary consumers (Finke and Denno, 2003; Rosenheim et al., 1993; Rosenheim, 1998) and has been demonstrated in some field studies (Finke and Denno, 2003). However, empirical evidence suggests that some types of IGP between predators and parasitoids may not be deleterious at the population level, but instead may enhance the top-down regulation imparted by the predator/parasite complex on the primary consumer (Colfer and Rosenheim, 1995; Gardiner and Landis, 2007; Samways, 1986). Recent reviews that looked at the consequences of IGP on biological control concluded that intraguild predation between a superior and intermediate predator often does not have a deleterious effect on biological control of the target prey in contrast with predicted theory, but instead may slightly enhance it (Janssen et al., 2006; Rosenheim and Harmon, 2006; Holt and Huxel, 2007; Vance-Chalcraft et al., 2007).

In our study, *Coccinella septempunctata* demonstrated non-discrimination when feeding on aphids or mummies, essentially feeding on a mixed diet. Waldbauer and Friedman in a 1991 review described the criteria for identifying self selection by insects for optimal diets. Hauge (1998) assumed that a mixture of prey species may provide greater nutritional diversity regardless of the degree of selectivity demonstrated by the predator in its prey choices. Thus, such a mixed diet would provide improved performance through lower mortality, faster development and larger adult body size and weight. Evans et al. (1999) described some benefits accrued by a coccinellid predator from consuming a mixture of essential and alternative food sources. Soares et al. (2004) showed that male *Harmonia axyridis* (Pallus) exhibited a constant preference for *Myzus persicae* (Sulzer) aphids when presented a mixed diet of *M. persicae* and *A. pisum*, while females exhibited a null switching behavior. They argued that the null switching behavior exhibited by female *H. axyridis* demonstrated self selection because the mixed diet was superior in terms of quality and balance for reproductive success. Prey of inferior quality has been shown affect several life history traits of coccinellids, including slower larval development, decreased adult longevity, fecundity and fertility, increase mortality and produced smaller adults (Blackman, 1967; Cabral et al., 2006) Such sub-optimal food for Coccinellidae can serve as a source of energy but does not improve life history parameters. The lack of discrimination that we observed for *C. septempunctata* larvae in our study would not be beneficial for optimal development when faced with a mixed diet of greenbugs and *L. testaceipes* mummies, but may allow for survival as a food source dwindles.

In contrast, *Hippodamia convergens* larvae occasionally rejected mummies as a food source. Crawley and Krebs (1992), using optimal foraging models, predicted that predators searching for prey should select the most profitable prey type and reject the unprofitable prey. Such behavior would minimize loss of opportunity time and maximize energy return (Stephens and Krebs, 1986). Therefore, if parasitism alters prey suitability and profitability, predators possessing the ability to detect, recognize and avoid a less suitable parasitized prey item would have an advantage for optimal development. However, if they exhibited strong null switching, it may be deleterious because as the preferred food source dwindles, they would not have a "mixed" diet that they could use to complete development.

Many *coccinellid* larvae are sensitive to differences in the nutritional value, toxicity, or suitability of prey which can result in lower survival, longer developmental times, decreased weight and size and reduced fecundity of emerging adults (Giles et al., 2002; Hodek and Honek, 1996; Phoofolo and Obrycki, 1998; Smith, 1965; Simelane et al., 2008). Our studies provide a starting point for conducting further research among the IGP relationships in the winter wheat system for greenbugs (prey), *L. testaceipes* (intermediate predator) and Coccinellidae (superior predator) in terms of nutritional quality of prey, interspecific differences in prey preferences among the Coccinellid fauna, and their ultimate effects on biological control of greenbug in winter wheat.

In summary, our results suggest that (1) *C. septempunctata* and *H. convergens* larvae are capable of and will readily consume *L. testaceipes* mummies as a food source, (2) an exclusive diet of *L. testaceipes* mummies would be deleterious to their survival, (3) *H. convergens* and *C. septempunctata* larvae differ slightly in demonstrating preference for feeding on mummies in laboratory choice tests, and (4) a mixture of *L. testaceipes* mummies and unparasitized greenbugs is a suitable but inferior (e.g. delayed development and smaller adult size) diet for these Coccinellidae species. While this study does not represent all of the possible interactions that may occur in sorghum and wheat fields in the Southern Plains, our results suggest that either (1) greenbugs possess essential nutrients which are required for growth, development and survival of Coccinellidae predators that are lacking in *L. testaceipes* mummies or (2) *L. testaceipes* mummies contain toxic materials that are detrimental to survival and development. Further studies, particularly those in sorghum and wheat fields, are needed to examine the overall impact of IGP by Coccinellidae on *L. testaceipes* and the effect of IGP on regulation of *S. graminum* populations by *L. testaceipes.*

Acknowledgments

We thank Doug Jones, Mpho Phoofolo and N.C. Elliott for technical support. The authors also appreciate the careful review of this manuscript by Alissa Berro, Louis Hesler and Mpho Phoofolo and two anonymous reviewers. This research was based on work in partial fulfillment of the requirements of M. Lebusa's M.S. degree and was supported in part by a grant from the AED Kellogg Foundation, under projects OKL02334 and OKL02455, and Bayer CropScience, BASF, Dow Agrosciences, FMC Corporation and Syngenta Corporation. This article is a joint contribution from the Oklahoma Agricultural Experiment Station, the Oklahoma Cooperative Extension Service, and has been approved for publication by the Director, Oklahoma Agricultural Experiment Station, Stillwater.

References

Blackman, R.L., 1967. The effects of different aphid foods on *Adalia bipunctata* L. and *Coccinella septempunctata* L. Annals of Applied Biology 59, 207–219.

Cabral, S., Soares, A.O., Moura, R., Garcia, P., 2006. Suitability of *Aphis fabae*, *Myzus persicae* (Homoptera: Aphididae) and *Aleyrodes proletella* (Homoptera: Aleyrodidae) as prey for *Coccinella undecimpunctata* (Coleoptera: Coccinellidae). Biological Control 39, 434–440.

Colfer, R.G., Rosenheim, J.A., 1995. Intraguild predation by coccinellid beetles on an aphid parasitoid , *Lysiphlebus testaceipes*. Proceedings of the Beltwide Cotton Conference 2, 1033–1036.

Colfer, R.G., Rosenheim, J.A., 2001. Predation on immature parasitoids and its impact on aphid suppression. Oecologia 126, 292–304.

Crawley, M.J., Krebs, J.R., 1992. Foraging Theory. In: Crawley, M.J. (Ed.), Natural Enemies. Blackwell Scientific Publications, Oxford, pp. 90–114.

Elliott, N.C., Webster, J.A., Kindler, S.D., 1994. Developmental response of *Lysiphlebus testaceipes* to temperature. The Southwestern Entomologist 24, 1–4.

Elliott, N.C., Royer, T.A., Giles, K.A., Kindler, S.D., Porter, D.R., Elliott, D.T., Waits, D.A., 2004. A web-based management decision support system for greenbugs in winter wheat. Online: Crop Management. Doi: 10.1094/CM-2004-1006-01-MG. Available from: http://www.plantmanagementnetwork.org/cm/ Plant ManagementNetwork.

Evans, E.W., Stevenson, A.T., Richards, D.R., 1999. Essential versus alternative food of insect predators: benefits of a mixed diet. Oecologia 121, 107–112.

Fernandes, O.A., Wright, R.J., Mayo, Z.B., 1998. Parasitism of greenbugs (Homoptera: Aphididae) by *Lysiphlebus testaceipes* (Hymenoptera: Braconidae) in grain sorghum. Journal of Economic Entomology 91, 1315–1319.

Finke, D.L., Denno, R.F., 2003. Intra-guild predation relaxes natural enemy impacts on herbivore populations. Ecological Entomology 28, 67–73.

French, B.W., Elliott, N.C., Kindler, S.D., Arnold, D.C., 2001. Seasonal occurrence of aphids and natural enemies in wheat and associated crops. The Southwestern Entomologist 26, 49–61.

Gardiner, M.M., Landis, D.A., 2007. Impact of intraguild predation by adult *Harmonia axyridis* (Coleoptera: Coccinellidae) on *Aphis glycines* (Hemiptera: Aphididae) biological control in cage studies. Biological Control 40, 386–395.

Giles, K.L., Dillwith, J.W., Berberet, R.C., Elliott, N.C., 2005. Survival, development and growth of *Coccinella septempunctata* fed *Schizaphis graminum* from resistant and susceptible winter wheat. The Southwestern Entomologist 30, 113–120.

Giles, K.L., Jones, D.B., Royer, T.A., Elliott, N.C., Kindler, S.D., 2003. Development of a sampling plan in winter wheat that estimates cereal aphid parasitism levels and predicts population suppression. Journal of Economic Entomology 96, 975–982.

Giles, K.L., Madden, R.D., Stockland, R.E., Payton, M.E., Dillwith, J.W., 2002. Host plants affect predator fitness vial the nutritional value of herbivore prey: investigation of a plant–aphid–ladybeetle system. BioControl 47, 1–21.

Hagen, K.S., 1986. Ecosystem analysis: plant cultivars (HPR), entomophagous species and food supplements. In: Boethal, D.J., Eikenbary, R.D. (Eds.), Interactions of Plant Resistance and Parasitoids and Predators of Insects. Wiley, New York, pp. 151–197.

Hauge, M.S., 1998. The influence of three cereal aphid species and mixed diet on larval survival, development and adult weight of *Coccinella septempunctata*. Entomologia Experimentalis et Applicata 89, 319–322.

Hodek, I., Honek, A., 1996. Ecology of Coccinellidae. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Holt, R.D., Huxel, G.R., 2007. Alternative prey and the dynamics of intraguild predation: theoretical perspectives. Ecology 88, 2706–2712.

Janssen, A., Montserrate, M., HilleRisLambers, R., de Roos, A.M., Palllini, A., Sabelis, M.W., 2006. Intraguild predation usually does not disrupt biological control. In: Brodeur, J., Bovine, G. (Eds.), Trophic and Guild Interactions in Biological Control. Springer, Dordrecht, The Netherlands, pp. 21–44.

Jones, D.B., 2005. Winter ecology of the parasitoid *Lysiphlebus testaceipes* Cresson. Ph.D. Dissertation. Ph.D. Dissertation, Oklahoma State University.

Jones, D.B., 2001. Natural enemy thresholds for greenbug, *Schizaphis graminum* (Rondani) on winter wheat. M.S. Thesis, Oklahoma State University.

Kring, T.J., Kring, J.B., 1988. Aphid fecundity, reproductive longevity, and parasite development in the *Schizaphis graminum* (Rondani) Hymenoptera: Aphididae) – *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Braconidae) system. The Canadian Entomologist 120, 1079–1083.

Lebusa, M.M. 2004. Suitability of greenbugs (*Schizaphis graminum*) parasitized by *Lysiphlebus testaceipes* as a food source for predatory Coccinellidae: *Coccinella septempunctata* and *Hippodamia convergens*. M.S. Thesis, Oklahoma State University.

Majerus, M.E.N., 1994. Ladybirds. New Naturalist series no 81. HarperCollins, London.

Meyhöfer, R., Hindayana, D., 2000. Effects of intraguild predation on parasitoid survival. Entomologia Experimentalis et Applicata 97, 115–122.

Meyhöfer, R., Klug, T., 2002. Intraguild predation on the aphid parasitoid *Lysiphlebus fabarum* (Marshall) Hymenoptera: Aphidiidiae): mortality risks and behavioral decisions made under the threats of predation. Biological control: Theory and Applications in Pest Management. 25, 239–248.

Meyling, N.V., Enkegaard, A., Br?dsgaard, H., 2004. Intraguild predation by *Anthocoris nemorum* (Heteroptera: Anthocoridae) on the aphid parasitoid *Aphidius colemani* (Hymenoptera: Braconicae). Biocontrol Science and Technology 14, 627–630.

Michaud, J.P., Grant, A.K., 2005. Suitability of pollen sources for the development and reproduction of *Coleomegilla maculata* (Coleoptera: Coccinellidae) under simulated drought conditions. Biological Control 32, 363–370.

Michels Jr., G.J., Elliott, N.C., Romero, R.L., French, W.B., 1997. Estimating populations of aphidophagous Coccinellidae (Coleoptera) in winter wheat. Environmental Entomology 26, 4–11.

Müller, C.B., Brodeur, J., 2002. Intraguild predation on biological control and conservation biology. Biological Control 25, 216–223.

Murdoch, W.W., Marks, R.J., 1973. Predation by coccinellid beetles: experiments on switching. Ecology 54, 160–167.

Obrycki, J.J., Giles, K.L., Ormord, A.M., 1998. Interactions between an introduced and indigenous coccinellid species at different prey densities. Oecologia 117, 279–285.

Phoofolo, M.W., Obrycki, J.J., 1998. Potential for intraguild predation and competition among predatory Coccinellidae and Chrysopidae. Entomologia Experimentalis et Applicata 89, 47–55.

Phoofolo, M.W., Giles, K.L., Elliott, N.C., 2007. Quantitative evaluation of suitability of the greenbug, *Schizaphis graminum*, and the bird cherry-oat aphid, *Rhopalosiphum padi*, as prey for *Hippodamia convergens* (Coleoptera: Coccinellidae). Biological Control 41, 25–32.

Polis, G.A., Meyers, C.A., Holt, R.D., 1989. The ecology and evolution of intraguild predation: potential competitors that eat each other. Annual Review of Ecology and Systematics 20, 297–330.

Raymond, B.A., Darby, C., Douglas, A.E., 2000. Intraguild predators and the spatial distribution of a parasitoid. Oecologia 124, 367–372.

Rice, M.E., Wilde, G.E., 1988. Experimental evaluation of predators and parasitoids in suppressing greenbugs (Homoptera: Aphididae) in sorghum and wheat. Environmental Entomology 17, 836–841.

Roger, C., Coderre, D., Vigneault, C., Boivin, G., 2001. Prey discrimination by a generalist coccinellid predator: effect of prey age or parasitism? Ecological Entomology 26, 163–172.

Rosenheim, J.A., 1998. Higher order predators and the regulation of insect herbivore populations. Annual Review of Entomology 43, 421–447.

Rosenheim, J.A., Harmon, J.P., 2006. The influence of intraguild predation on the suppression of a shared prey population: an empirical reassessment. In: Brodeur, J., Bovine, G. (Eds.), Trophic and Guild Interactions in Biological Control. Springer, Dordrecht, The Netherlands, pp. 1–20.

Rosenheim, J.A., Wilhoit, L.R., Armer, C.A., 1993. Influence of intraguild predation among generalist insect predators on the suppression of an herbivore population. Oecologia 96, 439–449.

Roy, H.E., Alderson, P.G., Pell, J.K., 2003. Effect of spatial heterogeneity on the role of *Coccinella septempunctata* as an intra-guild predator of the aphid pathogen *Pandora neoaphidis*. Journal of Invertebrate Pathology 82, 85–95.

Royer, T.A., Giles, K.L., Elliott, N.C., 2004. Glance ‘n Go sampling for greenbugs in winter wheat, Spring edition, L-306 and Fall edition, L-307. Oklahoma Cooperative Extension Service, Stillwater, OK.

Samways, M.J., 1986. Combined effect of natural enemies (Hymenoptera: Aphelinidae and Coleoptera: Coccinellidae) with different niche breadths in reducing high populations of red scale, *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae). Bulletin of Entomological Research 76, 671–683.

SAS Institute, 2004. PROC user’s manual, version 9.1 ed. SAS Institute, Cary, NC.

Simelane, D.O., Steinkraus, D.C., Kring, T.J., 2008. Predation rate and development of *Coccinella septempunctata* L. influenced by *Neozygites fresenii*-infected cotton aphid prey. Biological Control 44, 128–135.

Smith, B.C., 1965. Effects of food on the longevity, fecundity, and development of adult coccinellids (Coleoptera: Coccinellidae). The Canadian Entomologist 97, 910–919.

Snyder, W.E., Ives, A.R., 2001. Generalist predators disrupt biological control by a specialist parasitoid. Ecology 82, 705–716.

Snyder, W.E., Ballard, S.N., Yang, S., Clevenger, G.M., Miller, T.D., Ahn, J.J., Hatten, T.D., Berryman, A.A., 2004. Complimentary biocontrol of aphids by the ladybird beetle *Harmonia axyridis* and the parasitoid, *Aphelinus asychis* on greenhouse roses. Biological Control 30, 229–235.

Soares, A.O., Coderre, D., Schanderl, H., 2004. Dietary self-selection behaviour by the adults of the aphidophagous lady beetle *Harmonia axyridis* (Coleoptera: Coccinellidae). Journal of Animal Ecology 73, 478–486.

Stephens, D.W., Krebs, J.R., 1986. Foraging Theory. Princeton University Press, Princeton, NJ, USA.

Takizawa, T., Yasuda, H., Agarwala, B.K., 2000. Effects of parasitized aphids (Homoptera: Aphididae) as food on larval performance of three predatory ladybirds (Coleoptera: Coccinellidae). Applied Entomology and Zoology 35, 467–472.

Vance-Chalcraft, H.D., Rosenheim, J.A., Vonesh, J.R., Osenberg, C.W., Sih, A., 2007. The influence of intraguild predation on prey suppression and prey release: a meta-analysis. Ecology 88, 2689–2696.

Waldbauer, G.P., Freidman, S., 1991. Self-selection of optimal diets by insects. Annual Review of Entomology 36, 43–63.

Wheeler Jr., A.G., Hayes, J.T., Stephens, J.L., 1968. Insect predators of mummified pea aphids. The Canadian Entomologist 100, 221–222.