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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)

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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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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*