Biological Control 58 (2011) 248–254

Contents lists available at ScienceDirect

Biological Control

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

Evidence for utilization of Diptera in the diet of field-collected coccinellid larvae from an antibody-based detection system

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ARTICLE INFO

Article history:
Received 8 October 2010
Accepted 27 May 2011
Available online 1 June 2011

Keywords:
Alternative prey
ELISA
Gut-content analysis
Intraguild predation
Predatory lady beetles

ABSTRACT

Aphidophagous coccinellid larvae have a wide range of potential prey in alfalfa and during times of low aphid abundance, larvae may supplement their diet with alternative prey. To understand the effects of the seasonal aphid availability on alternative prey use, an order-specific monoclonal antibody, *DrosW-VI-B8*, was used to examine the frequency of dipteran predation by these important natural enemies. Over 400 larvae were hand-collected from alfalfa and, in parallel, arthropod abundance was recorded. *Harmonia axyridis* and *Coccinella septempunctata* larvae were abundant early in the season when aphid populations were at their peak and *Coleomegilla maculata* larvae were collected later in the season when potato leafhoppers were abundant in the alfalfa. A relatively low proportion of field-collected *H. axyridis*, *C. septempunctata*, and *C. maculata* tested positive for dipteran proteins throughout the season. Similar to prior studies examining stage differences in coccinellid food breadth, older instars tested positive for dipteran proteins (3rd instar, 6% positive; 4th instar, 7% positive) but no early instars screened positive. This study provides a valuable insight into the trophic linkages that exist between coccinellid larvae and Diptera.

1. Introduction

The order Diptera is a species-rich group that is a prey source for many terrestrial predators, such as spiders (Nentwig, 1983; Harwood et al., 2007), predatory mites (Castilho et al., 2009; Jess and Schweizer, 2009), and entomophagous nematodes (Royer et al., 1996; Corlay et al., 2007; Jess and Schweizer, 2009). The use of Diptera as an alternative non-pest prey item for many predators in agricultural food webs has not been documented, despite their abundance (Jones, 1976; Delettre and Lagerlof, 1992; Nielsen et al., 1994; Nielsen and Nielsen, 2002; Harwood et al., 2007). Aphidophagous dipterans, including species in the families Syrphidae, Cecidomyiidae, and Chamaemyiidae, are often correlated with aphid abundance and likely interact with other aphid predators such as Coccinellids (Neuenschwander et al., 1975; Frazer et al., 1981; Evans and Youssef, 1992; Elliott et al., 2002; Moser, 2003; Nakashima and Akashi, 2005; Pons et al., 2005; Alhmedi et al., 2008; Kovanci et al., 2007; Brewer and Noma, 2010; Dib et al., 2010). Based on gut dissections of adult beetles, dipteran larvae have been identified in the gut contents of the Coccinellid, *Coccinella septempunctata* L., collected from various agricultural systems (Triltsch, 1999). However, the frequency of dipteran predation by predatory Coccinellidae larvae is unknown.

Aphidophagous Coccinellids are important natural enemies in many agro-ecosystems and several Coccinellid species that feed on aphids are also intraguild predators (Lucas et al., 1998; Dixon, 2000; Moser, 2003; Gardiner and Landis, 2007; Moser and Obrycki, 2009; Alhmedi et al., 2010). For predators that feed on aphids, consumption of a broad-range of food sources can be important because the abundance of their primary prey, aphids, is ephemeral (Dixon, 2000) and aphids are often a low quality food source (Triltsch, 1999; Toft, 2005; Lundgren et al., 2009, 2011; Lundgren and Weber, 2010). Furthermore, dietary diversification by predators has resulted in improved nutrition and has increased fitness for many species (Oelbermann and Scheu, 2002; Toft, 2005; Harwood et al., 2009; Moser and Obrycki, 2009; Lundgren, 2009a).

Techniques used to identify predator-prey interactions include laboratory and field-cage studies, field observations, gut dissections, and molecular techniques, such as polymerase chain reaction (PCR) and enzyme-linked immunosorbent assays (ELISAs) (see review Weber and Lundgren, 2009). There are limitations with most of these techniques and the combined use of multiple techniques may be the most appropriate approach. For example, laboratory studies can identify potential predator-prey interactions but because these studies do not simulate the environmental complexity found in the field, additional field-based methods can be utilized to examine specific interactions identified in the laboratory. Predator-gut content analysis using monoclonal antibodies (MAbs) allows for screening numerous individuals for a particular prey protein and is extremely effective at detecting infrequent predation events

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doi:10.1016/j.biocontrol.2011.05.014

and alternative prey use (Greenstone and Morgan, 1989; Symondson et al., 2000; Harwood et al., 2004; Unruh et al., 2008). For example, the use of a MAb detected low levels of dipteran predation (16%) by the linyphiid spider, *Erigone autumnalis*, in alfalfa (Harwood et al., 2007).

Our research objective is to determine the frequency of dipteran predation by predatory larvae of three Coccinellid species, *Harmonia axyridis* Pallas, *Coleomegilla maculata* DeGeer, and *C. septempunctata*. We used indirect ELISAs with Mabs to test for dipteran proteins within field-collected Coccinellids. We also measured the dipteran protein degradation rates in laboratory feeding trials. We hypothesized that Diptera may serve as an alternative prey item in alfalfa when aphid populations were low or declining.

2. Materials and methods

2.1. Analysis and ELISA protocol

The *DrosW-VI-B8* antibody was developed from a mixture of Diptera families that were collected from alfalfa in Kentucky, our target system. The immunization protocols and developmental procedures are described in details in Harwood et al., 2007. The *DrosW-VI-B8* antibody was tested with 22 dipteran species from 13 different families, including Syrphidae, Drosophilidae, and Tachinidae. A total of 82 non-dipteran controls from 15 different families, including *Acyrthosiphon pisum* Harris (Hemiptera: Aphididae), *Empoasca fabae* Harris (Homoptera: Cicadellidae), *C. septempunctata*, *H. axyridis*, and *C. maculata*, were tested from the field site for cross-reactivity and no false-positives were detected (Harwood et al., 2007).

Whole-body homogenates of predators were prepared following protocols of Harwood et al. (2007) and Harwood (2008). Field-collected and laboratory-reared larvae were individually weighed and homogenized to a stock concentration of 1:20 (mg: l) in phosphate-buffered saline (PBS) and centrifuged at 8000 g for 15 min. The resulting supernatant was removed and stored at -20°C until ELISA analysis; particulate matter was discarded. A stock concentration of 1:100 (mg:μl) in PBS was used for larvae that weighed less than 0.0015 g. Samples, in duplicate, were coated directly onto Fisherbrand™ 96-well polystyrene micro-titration plates (Fisher Scientific L.L.C., Pittsburgh, PA, USA) at a 1:20,000 concentration, diluted in PBS. After incubation for 2 h, plates were washed and 200 μl of the *DrosW-VI-B8* monoclonal antibody, diluted 1:1000 in PBS Tween (0.05% polyoxyethylene-20 sorbitan monolaurate; Sigma-Aldrich, St. Louis, MO, USA), was added to one of the two (duplicate) wells; the second well received 200 μl of PBS Tween (without antibody). After the subsequent incubation and wash, 200 μl of ImmunoPure goat anti-mouse IgG horseradish peroxidase enzyme conjugate (dilution 1:4000 PBS-Tween; Pierce Biotechnology, Rockford, IL, USA) was added to each well. After the final incubation and wash, 200 μl of the enzyme substrate, *o*-phenylenediamine (Sigma-Aldrich, St. Louis, MO, USA) in a citrate-phosphate buffer, was added to the wells. The reaction was stopped by addition of 50 μl 2.5 M H_2SO_4 and a Thermo Labsystems Multiskan Plus spectrophotometer (Thermo Electron, Waltham, MA, USA) was used to measure the absorbance readings (recorded at 492 nm). The absorbance from duplicate wells was subtracted from wells containing antibodies to eliminate non-specific binding effects (Symondson et al., 2000). In parallel to the coating of ELISA plates with field-collected or feeding trial Coccinellids, positive and negative controls were included on all plates (after Harwood et al., 2007); positive controls ($n = 8$ samples per plate) were *Drosophila melanogaster* and negative controls ($n = 8$ samples per plate) were the highest cross-reacting non-dipteran, *Graminella nigrifrons* (Forbes) (Homoptera: Cicadellidae). The threshold for positive reactivity was assessed when Coccinellids elicited an absorbance value greater than the mean + 3.0 SD of the absorbance value recorded by these negative controls.

2.2. Antigen decay rates

To determine dipteran protein degradation rates within each Coccinellid species collected in the field (*H. axyridis*, *C. maculata*, and *C. septempunctata*), larvae of each species were fed dipteran prey, *Musca domestica* L. (Diptera: Muscidae) or *Drosophila melanogaster* Meigan (Diptera: Drosophilidae) and frozen at several times post-feeding, and screened by ELISA, as above. Prior to exposure to *M. domestica*, Coccinellid larvae (*H. axyridis* 2nd, 3rd, and 4th instars; *C. maculata* 2nd, 3rd, and 4th instars; *C. septempunctata* 2nd and 3rd instars) were starved for 24 h. Larvae were individually supplied with 3 sliced *M. domestica* pupae (Oregon Feeder Insects Company City, Payette, ID, USA) and allowed to feed on pupae for up to 30 min under laboratory conditions. Larvae were excluded from the experiment if they fed on the pupae for less than 10 min during the 30 min feeding period. Prior to exposure to *D. melanogaster* larvae, *H. axyridis*, *C. septempunctata*, and *C. maculata* 3rd instars were also starved for 24 h. Each Coccinellid larva was allowed to feed on up to 3 *D. melanogaster* larvae for 2 h. Given that starvation may artificially increase the rate of prey digestion and thus reduce prey detection with ELISA (Symondson and Liddell, 1995; Symondson et al., 2000), larvae were supplied with an *ad libitum* supply of *A. pisum* immediately following feeding on *M. domestica* or *D. melanogaster*. Larvae were frozen in groups ($n = 10$) at 0, 1, 2, 3, and 4 h post-feeding; preliminary screening indicated that dipteran proteins were not detectable after 5 h (S. Moser, unpublished data). This detection period is shorter than previously reported for linyphiid spiders (Harwood et al., 2007).

2.3. Collection of Coccinellid larvae and prey items

Field census and sampling were conducted from May–August 2006 in alfalfa, *Medicago sativa* L. (Fabales: Fabaceae), located at the University of Kentucky Spindletop Research Station, Lexington, KY, USA (Universal Trans-Mercator Grid: 4224676 N, 689850 E, Zone 16, field about 4000 m^2). An uncut alfalfa border (about 2 m wide × 50 m and 83 m long) was maintained around the perimeter of the field after the first cutting (week of May 31).

Sweep-net samplings were conducted to detect the presence of Coccinellid larvae (20 sweeps per sample, three samples from random locations); larvae were hand-collected by visual census in alfalfa if at least one larva was found in the sweep samples (10 of 17 weeks). An individual larva was kept in a 1.5-ml microcentrifuge tube and immediately stored in a field freezer (Engel portable freezer, 34 qt). Hand-collection was conducted for 4 h, starting at 9:00 am, or until 50 larvae were collected on each sampling date. Larvae were collected by hand to prevent contamination through reflex-bleeding and artificial predator-prey interactions, which have potential to occur with other sampling methods.

To examine the abundance of aphids (pea aphids and spotted aphids) and alternative prey (dipterans and leaf hoppers), four whole-alfalfa plants were cut randomly at ground surface, placed in a plastic bag, and immediately frozen in the field freezer. Prey samples and individual larvae were transferred from the field freezer into a −20 °C laboratory freezer until analyzed.

2.4. Data analysis

For the analyses of antigen decay rates, a logit analysis (dose-response model, binomial distribution, logit link function) was conducted for each Coccinellid species to compare dipteran protein detection (proportion positive) to the factors of time post-feeding, larval instar, and prey life stage (larvae or pupae) in the laboratory