Supplemental Foods Affect Energetic Reserves, Survival, and Spring Reproduction in Overwintering Adult Hippodamia convergens (Coleoptera: Coccinellidae)

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Abstract

For insects that overwinter as adults, winter food resources may affect subsequent spring reproduction and abundance. We tested if provision of food supplements to overwintering adult Hippodamia convergens (Guerin) increased energy reserves, winter survival, and spring reproduction. During 2015–2016, H. convergens adults were placed in field cages in December; adults in each cage received water, Acyrthosiphon pisum (Harris) (Hemiptera: Aphididae), Ephesia kuehniella (Zeller) (Lepidoptera: Pyralidae) eggs, bee pollen, wheast protein, sugar, honey, or no food (control). In 2016–2017, treatments were reduced to sugar, bee pollen, A. pisum with E. kuehniella eggs, and no food (control). Adults were sampled to quantify weight, lipid, carbohydrate, and protein content. In 2015–2016, A. pisum and E. kuehniella eggs increased adult weight and protein content, but adult carbohydrate content was reduced by A. pisum and wheast protein treatments. Adults receiving honey and sugar supplementation had higher lipid and carbohydrate content relative to controls. The number of live individuals at the end of the experiment in March 2016 did not differ among treatments. In 2016–2017, winter prey supplements had the greatest effect on protein content, weight, and number of live adults recovered, whereas sugar supplementation increased lipid and carbohydrate content, and number of live adults recovered. Spring reproduction of surviving pairs was evaluated among treatments in March 2017. Prey supplementation in 2016–2017 increased the number of eggs laid and decreased preoviposition period, and food treatment did not affect fertility. Our results indicate that prey and sugar resources improve the overwintering success and spring reproduction of H. convergens.

Key words: overwintering, food supplementation, survival, augmentative biological control, adult diapause

For North American lady beetles and many other temperate zone insects, finding winter habitat with suitable abiotic (Desender 1982, Sotherton 1984, Thomas et al. 1992, Lys and Nentwing 1994, Pfiffner and Luka 2000, Raymond et al. 2014) and biotic conditions (Thomas et al. 1991, 1992; Lorenzon et al. 2015) is crucial for their survival. A fundamental understanding of these factors can improve biological control by increasing the fitness of overwintering natural enemies in the spring. The convergent lady beetle, Hippodamia convergens (Guerin), a common and economically important predator in North America, feeds on a variety of prey and plant resources (Hagen 1962, Obrycki and Kring 1998, Lundgren 2009). Many Coccinellidae overwinter as adults in a state of reproductive diapause, and due to seasonal changes in food availability, may go long periods with little to no food. Thus, storing and conserving energy is a critical adaptation for surviving the winter and resuming reproduction in the spring (Hahn and Denlinger 2011).

Lipids, carbohydrates, and proteins are important energy reserves for overwintering Coccinellidae (Hagen 1962, Sakurai 1969, Watanabe 2002, Raak-van den Berg et al. 2012, Awad et al. 2013). Lipids are the primary energy store in diapausing Coccinellidae (Hagen 1962, Raak-van den Berg et al. 2012), whereas carbohydrates are used for the synthesis of cryoprotectants and metabolic energy (Sakurai 1969, Watanabe 2002). The role of proteins in overwintering Coccinellidae is not fully understood, although these stored proteins are used for vitellogenin production in spring (Sakurai et al. 1987, Awad et al. 2013). Energy reserves stored for winter metabolism may also be used as energy for spring reproduction. For example, lipids accumulated by adult Culex pipiens L. (Diptera: Culicidae) prior to winter are utilized in spring egg production (Zhou and Miesfeld 2009). Thus, measuring these energy reserves can indicate the condition of the insect (Sakurai 1969, Jean et al. 1990, Watanabe 2002, Labrie et al. 2008).
Although metabolism is typically reduced in the winter, temperature can influence the rate of energy expenditure by overwintering insects (Jean et al. 1990, Thomas et al. 1992, Williams et al. 2003). Lipid depletion and mortality of overwintering Coleomegilla maculata (Degener) (Coleoptera: Coccinellidae) increased exponentially above −0.5°C in laboratory experiments (Jean et al. 1990). However, elevated winter temperatures may indirectly benefit insects in the field by providing an opportunity to feed and replenish metabolic reserves for insects that overwinter in a mobile stage (Danks 1991, Thomas et al. 1992, Dennis et al. 1994, De Block et al. 2007, Eitzinger and Traugott 2011). For example, winter survival of adult Demetrias atricapillus (L.) (Coleoptera: Carabidae) increased when prey was present in overwintering sites (Thomas et al. 1992) or artificially added (Dennis et al. 1994). In the laboratory, overwintering adult H. convergens fed at temperatures above 5°C; the rate of consumption increased with temperature (Roach and Thomas 1991). Winter survival and vernal fecundity of insects can also depend on the type of food available. Postdiapause reproduction and survival of H. axyridis was improved by the addition of ad libitum aphid prey compared with a diet of sugar or sugar with <10 aphids per day (Reznik and Vaghina 2013).

The mean daily maximum temperature of 6.3°C from December 1 to March 31 in central Kentucky (data from NOAA National Climatic Data Centre [USA] www.ncdc.noaa.gov; previous 30 yrs) is suitable for adult activity and feeding by H. convergens (Roach and Thomas 1991). Thus, overwintering lady beetles have periodic opportunities to feed in Kentucky, making this region a suitable location to test the effect of food supplementation on overwintering adults and subsequent spring reproduction. The objective of this study was to quantify the effects of supplemental foods on overwintering adult H. convergens energy-reserve content, survival, and spring reproduction.

**Materials and Methods**

2015–2016 Field Experiment

*Hippodamia convergens* adults were collected from overwintering aggregations in Arizona in October 2015 by ARBICO-Organics (ARBICO-Organics, Oro Valley, AZ) and shipped to Lexington, KY, on 10 December 2015. *Hippodamia convergens* diapause lasts from October to at least January (Hagen 1962). Once received, adults were stored at 4°C with moist paper towels until used in the experiment on 12 December 2015.

Field sites were located at the University of Kentucky’s Spindletop Research Farm, Fayette Co., Lexington, KY (N 38° 6’ 15.9” W 84° 25’ 57.9”). Overwintering sites for *H. convergens* in Kentucky are unknown, but large (>6 m tall) hardwood trees were selected as proxies because of previous observations of adult *C. maculata* overwintering around the base of hardwood trees at this location. Each of the five replicate sites was a minimum of 180 m from the nearest neighboring replicate site. At each site, eight overwintering cages (one cage per treatment; see description below) were evenly spaced along a semicircle with a 3.5-m radius from the base of the tree, buried 12 cm into the ground and extending 8 cm above the ground (Fig. 1a).

Overwintering cages were 10-cm-diameter PVC pipe, cut into 20-cm-length sections. Lunate 280 micron mesh covered both ends, and hardware cloth (6.35 mm3 mesh) covered the top end to prevent entry by vertebrates. Petri dishes (5.5 x 1.5 cm) were glued to the inside of cages 2.5 cm from the top for feeding trays. Cages were filled with untreated cypress mulch up to the feeding tray to provide substrate for *H. convergens* (Jean et al. 1990). On 12 December 2015, *H. convergens* were separated into 40 groups of 300 and placed into cages. The first 12 groups of 300 adults were individually counted and weighed. The remaining 28 groups were estimated by total weight, based on the mean weight (4.7 ± 0.7 g) of the first 12 groups. Temperatures in the cages were recorded hourly using iButtons (Maxim Integrated, San Jose, CA). Individual recorders were wrapped in parafilm to protect them from water and placed in two randomly selected cages per site. Temperature recorders were placed on the surface of the cypress mulch substrate within each cage.

At each field site, cages were randomly assigned one of eight food supplement treatments, so that each treatment was represented once at each replicate site. Food supplements were as follows: no food (control), 10 ml water, 10 ml 15% sucrose solution (referred to as sugar), 10 ml honey, 5 g bee pollen substitute (BEE PRO, Mann Lake Ltd, Hackensack MN; referred to as pollen), 5 g wheat protein (Beneficial Insectary Inc., Redding, CA), 3 g previously frozen Ephestia kuehniella eggs (Zeller) (Lepidoptera: Pyralidae), which were stored at ~20°C (Beneficial Insectary Inc.), or 2.5 g live pea aphids [Acyrthosiphon pisum (Harris)] (Hemiptera: Aphididae) reared on fava beans [Vicia faba L]. Water, sugar, and honey were applied to a 5 x 1 cm sponge to prevent adults from drowning. Pollen and wheat protein were mixed with 2 ml of water to form a paste. These food treatments are established food sources for Coccinellidae, either in the wild or for laboratory rearing (Lundgren 2009, Moser and Obrycki 2009, Choate and Lundgren 2013). Aphids were observed to move around the cage once placed on the food trays. Umbrellas for food trays were made out of 5-cm petri dish lids with 2.5-cm plastic legs holding them over the food tray to shield the food trays from rainfall.

![Fig. 1.](https://academic.oup.com/ee/article-abstract/49/1/1/5636802)
Caged *H. convergens* were given food supplementation on days when temperatures were predicted to exceed 4°C (forecasts from NOAA National Weather Service [USA], [www.weather.gov](http://www.weather.gov)), but no more than once every 7 d. The selection of 4°C as a threshold temperature was based on observations that *C. maculata* were active around overwintering sites at temperatures > 4°C in Kentucky (personal observation). Live, active *H. convergens* were sampled from cages on 31 December 2015, 21 January 2016, 18 February 2016, and 11 March 2016. On the first sampling date, 20 adults were sampled, but the sampling size was reduced to 10 on subsequent sampling dates due to concerns of depleting the number of live individuals before the end of the experiment. *Hippodamia convergens* collected from cages were immediately placed in ice chests and transferred to a −80°C freezer. Experiments were terminated after five consecutive days in March had a maximum temperature over 15°C. At the end of the field portion of the experiment, on 11 March 2016, all *H. convergens* adults were collected, and the number of live *H. convergens* was used as a proxy for survival for each treatment cage. True survival could not be calculated because removing live *H. convergens* throughout the experiment prevented accurate calculation of survival.

### 2016–2017 Field Experiment

Experimental procedures were similar to 2015–2016 except for three modifications: the number of treatments was reduced from eight to four (based on the results of 2015–2016 experiment), the number of adults per cage was increased from 300 to 360, and the number of sampling dates was reduced from four to three. Water, honey, and wheasen protein treatments were removed in 2016–2017 and the *E. kuehniella* eggs and aphid treatment were combined (1 g of *E. kuehniella* eggs and 2.5 g aphids; referred to as prey). Sugar and bee pollen were given to *H. convergens* in the same quantity and manner as the previous year. Each treatment had one replicate at each of the five sites. Cage spacing from the tree remained the same as in 2015–2016 ([Fig. 1b](#)), but only four cages were buried at each location (one cage per treatment).

*Hippodamia convergens* adults were received from ARBICO-Organics on 1 December 2016 and stored at 4°C. The number of *H. convergens* per cage was increased to 360, and the number of live adults removed per sampling period was increased to 30. There were three sampling dates, 2 December 2016 (the day *H. convergens* were placed in field cages), 31 January 2017, and 29 March 2017 (the final day of the experiment). Sample beetles were handled in the same manner as the previous year.

An iButton temperature logger was placed in one cage at each site. Soil surface temperature was measured by a second logger placed in a cotton cloth bag and staked under a 15 × 15 cm section of hardware cloth at the peak of the site’s semicircle ([Fig. 1b](#)). Food treatments were replenished (no more than once every 7 d) when the temperature was predicted to be ≥15°C. The increased temperature threshold was used to more reliably select periods when *H. convergens* adults would be actively feeding. Roach and Thomas ([1991](#)) observed that 5% of *Heliothis sea* (Boddie) (Lepidoptera: Noctuidae) eggs were consumed in 24 h by overwintering *H. convergens* at 5 and 10°C, whereas 25% of eggs were consumed at 15°C. On 29 March 2017, adults were collected and the number alive in each cage was counted.

### Energy-Reserve Analysis

Adults collected from cages and stored in −80°C were weighed (while frozen) on an electronic balance to 0.1 mg (XSE105, Mettler-Toledo, LLC, Columbus OH) and sexed using a stereomicroscope (males have an indentation on their fifth ventral abdominal segment). Sexes were analyzed separately, although due to random sampling, the sex ratio was not the same among cages. Energy-reserve content (lipids, carbohydrates, and proteins) were quantified in separate samples of adults, with five adults for each energy reserve analyzed per cage per sampling date from the 2015 to 2016 experiment and 10 adults per sample analyzed per energy reserve in 2016–2017 experiment. Sex ratios of samples within each set of 5 and 10 were random.

Initially, analyses were conducted on individual adults (sampling unit), and the results averaged within each cage (experimental unit) for protein, carbohydrate, and lipid content. However, this approach was found to be time intensive, and an experiment was done to compare energy-reserve extractions on individual adults and pooled adults from a cage, testing if the methods yielded the same results. Fifty-five female *H. convergens* (survivors from 2015 to 2016 experiment that were not used for energy-reserve analysis) were separated into 11 groups of five adults. Adults were individually homogenized, and then were either placed into individual vials or into pooled vials (five adults combined into one vial per group), yielding 11 groups of individuals (five vials per group) or 11 pooled vials. Protein, lipid, and carbohydrate contents were then measured according to measurements of concentrations were then taken according to protein, lipid, or carbohydrate methods (see below). Energy-reserve content was statistically compared using an equivalence test (SAS Institute Inc. [2014](#)) to determine how similar the two methods were for estimating lipid, carbohydrate, and protein content. Lipid (upper $t_9 = 6.1$, $P < 0.0001$, lower $t_9 = −2.35$, $P = 0.02$), carbohydrate (upper $t_9 = 3.23$, $P = 0.005$, lower $t_9 = −2.49$, $P = 0.02$), and protein (upper $t_9 = 1.98$, $P = 0.04$, lower $t_9 = −2.02$, $P = 0.04$) measurements were similar whether individually measured or pooled, meaning measurements from the initial individual analysis method were equivalent to pooled sample measurements. Adults from 11 March 2016 and 2 December 2016 were measured individually (the initial adults analyzed); adults from all other sample dates were pooled into groups by field cage and sex.

### Lipid Analysis

Lipids were extracted using modified methods of Folkh *et al.* ([1957](#)). *Hippodamia convergens* were homogenized in 1 ml of 2:1 chloroform: methanol solution, agitated for 15 min at 300 rpm and then centrifuged at 13,000 × g for 5 min at 4°C. The supernatant was transferred to a new tube and combined with 400 µl ddH2O. Tubes were incubated for 5 min at room temperature (−22°C), after which the mixture was partitioned into a lower chloroform phase and an upper aqueous phase.

12.5 µl of the lower chloroform phase was transferred to individual glass tubes. A standard consisting of 1 mg/ml canola oil in chloroform was used to generate an eight-point standard curve containing 0- to 500-µg lipid. Samples and standards were then heated at 110°C until the liquid evaporated and only lipid remained. After evaporation, lipid was quantified using sulfo-phospho-vanillin methods (van Handel [1985](#)). Dried lipid samples were resuspended in 100 µl of sulfuric acid and heated at 90°C for 10 min. Afterward, 1 ml of vanillin phosphoric acid reagent (120 mg of vanillin dissolved in 20 ml of ddH2O and 80 ml of phosphoric acid; vanillin Product TCH0264; VWR, Radnor, PA) was added to each sample and standard. Color was allowed to develop for 20 min at room temperature. Samples and standards were loaded in triplicate into 96-well plates, 100 µl per well. Optical density at 525 nm was measured by a spectrophotometer (ClarioStar Microplate reader, BMG Labtech, Offenburg, Germany).
Carbohydrate Analysis

Carbohydrate content was quantified using the anthrone method modified from van Handel (1985). Individual *H. convergens* were homogenized in 0.5 ml phosphate-buffered saline containing 0.05% Tween (TWEEN 20, SKU # 00-3005; ThermoFisher, Waltham, MA; Gefen et al. 2006, Tennen et al. 2014). Samples were then centrifuged at 13,000 × g at 4°C for 5 min. After centrifuging, 50 µl of each sample’s liquid phase was removed and combined with 50 µl ddH2O. Samples were compared with an eight-point standard curve of glucose ranging from 0 to 1,000 µg/ml (d(+)-glucose solution, Product # G8644, Sigma–Aldrich, St. Louis, MO). To develop color, 400 µl of anthrone reagent (140 mg of anthrone dissolved in 72 ml H2SO4, and 28 ml H2O; anthrone reagent, Product # 319899, Sigma–Aldrich) was added to each sample and standard. Tubes were then vortexed and heated at 100°C for 17 min to facilitate color development. Samples and standards were loaded in triplicate into 96-well plates, 125 µl per well. Optical density at 625 nm was measured by a spectrophotometer (ClarioStar Microplate reader, BMG Labtech, Offenburg, Germany).

Protein Analysis

Quantification of proteins was accomplished with the BCA protein assay kit (Product #P23227; ThermoFisher) using the manufacturer’s protocol. Adults were homogenized in 0.5 ml of RIPA buffer (Product #8990; ThermoFisher) and then centrifuged at 14,000 × g for 5 min. After centrifuging, 25 µl of the supernatant was removed and combined with 75 µl of H2O. Samples were compared with an eight-point standard curve of 0–2,000 µg/ml bovine serum albumin. Color was developed by combining 25 µl of sample with 200 µl of the working reagent from the BCA protein assay kit in one well of a 96-well plate. Samples were loaded into wells in triplicate. Well plates were agitated for 30 s and then heated at 37°C for 30 min. Optical density at the 563 nm was measured using a spectrophotometer (ClarioStar Microplate reader, BMG Labtech).

Fecundity and Fertility Experiment

Live beetles collected from cages at the termination of the 2016–2017 field experiment (29 March 2017) were sexed, and three mating pairs per field cage were established (60 pairs total). Pairs were kept in 0.24-liter cardboard containers at 22°C and a photoperiod of 16:8 (L:D) h with a plastic vial containing distilled water with a cotton ball stopper to supply moisture. Folded pieces of paper were added as an egg-laying substrate. Pairs were fed A. pismum ad libitum daily and given 5-cm sections of V. faba stems. Oviposition was monitored daily. Eggs laid were removed from containers, counted, and placed in 12-ml plastic vials with a cotton stopper and kept under the same conditions as the adults. Eggs were checked daily for eclosion or until 1 wk passed, and no larvae were observed. Fecundity was defined as the total number of eggs laid by a female over 30 d and fertility as the proportion of eggs laid within individual egg masses that hatched.

Data Analysis

Differences among treatments in the number of live *H. convergens* were tested by mixed-model analysis of variance (ANOVA) with treatment as fixed effect and site as random effect. Initially, energetic reserve contents were corrected with weight, analyzing mg energetic reserve per mg adult body weight. This, however, was found to mask the differences among treatments as beetles with more energetic reserves weighted more. To avoid masking treatment effects, all energy-reserve contents are expressed as mg of nutrient per individual *H. convergens*. The experimental units for energy reserves were averages of 5 (2015–2016) or 10 (2016–2017) individuals per cage per sampling period, grouped by sex within groups of 5 or 10 adults. A repeated-measures mixed-model ANOVA was used to test the effects of food supplement on energy-reserve content; each energy reserve was tested separately. Energy-reserve content was the dependent variable, food supplement, sampling date (time), and sex were included as fixed effects. Site and cage were included as random effects. Differences between treatments and controls were assessed with Dunnett’s post hoc test on the final sampling date, 3 November 2016 and 29 March 2017. Weights of *H. convergens* were obtained from adults used in the energy-reserve assays. Weights were analyzed in the same manner as the energy reserves. Temperature was compared between cages and surface in 2016–2017 with a repeated-measures ANOVA. Recorder location and site were treated as fixed effects.

Days to produce the first viable egg mass (preoviposition time) and five viable egg masses were analyzed with Cox’s proportional hazard model. Time to five viable egg masses was used as a metric for the level of fertility of a female. Fecundity (total number of eggs per female over 30 d) and fertility (number of viable eggs produced per egg mass per female) were averaged within field cages and analyzed with one-way ANOVA. Treatment was a fixed effect and field cage site a random effect. Dunnett’s post hoc test was used to determine the differences in effect between treatments and the control. Cox’s proportional hazard model was done in JMP (2015) (JMP 12.1.0). All other tests were done in R v. 3.2.4 (R Core Team 2017) with packages ‘nlme’ (Bates et al. 2017) and ‘multcomp’ (Hothorn et al. 2008). Voucher specimens of *H. convergens* are in the University of Kentucky Insect Museum. Data are deposited in the UKnowledge repository: https://doi.org/10.13023/mj65-sd73.

Results

Energy Reserves

*Hippodamia convergens* were fed seven times in 2015–2016 and nine times in 2016–2017. Temperature did not differ among cages or locations in either year (Supp Tables S1 and S2 [online only]). All food supplements were observed to be eaten by the adults at least once in both years. The number of individuals that fed and the amount eaten were not quantified. In 2015–2016, lipid (mg lip per adult; F1,193 = 24.23, P < 0.001) and carbohydrate content (mg carbohydrate per adult; F1,179 = 10.94, P < 0.001) decreased significantly from 31 December 2015 to 11 March 2016 (Table 1). A similar trend was observed in 2016–2017, lipid (F2,62 = 19.97, P < 0.001), and carbohydrate content (F2,44 = 33.54, P < 0.001) decreased from 2 December 2016 to 29 March 2017 (Table 2).

Protein content (mg protein per adult) in 2015–2016 increased, decreased from 2 December 2016 to 29 March 2017 (Table 2).

Table S3A (online only).

**Table 1.** Treatment effects did not differ between sexes. ANOVA tables for 2016–2017: F1,35 = 4.59, P = 0.02; Table 2. In both years, lipid content (2015–2016: F2,99 = 22.39, P < 0.001; 2016–2017: F2,99 = 35.23, P < 0.001), carbohydrate content (2015–2016: F2,179 = 17.03, P < 0.001; 2016–2017: F2,179 = 17.98, P < 0.001), and protein (2015–2016: F2,179 = 17.64, P < 0.001; 2016–2017: F2,179 = 4.59, P = 0.04) were higher in females than in males; however, treatment effects did not differ between sexes. ANOVA tables for 2015–2016 and 2016–2017. Energy reserves are reported in Supp Table S3A (online only).
### Materials and Methods

Data are grouped by sampling date, winter food-supplementation treatment, and sex. Numbers are mean energy reserves or mean adult weight ± SE. *Asterisk* (*) indicates significant difference (P < 0.05) from controls according to Dunnett's post hoc test on 11 Mar. 2016. NA are samples lost due to measurement error. Empty cells are because protein was not measured on *H. convergens* sampled on those dates, see Materials and Methods. NA (not applicable).
On the final sampling date of 11 March 2016, adult lipid content was significantly affected by food supplements ($F_{7,52} = 14.73, P < 0.001$); prey ($P = 0.001$) and sugar ($P < 0.001$) supplemented beetles had higher lipid contents than the unfed control adults. Carbohydrate content on 11 March 2016 was significantly different ($F_{7,58} = 3.81, P < 0.001$) among treatments; aphid ($P < 0.001$) and wheat protein ($P < 0.001$) supplemented adults had lower carbohydrate content than controls, whereas sugar was marginally higher ($P = 0.06$) than controls. *Ephesia kuehniella* eggs, pollen, water, and honey-supplemented adults had similar carbohydrate content relative to controls. On 29 March 2017, carbohydrate content was increased ($F_{2,19} = 4.47, P = 0.02$) in sugar ($P = 0.02$) supplemented individuals relative to controls. The carbohydrate content of *H. convergens* supplemented with prey had a trend of being higher than controls ($P = 0.08$). On 11 March 2016, protein content was also affected by treatments ($F_{7,52} = 8.07, P < 0.001$). Aphid ($P < 0.001$) and *E. kuehniella* eggs ($P < 0.001$) had higher protein content compared with controls. On 29 March 2017, protein content was significantly higher ($F_{2,19} = 10.81, P < 0.001$) in prey-supplemented adults ($P = 0.001$) relative to controls. ANOVA tables of energetic reserves on the final sampling dates, 11 March 2016 and 27 March 2017, are reported in Supp Table S3B (online only).

### Survival
Total number of adults present in cages could not be determined due to decomposition of adults in both years, so only numbers of live adults were counted. In 2015–2016, supplemental food had no effect on the number of adults found alive on the final sampling date, 11 March 2016 (Fig. 2). Two cages were excluded from analysis because the low number of individuals alive (two and nine, both aphid treatments) was likely due to escape of *H. convergens*. In 2016–2017, supplemental food significantly affected the number of *H. convergens* alive on 29 March 2017 ($F_{1,19} = 5.28, P = 0.02$). The number of live adults in the prey and sugar treatments was higher relative to controls ($P = 0.009$ and $P = 0.06$ respectively; Fig. 3).

### Fecundity and Fertility
Over the 30 d of the reproductive experiment, 33 of 60 (55%) females produced eggs and 21 of the 33 (64%) females produced five egg masses. Mortality of females prior to oviposition varied among treatments. Only one of the 15 prey-supplemented females died prior to oviposition; however, 7 of the 15 sugar-fed females, 10 of the 15 pollen supplemented females, and 9 of the 15 control females died.

### Table 2. Energy-reserve content (lipid, carbohydrate, or protein) and weights (mg) of overwintering *Hippodamia convergens* in 2015–2016

<table>
<thead>
<tr>
<th>Sample date</th>
<th>Treatment</th>
<th>Sex</th>
<th>Lipid</th>
<th>Carbohydrate</th>
<th>Protein</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 Dec. 2016</td>
<td>Control</td>
<td>F</td>
<td>3.08 ± 0.52 [5]</td>
<td>0.19 ± 0.01 [5]</td>
<td>1.74 ± 0.06 [5]</td>
<td>20.10 ± 0.47 [5]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M</td>
<td>1.73 ± 0.39 [5]</td>
<td>0.14 ± 0.01 [5]</td>
<td>1.43 ± 0.06 [5]</td>
<td>15.79 ± 0.30 [5]</td>
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<tr>
<td></td>
<td>Pollen</td>
<td>F</td>
<td>3.26 ± 0.30 [5]</td>
<td>0.17 ± 0.01 [5]</td>
<td>1.53 ± 0.06 [5]</td>
<td>19.54 ± 0.39 [5]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M</td>
<td>1.99 ± 0.26 [5]</td>
<td>0.14 ± 0.01 [5]</td>
<td>1.35 ± 0.08 [5]</td>
<td>15.97 ± 0.46 [5]</td>
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<tr>
<td></td>
<td>Prey</td>
<td>F</td>
<td>3.52 ± 0.52 [5]</td>
<td>0.18 ± 0.01 [5]</td>
<td>1.57 ± 0.07 [5]</td>
<td>20.36 ± 0.82 [5]</td>
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<td></td>
<td></td>
<td>M</td>
<td>1.87 ± 0.41 [5]</td>
<td>0.15 ± 0.02 [5]</td>
<td>1.37 ± 0.05 [5]</td>
<td>16.61 ± 0.49 [5]</td>
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<td>Sugar</td>
<td>F</td>
<td>3.31 ± 0.29 [5]</td>
<td>0.18 ± 0.01 [5]</td>
<td>1.67 ± 0.07 [5]</td>
<td>19.46 ± 0.62 [5]</td>
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<td>M</td>
<td>2.62 ± 0.59 [5]</td>
<td>0.14 ± 0.01 [5]</td>
<td>1.46 ± 0.06 [5]</td>
<td>16.79 ± 0.71 [5]</td>
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<td>M</td>
<td>1.48 ± 0.40 [5]</td>
<td>0.18 ± 0.01 [5]</td>
<td>1.08 ± 0.11 [5]</td>
<td>16.89 ± 1.09 [5]</td>
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<td>Pollen</td>
<td>F</td>
<td>2.78 ± 0.31 [5]</td>
<td>0.21 ± 0.01 [5]</td>
<td>1.25 ± 0.13 [5]</td>
<td>18.48 ± 1.06 [5]</td>
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<td></td>
<td>Prey</td>
<td>F</td>
<td>1.80 ± 0.10 [5]</td>
<td>0.20 ± 0.01 [5]</td>
<td>1.31 ± 0.19 [5]</td>
<td>20.18 ± 1.18 [5]</td>
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<td>M</td>
<td>1.30 ± 0.45 [5]</td>
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<td>22.00 ± 5.88 [5]</td>
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<td></td>
<td>M</td>
<td>1.48 ± 0.26 [5]</td>
<td>0.22 ± 0.02 [5]</td>
<td>1.26 ± 0.22 [5]</td>
<td>15.66 ± 0.27 [5]</td>
</tr>
<tr>
<td></td>
<td>Pollen</td>
<td>F</td>
<td>0.93 ± 0.15 [5]</td>
<td>0.06 ± 0.01 [3]</td>
<td>1.04 ± 0.48 [3]</td>
<td>17.86 ± 0.52 [5]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M</td>
<td>0.03 ± 0 [1]</td>
<td>0.08 ± 0.01 [2]</td>
<td>0.39 ± 0.29 [2]</td>
<td>14.58 ± 0.31 [3]</td>
</tr>
<tr>
<td></td>
<td>Prey</td>
<td>F</td>
<td>0.78 ± 0.15 [3]</td>
<td>0.08 ± 0.02 [5]</td>
<td>1.00 ± 0.38 [4]</td>
<td>17.37 ± 0.74 [4]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M</td>
<td>0.10 ± 0 [1]</td>
<td>0.08 ± 0.01 [2]</td>
<td>0.62 ± 0 [1]</td>
<td>14.25 ± 0.80 [2]</td>
</tr>
<tr>
<td></td>
<td>Sugar</td>
<td>F</td>
<td>1.64 ± 0.28 [5]*</td>
<td>0.16 ± 0.05 [5]</td>
<td>2.41 ± 0.31 [5]*</td>
<td>20.88 ± 0.52 [5]*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M</td>
<td>1.30 ± 0.39 [5]*</td>
<td>0.10 ± 0.02 [4]</td>
<td>1.61 ± 0.44 [5]*</td>
<td>17.27 ± 0.50 [5]*</td>
</tr>
<tr>
<td></td>
<td>Sugar</td>
<td>F</td>
<td>2.62 ± 0.17 [5]*</td>
<td>0.17 ± 0.02 [5]*</td>
<td>0.73 ± 0.14 [4]</td>
<td>19.58 ± 0.23 [5]*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M</td>
<td>1.74 ± 0.25 [3]*</td>
<td>0.19 ± 0 [1]*</td>
<td>0.59 ± 0.34 [4]</td>
<td>15.23 ± 0.19 [3]*</td>
</tr>
</tbody>
</table>

Data are grouped by sampling date, winter food-supplementation treatment, and sex. Numbers are mean energy reserves or mean adult weight ± SE. [N] is sample size for each energy reserve and adult weight. Asterisk (*) indicates significant difference ($P < 0.05$) from controls according to Dunnett’s post hoc test on 29 Mar. 2017.
prior to oviposition. The number of eggs produced was significantly different ($F_{4,56} = 11.23, P < 0.001$) with prey-supplemented females laying more eggs ($P < 0.001$). Although prey-supplemented females produced more eggs, the fertility of the eggs produced was similar among treatments (Table 3). Females that died or did not oviposit during the 30-d experiment were included as censored data for time to first and fifth egg mass. Time to first egg mass (preoviposition period; likelihood ratio: $23.8, df = 3, P < 0.001$) and to fifth egg mass (likelihood ratio: $24.9, df = 3, P < 0.001$) differed among treatments (Table 3). The mean preoviposition period for prey-supplemented females was 5.5 d shorter than control, 4.4 d shorter than pollen, and 2.1 d shorter than sugar-fed females.

**Discussion**

Winter dormancy represents a substantial portion of an insect’s life cycle in many temperate regions. Predatory insects, which overwinter as mobile life stages, may feed under natural winter conditions (Thomas et al. 1992, Dennis et al. 1994, De Block et al. 2007, Eitzinger and Traugott 2011), but the effects of different winter food resources on winter biology have not been investigated. This field experiment was replicated over two winters to test the impact of food supplements to overwintering *H. convergens* adults on winter survival, energy reserves, and spring reproduction. We documented that aphids, *E. kuehniella* eggs and to a lesser extent sugar supplements, were beneficial for *H. convergens* because these treatments increased winter survival, spring energy-reserve contents, weight, and reproduction.

**Energy Reserves**

Lipid and carbohydrate content decreased over the course of the experiment in both years, whereas protein content decreased only in 2016–2017 (Tables 1 and 2). The composition (protein, carbohydrate) of food supplements was largely predictive for which energy reserve was affected. Sugar supplementation had the greatest positive impact on lipid and carbohydrate content in both years, and prey resources increased protein content of adults in both years. Prey and sugar (honey, sugar water, nectar) are common food resources provided for laboratory rearing and naturally occurring in the field (Evans and Gunther 2005, Lundgren 2009, Reznik and Vaghina 2013); we observed that these nutrients are also beneficial for overwintering *H. convergens*. Pollen and wheat protein have been used to rear *Coccinellidae* ( Nichols and Neel 1977, Michaud and Qureshi 2005); however, neither food resource affected *H. convergens* energy reserves in the present study. Thus, it appears that pollen and wheat protein on their own are not a sufficient resource for *H. convergens* to maintain energy reserves during overwintering.

The carbohydrate content of *H. convergens* in the no food control treatment at the end of 2015–2016 was unexpectedly equal to or greater than other food supplement treatments, including aphids. Foods such as aphids, even in very low abundance, may increase the rates of post-diapause development in overwintering adult *Coccinellidae* (Reznik and Vaghina 2013). Aphid fed adults in 2015–2016 possibly had a higher rate of postdiapause development compared with controls, which could lead to increased metabolism and further depletion of energy reserves (Kostal 2006). Sugar and honey supplements improved or maintained carbohydrate stores relative to unfed *H. convergens*.

**Weight**

Prey supplementation led to a relative increase in adult weight at the end of the experiment in both years (Tables 1 and 2). Given the energy reserve results, it would be expected that sugar supplementation would also increase weight; however, this was not observed. Prey is required for *H. convergens* for reproduction, whereas sugar is only beneficial for extending the life span of *H. convergens* in the absence of prey (Hagen 1962, Michaud and Qureshi 2006). The amount of food supplement consumed was not monitored in our experiment, although consumption of supplemental food treatments was observed at least once for all treatments on sampling days and days when food supplements were distributed. Increased weight (or lack of winter weight decrease) of adults supplied prey possibly occurred because prey resources were consumed more readily. Sugar may have only maintained energy reserves, not greatly affecting *H. convergens* overall weight. A similar result was reported by Thomas et al. (1992), in which winter weight loss in the carabid *D. atricapillus* could be offset when prey was supplied.

**Survival**

Based on energy-reserve analysis of the 2016–2017 experiment, it is expected that treatments resulting in higher lipid or carbohydrate content would have a higher number of individuals alive at the end of the experiment. However, the prey supplementation treatment,
which had lower carbohydrate and lipid content than other treatments, had the most living adults in 2016–2017. We are unsure how to explain the unexpected results, but as mentioned before, aphids and *E. kuehniella* eggs are considered critical for *H. convergens* reproduction and probably increased survival by improving overall adult condition. Prey diet also positively affected adult weight at the end of the experiment, body weight may positively correlate with higher survival. Also, prey diet may have benefited adults in ways not measured by the energetic reserve analysis here.

The significant difference in number of adults recovered only in 2016–2017 is possibly due to a difference in the length of the experiment. The experiment in 2016–2017 went 18 d further into the spring than the 2015–2016. Using 12°C as a cumulative degree-day threshold (Obrycki and Tauber 1982), from 1 January to 11 March in both years, cumulative DD$_{12}$ were similar, 25 for 2015–2016 and 21 for 2016–2017. From 12 to 29 March, 2016–2017 *H. convergens* adults in the field accumulated an additional 27 cumulative DD$_{12}$, doubling the DD$_{12}$. Experiencing higher temperatures will lead to an increase in metabolism and without the ability to replace those resources, mortality can increase (Jean et al. 1990). More DD$_{12}$ likely also led to an increase in consumption of energy reserves by *H. convergens* adults, further increasing the difference between treatments and years (Roach and Thomas 1991).

### Fecundity and Fertility

Providing prey to overwintering adult *H. convergens* enhanced spring reproduction, reducing the preoviposition period and increasing the number of females who survived to produce eggs (Table 3). It is important to note that all treatment groups were fed aphids ad lib after the winter, while reproduction was being measured. Feeding aphids to adults during the reproductive experiment could possibly reduce differences between treatments, as aphid feeding cues ovarian development within 8 d (Davis and Kirkland 1982). Lipid and carbohydrate content were higher in sugar-supplemented individuals. However, there was a surprisingly high mortality rate of overwintering beetle aggregations with access to food may fare better. There are opportunities for *H. convergens* to replenish their energy reserves during winter at mid and southern latitudes, like Kentucky, which may increase their survival and spring reproduction in the central United States. It is likely that wild *H. convergens* do have some level of prey available, while overwintering in Kentucky and by March may have access to nectar from emerging wild flowers. This experiment highlights the importance of biotic resources in winter habitat for *H. convergens*. Early spring predation of aphids is critical for slowing the growth of aphid populations (Atthey et al. 2016), and supplemental food resources that increase energy reserves, winter survival, and spring reproduction may lead to greater abundance and faster reproduction of this aphidophagous insect species.

### Supplementary Data

Supplementary data are available at *Environmental Entomology* online.

### Acknowledgments

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### References Cited


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**Table 3.** Proportion of females out of 15 pairs per treatment that laid at least one egg mass and laid five egg masses during 30-d experiment

<table>
<thead>
<tr>
<th>Food supplement</th>
<th>Laid at least one egg mass</th>
<th>Laid at least five egg masses</th>
<th>Mean eggs laid per female</th>
<th>Days to first egg mass</th>
<th>Proportion of eggs hatched</th>
<th>Days to five egg masses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.33</td>
<td>0.13</td>
<td>13.3 ± 5.9</td>
<td>9.6 ± 2.4 [5]</td>
<td>0.49 ± 0.1 [5]</td>
<td>13.0 ± 0 [2]</td>
</tr>
<tr>
<td>Pollen</td>
<td>0.27</td>
<td>0.27</td>
<td>13.1 ± 9.4</td>
<td>8.5 ± 2.9 [4]</td>
<td>0.37 ± 0.2 [4]</td>
<td>10.0 ± 0.5 [4]</td>
</tr>
<tr>
<td>Sugar</td>
<td>0.46</td>
<td>0.46</td>
<td>24.1 ± 8.8</td>
<td>6 ± 0.9 [7]</td>
<td>0.49 ± 0.1 [7]</td>
<td>12.0 ± 2 [7]</td>
</tr>
<tr>
<td>Prey</td>
<td>0.93</td>
<td>0.93</td>
<td>76.7 ± 9.1</td>
<td>4.1 ± 0.4 [14]</td>
<td>0.61 ± 0.04 [14]</td>
<td>9.5 ± 1.1 [14]</td>
</tr>
</tbody>
</table>

Stats comparison

\[ \chi^2 = 19.66, df = 3, P < 0.001 \]

\[ \chi^2 = 20.4, df = 3, P < 0.001 \]

\[ F_{1,4} = 11.23, P < 0.001 \]

\[ F_{2,24} = 0.28, P = 0.88 \]

\[ \chi^2 = 24.9, df = 3, P < 0.001 \]