Crop colonisation, feeding, and reproduction by the predatory beetle, *Hippodamia convergens*, as indicated by stable carbon isotope analysis

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Abstract. 1. Habitat management to enhance natural enemy populations in agricultural systems may help regulate levels of crop pests, but little research addresses the behaviour of immigrating beneficial insects.

2. Stable carbon isotopes were used in complementary laboratory and field studies to examine colonisation behaviour of an ephemeral agricultural habitat by the lady beetle, *Hippodamia convergens* Guérin-Méneville.

3. Under laboratory conditions, *H. convergens* carbon isotope ratios, δ¹³C, changed after its food supply was shifted from a C₄ to a C₃-based diet of aphids produced on grain sorghum or cotton respectively. Final isotope ratios of adult *H. convergens* were closer to that of the new C₃-based diet, with most change in δ¹³C occurring within 3 days after the diet shift.

4. The carbon isotope ratios of lady beetle adults collected in cotton fields suggested that grain sorghum was a continuous source for *H. convergens* until many nearby sorghum fields matured and senesced.

5. When cotton aphid (*Aphis gossypii* Glover) prey were absent, carbon isotope ratios of beetle populations did not change over time and virtually no egg production by *H. convergens* was detected. This indicates that beetles were feeding little on non-aphid resources originating in cotton.

6. With cotton aphids present, beetle isotope ratios decreased towards the carbon isotope ratio of cotton, indicating adult feeding in cotton. As a result, egg masses produced had carbon isotope ratios in the C₃ range of values.

7. The results suggest that some predator species may be retained in habitats without large prey populations, a quality essential in controlling pests in agricultural systems.

Keywords. Aphididae, Coccinellidae, cotton, lady beetle, sorghum.

Introduction

Prominent differences between natural and cultivated habitats include an increased frequency of disturbance from pesticide use, along with artificially reduced plant diversity and persistence in the latter (Levins & Wilson, 1980; Hobbs & Huenneke, 1996). These contrasts produce an overall reduction in the diversity and abundance of arthropod predators and parasitoids that help to control herbivorous pests (Russell, 1989). Consequently, outbreaks of pest populations are more frequent in agriculture than in natural systems. One ecologically based strategy to address pest problems is to promote earlier or greater colonisation by natural enemies through specific habitat management techniques (Landis *et al.*, 2000). Several such strategies have been proposed, but little research has been directed at the explicit process of natural enemy colonisation and the behaviour of predators or parasitoids after entering a new habitat.
Carbon isotope analysis can be used to elucidate both the process of natural enemy colonisation and the behaviour of predators and parasitoids after entering a new environment. To use this method, the stable carbon isotope ratios ($^{13}$C:$^{12}$C) of plants acting as a source of natural enemies and the habitat being colonised must be distinct, as is the case with plants using exclusively C$_3$ or C$_4$ photosynthetic pathways to fix inorganic carbon. The mean ratios of the two non-radioactive isotopes of carbon [expressed as $\delta^{13}$C, a parts per thousand (‰) difference relative to a reference material] of C$_3$ and C$_4$ plant species are $\sim$28‰ and $\sim$14‰ respectively (O’Leary, 1988). These values are transferred with little distortion to herbivores and to the predators or parasitoids that consume these plant-feeding pests (DeNiro & Epstein, 1978; Petelle et al., 1979; Ostrom et al., 1997; Oelbermann & Scheu, 2002). This process effectively marks all herbivorous insects and their natural enemies, but any change from a C$_3$- to a C$_4$-based diet (or vice versa) causes a shift in carbon isotope ratio approaching the $\delta^{13}$C value of the new diet (Ostrom et al., 1997; Markow et al., 2000; Oelbermann & Scheu, 2002).

In this study, stable isotope analysis was used to examine the process of crop colonisation and subsequent feeding and reproduction by the predatory lady beetle Hippodamia convergens Guérin-Méneville in an agroecosystem including the isotopically distinct crops cotton (Gossypium hirsutum L.) and grain sorghum [Sorghum bicolor (L.) Moench]. This native coccinellid is widely distributed in North America and is the most abundant predator species in some agroecosystems (Gordon, 1985; Prasifka et al., 1999; Mohamed et al., 2000; Wright & DeVries, 2000). Although it consumes prey from several insect orders, the convergent lady beetle is most closely associated with aphids (Homoptera: Aphididae), consuming 300 or more as larvae and continuing to feed throughout adulthood (Michels & Behle, 1991).

Hippodamia convergens adults move within and between habitats, with adults often aggregating at areas of high aphid density where females generally deposit their eggs (Hodek & Honěk, 1996; Elliott & Kiechlefer, 2000). To address the hypothesis that H. convergens from grain sorghum colonise nearby cotton fields (Fye, 1971; Lopez & Teetes, 1976; Prasifka et al., 1999) and to explore the behaviour of colonising beetles, study objectives were to determine (1) how rapidly lady beetle carbon isotope ratios change after a dietary shift between C$_3$ and C$_4$ resources, (2) over what period H. convergens adults in grain sorghum colonise nearby cotton, (3) the extent of adult feeding after colonisation of cotton, and (4) whether prey resources from grain sorghum contribute directly to egg production by H. convergens in cotton.

Materials and methods

Laboratory diet experiment

Two food sources with distinct $\delta^{13}$C values were given to adult H. convergens to examine how quickly carbon isotope ratios changed after a shift in the isotopic composition of the beetles’ diet. Lady beetles obtained from a commercial insectary (Rincon-Vitova Insectaries Inc., Ventura, California) were separated into groups of five adults with at least one female per group and placed into Petri dishes inside an environmental chamber. Lighting in the chamber was set to a LD 16:8 h photoperiod, while temperature and relative humidity were monitored for the duration of the diet experiment with a HOBO® H8 Pro Series data logger (Onset Computer Corporation, Bourne, Massachusetts) recording at 1-h intervals (daily means $\pm$ SD: 26.7 $\pm$ 0.8°C, 57.0 $\pm$ 11.3% RH; $n$ = 38 days). Water was provided using dental wicks saturated with reverse-osmosis filtered water. These beetles were fed ad libitum a diet of greenbug, Schizaphis graminum (Rodani), reared on greenhouse-grown grain sorghum plants. To produce a group of H. convergens with uniform carbon isotope ratios, 35 eggs laid by the insectary-supplied beetles (from at least six different females) were separated individually into new Petri dishes and provided the same diet of S. graminum and water until pupation.

Upon emergence, a sample of adult beetles ($n$ = 5) was removed, labelled, and placed into a freezer for preservation as control samples. Remaining beetles were fed on S. graminum for another 3 days, at which point a second group of control samples ($n$ = 4) was collected. The diet provided to H. convergens adults was then switched to cotton aphid, Aphis gossypii Glover, which was reared on potted cotton plants grown in a greenhouse. Samples of four to five of the remaining beetles were removed 1, 3, 5, 7, and 14 days after the diet was changed. Four beetles that died before their scheduled removal were discarded. To establish the carbon isotope ratios of plants and aphids in the diet experiment, aphid and plant samples were also collected. Plant samples were cut from upper leaves of cotton and sorghum plants with care taken to avoid main leaf veins. Aphids were collected in groups of 10 or more by disturbing aphid colonies and collecting fallen or walking aphids with fine point forceps. As with the lady beetle samples, aphid and plant material was preserved by freezing before sample preparation and analysis.

Field collections of Hippodamia convergens eggs and adults

In 2001 and 2002, four sites were selected near Ballinger, Texas (Runnels County). Each site was made up of a pair of commercially managed cotton and grain sorghum fields that shared one lengthwise border and were oriented with parallel rows. In each cotton field, a plot 100 $\times$ 40 m ($l \times w$) was marked with landscaping flags, with plots located at least 100 m from the field borders not shared with grain sorghum (Fig. 1). These plots were then divided into subplots positioned 10, 20, and 50 m from the interface of the cotton and grain sorghum fields.

Sampling of H. convergens adults and egg masses in cotton began 3 weeks after crop emergence, when adjacent grain sorghum fields reached the soft-dough stage of
development (Vanderlip, 1993). At each site, cotton plants in all subplots were sampled for *H. convergens* eggs and adults by visual inspection with 40 person-minutes of effort expended per subplot. Adults found in a single subplot were collected into a double-chambered aspirator whose removable inner chamber (a 2-dram screw cap vial) was used for storage of samples.

Egg masses were collected by removing the portion of plant tissue on which they were found (usually the lower leaf surface) and placing them into 2-dram vials. Collections of eggs and adults continued for 6–7 weeks, until the grain sorghum at each site reached physiological maturity and senesced. For reference, samples of green leaf tissue from cotton and sorghum plants at each site were also collected as in the laboratory experiment. All plant and insect material collected was preserved on dry ice until it could be returned to the laboratory for storage in a freezer.

In addition to insect and plant materials collected, the levels of both convergent lady beetles and cotton aphids were monitored in each cotton field as part of a separate study. The numbers of lady beetles per 100 plants and cotton aphids per 100 leaves were determined by visually inspecting 40 plants per field spread among 13 arbitrarily selected locations; however, plant inspections were not conducted in the plots from which *H. convergens* eggs and adults were collected, but from areas at least 60 m from all field borders in an effort to represent an overall aphid level for each cotton field. These monitoring efforts were always conducted within 2 days of field collections of beetle adults and eggs.

### Sample preparation and analysis

All collected material from laboratory and field experiments was washed twice in reverse-osmosis filtered water. Accurate isotope analysis often requires homogenisation by grinding larger samples of solids into a fine powder and subsampling, but this was not always necessary because of the relatively small sample masses. *Hippodamia convergens* adults were sliced in half along the anterior–posterior axis with a surgical scalpel to produce samples of size appropriate for isotope analysis (≤ 3 mg). Because of the large number of beetles collected in the field, a randomly selected group of 200 *H. convergens* adults were prepared and analysed for each year. Egg masses were treated as whole clusters after using a scalpel to separate eggs from plant material. Aphids were used in groups of about 10 individuals. All of the samples were then dried for 72 h at 65°C before being massed to an accuracy of ± 1 μg and packaged into tin sample capsules (Costech Analytical Technologies, Valencia, California). Plant samples were large enough to require homogenisation. After drying, leaf tissues were pulverised to a powder with a Wig-L-Bug mill (Spex Certiprep, Metuchen, New Jersey) before enclosing a subsample of desired mass (2–3 mg) into a sample capsule.

Sample carbon isotope ratios were determined at the University of Georgia’s Stable Isotope Laboratory via a combustion–gas chromatography–mass spectrometry process. In this process the gases produced by flash combustion of samples are sent to a gas chromatograph where carbon dioxide (CO₂) is separated out and sent to a mass spectrometer. The mass spectrometer separates CO₂ molecules based on their charge-to-mass ratio, producing a ratio of ¹³C:¹²C for each sample. These isotope ratios are expressed as δ¹³C, where:

\[
\delta^{13}C = \left[ \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right] \times 1000
\]

and the *R*<sub>sample</sub> and *R*<sub>standard</sub> are the ratios of ¹³C:¹²C for an individual sample and the analytical standard (Pee Dee Belemnite). This expresses isotope composition on a relative scale, but the use of an analytical standard does allow for conversion to absolute values.

### Statistical analysis

All statistical analyses were conducted using SAS software (SAS Institute Inc., 1999). For the laboratory diet experiment, mean carbon isotope ratios (δ¹³C ± SD) and shifts between trophic levels (Δδ¹³C) were calculated for each sample type. The changes in beetle isotope ratios...
over time were presented graphically but not analysed statistically. For field studies, a separate but identical analysis was conducted for each sample type (adults and eggs) in each year, with each site representing one replicate or \textit{H. convergens} population. To determine whether the carbon isotope ratios differed between sampling dates, a repeated-measures ANOVA (PROC MIXED) was used, with date treated as a repeated measure and the square root of the number of samples analysed (per field-date combination) used as a weighting factor. When variation in isotope ratios was shown by the repeated-measures analysis, pairwise comparisons of adjusted means (produced using the LSMEANS option) were made using the Tukey–Kramer test to control the experiment-wise error rate.

\section*{Results}

\subsection*{Changes in isotope ratio following a dietary shift}

Cotton and grain sorghum plants grown for the diet-switching experiment showed distinct isotope ratios which were transferred to the respective aphid species reared on each crop, and both groups of \textit{H. convergens} control samples reflected the $\delta^{13}$C values of the greenbug diet on which they were reared (Table 1). Mean differences, or isotopic shifts ($\Delta\delta^{13}$C) between trophic levels ranged from $+1.1\%e$ (grain sorghum to greenbugs, greenbugs to \textit{H. convergens}) to $-1.3\%e$ (cotton to cotton aphids). The $\delta^{13}$C values of \textit{H. convergens} changed greatly after the original C4-based diet (greenbugs) was changed to a C3-based resource (cotton aphids), moving from $-10.4 \pm 0.3\%e$ to $-18.8 \pm 2.3\%e$ (mean $\pm$ SD) in 3 days (Fig. 2). After feeding on cotton aphids exclusively for 14 days, \textit{H. convergens} $\delta^{13}$C values reached $-22.2 \pm 2.0\%e$ but were still enriched in $^{13}$C relative to their cotton aphid prey ($-28.4 \pm 1.0\%e$).

\subsection*{Field collections: general results}

The carbon resource bases for field-collected samples appeared to be similar to those used in the laboratory experiment, with mean $\delta^{13}$C values of field-collected cotton and grain sorghum ($-27.4\%e$ and grain sorghum ($-12.7\%e$) plants within 0.2\%e of those grown in greenhouse conditions. Mean population levels for \textit{H. convergens} adults peaked late in the 6–7-week sampling interval in both years, but appeared to be higher overall during 2001 (Fig. 3). Population trends of cotton aphids showed a distinct difference between years; \textit{A. gossypii} were undetectable in the four study fields during 2001, but were present on all sample dates in 2002 (Fig. 4).

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|c|}
\hline
\textbf{Sampled organisms} & \textbf{$n$} & \textbf{$\delta^{13}$C} \textbf{$\pm$ SD} & \textbf{$\Delta\delta^{13}$C} \\
\hline
Cotton & 4 & $-27.2 \pm 0.4$ & \\
Cotton aphids & 4 & $-28.4 \pm 1.0$ & $-1.3$ \\
Grain sorghum & 4 & $-12.7 \pm 0.1$ & \\
Greenbugs & 4 & $-11.6 \pm 0.3$ & $1.1$ \\
Convergent lady beetles$^1$ & 9 & $-10.4 \pm 0.3$ & $1.1$ \\
\hline
\end{tabular}
\caption{Carbon isotope ratios (mean $\delta^{13}$C $\pm$ SD) and isotope ratio shifts ($\Delta\delta^{13}$C) between trophic levels for plant and insect species used in diet-switching experiment.}
\end{table}

$^1$Control beetles collected at emergence and 3 days after pooled because of similarity ($t_7 = 1.68$, $P = 0.14$, independent samples $t$-test).

\begin{figure}
\centering
\includegraphics[scale=0.5]{Fig2.png}
\caption{Carbon isotope ratios (mean $\delta^{13}$C $\pm$ SD) of laboratory-reared \textit{Hippodamia convergens} adults before and after a change in diet from a C4-based resource (greenbugs reared on grain sorghum) to one based on C3 plants (cotton aphids reared on cotton).}
\end{figure}

\begin{figure}
\centering
\includegraphics[scale=0.5]{Fig3.png}
\caption{Density of convergent lady beetle populations at field sites in 2001 and 2002. Data are presented as beetles per 100 cotton plants (mean $\pm$ SE) with separate field sites used as replicates.}
\end{figure}
ratios from early collections of H. convergens in 2002 appeared slightly higher (less negative) than in 2001 (Fig. 5), but significantly increased during the sampling period ($F_{5,13} = 5.25$, $P < 0.01$). Tukey–Kramer means separation shows $\delta^{13}C$ values higher from 3 July to 17 July compared with 7 August, with carbon isotope ratios on other dates similar to both early and late sample periods. Plots of individual H. convergens isotope ratios over time (Fig. 6) show the considerable overall variation in beetle $\delta^{13}C$ values not apparent by examining population means.

Discussion

Changes in isotope ratio following a dietary shift

The rapid change in H. convergens carbon isotope ratios suggests that individual beetles moving from grain sorghum to cotton should start to reflect the isotope ratio of their new C₃ habitat within 2 weeks if adequate food is available. Though the mean isotope ratio of H. convergens moved toward that of its cotton aphid diet, after 2 weeks it remained approximately 6.2‰ richer in $^{13}C$ than the aphid diet. Similar results were obtained in a diet-switching experiment on the related coccinellid Hippodamia variegata (Goeze), which showed a distinct transition towards the $\delta^{13}C$ value of its new synthetic diet 2–6 days after the isotopic composition of its diet was changed (Ostrom et al., 1997). At 4 weeks after the diet switch, adult H. variegata isotope ratios remained about 3‰ higher than its artificial diet. It has been shown that specific tissues assimilate ingested carbon at different rates (Tieszen et al., 1983), and this phenomenon likely explains the pattern of a period
of relatively rapid carbon isotope transition followed by a slower progression towards the isotope ratio of the new diet (Fig. 2). For example, the hardened forewings (elytra) of beetles may reflect both larval and adult dietary history, as thin layers of carbon are periodically added to the adult exoskeleton (Hepburn, 1985; Tallamy & Pesek, 1996).

*Carbon isotope ratios of field-collected Hippodamia convergens adults and eggs*

Since selected fields were kept weed-free through regular cultivation, mean isotope ratios intermediate to cotton and grain sorghum values indicate recent immigration from nearby grain sorghum by a portion of the *H. convergens* population in cotton. In both years isotope ratios suggested that colonisation of cotton by adult beetles started before sampling began (Fig. 5), when cotton plants were rather small (two to four true leaves unfurled). Further, beetles with relatively high (−16.0‰) isotope ratios were collected from cotton in all sample dates in both years (Fig. 6), and populations reached their highest levels during the penultimate sample date (Fig. 3). Combined, these observations suggest that adult movement of *H. convergens* from grain sorghum continued for the duration of sample collection. These conclusions are supported by results from a concurrent mark–capture movement study that included two of the four fields used in isotope collections (Prasifka et al., 2004b).

Using δ13C values to examine adult feeding in cotton yielded unexpected results. Adult beetle populations showed relatively constant δ13C values over time in 2001, but beetle feeding in cotton should decrease isotope ratios over time. Two simple, non-exclusive hypotheses may explain this result. First, evidence suggests that adult *H. convergens* colonised cotton from grain sorghum throughout the study period (see beetles > −16.0‰ in Fig. 6). This constant addition of individuals with high (C4) isotope ratios could partially offset the expected changes towards more negative (C3) carbon isotope values that would result from *H. convergens* feeding in cotton. Second, because the expected change in isotope ratios is based on dietary intake of carbon, the constancy of isotope ratios could indicate that lady beetles in cotton were eating very little. The absence of detectable cotton aphid populations in 2001 (Fig. 4) also supports the hypothesis that lady beetle feeding was greatly reduced in 2001. Nevertheless, potential alternate prey [nymph and adult populations of the cotton fleahopper, *Pseudatomoscelis seriatus* (Reuter)] were abundant in cotton throughout the area in 2001 (Prasifka et al., 2004a). A competing hypothesis that beetles may have moved continuously between cotton and sorghum during 2001 (at similar rates, feeding in both locations) was also considered after data analysis was completed; however, because aphid populations in sorghum decline markedly by mid to late June (Krauter et al., 2001) and concurrent mark–capture results (Prasifka et al., 2004b) indicated movement by *H. convergens* from cotton to sorghum was uncommon, this possibility was eliminated.

Isotope ratios for adult *H. convergens* in 2002 allowed for a more straightforward interpretation, decreasing throughout the sample dates, indicating active feeding on prey in cotton. Most likely this is a result of feeding on cotton aphids, which were present throughout the duration of the study that year (Fig. 4). Even with their favoured prey (aphids) available, mean isotope ratios of lady beetle populations changed slowly relative to the results of the diet-switching experiment. It is unknown whether the slow overall change in δ13C values is a result of continued immigration from grain sorghum or aphid consumption at a much lower rate compared to *ad libitum* feeding in the laboratory.

Because *H. convergens* egg masses are produced from prey recently consumed by adult females, any egg masses with high (<−16.0‰) isotope ratios are interpreted as eggs produced from resources consumed chiefly in grain sorghum. In 2001, egg masses were extremely rare in studied cotton fields. The absence of detectable cotton aphid populations (Fig. 4) and the constancy of adult *H. convergens* carbon isotope ratios over time suggest that insufficient prey resources may have been available for egg production in cotton. In 2002, lady beetle egg production in cotton was greatly increased, and mean isotope ratios were consistently within the range of C3 plants. Two egg masses collected had isotope ratios in the C4 range, but this represented a very small fraction (3%) of all samples collected. In general, results from both years suggest that the direct contribution of prey resources in grain sorghum to egg production of *H. convergens* in cotton was negligible.

**Summary and conclusions**

This study used stable carbon isotopes to study movement and reproduction in a predatory insect, and the inferences regarding beetle movement agree with those of a concurrent study using more traditional mark–capture techniques (Prasifka et al., 2004b). In both study years, movement of *H. convergens* adults from grain sorghum into nearby cotton began at the earliest stages of cotton growth, and appeared to continue for several weeks. During 2001 *H. convergens* adults remained in cotton fields but fed very little, possibly due to the absence of their preferred cotton aphid prey. In 2002, aphids were more abundant and feeding by *H. convergens* adults resulted in a slow shift of population carbon isotope ratios towards C3 values. Egg production by lady beetles in cotton appeared to be almost entirely based on resources consumed in cotton.

Compared with crop colonisation by pests, the establishment of predator and parasitoid populations in agriculture tends to be late (Price, 1976). This may be attributed to simple differences between herbivores and their natural enemies; fields of young crops represent an abundant and homogenous food supply for herbivores, but natural enemies are dependent on populations of prey (or hosts) that are both spatially and temporarily variable (Price, 1976;
O’Neil & Wiedenmann, 1987). However, natural enemies may still be attracted to crop habitats when prey are absent (Price, 1986). In such cases, the challenge then becomes maintaining an adequate number of natural enemies in the crop in spite of low prey availability. The retention and continuous colonisation by H. convergens in 2001 suggests that this is possible. If H. convergens adults have experienced poor foraging success prior to moving into cotton, the apparently low-quality habitat in cotton may still be above the marginal value necessary to retain them (Krebs, 1978). Retention of adult beetles in cotton could also reflect the convergent lady beetle habit of subsisting on alternate resources (including nectar and pollen) when aphid prey are scarce (Hagen, 1962; Hodek & Honěk, 1996). This type of diet breadth is suggested to be particularly desirable for natural enemies in ephemeral environments (Gilstrap, 1997), but how frequently other natural enemy species show similar habitat fidelity under adverse food conditions is unknown.

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