Bulletin of Entomological Research (2009) **99**, 347–358
doi:10.1017/S0007485308006378

First published online 21 January 2009
Printed in the United Kingdom

Investigating the diet of the omnivorous mirid *Dicyphus hesperus* using stable isotopes

J.A. Bennett[1†], D.R. Gillespie[2] and S.L. VanLaerhoven[1*]

[1]Department of Biology, University of Windsor, 401 Sunset Ave., Windsor, ON, Canada N9B 3P4: [2]Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, Agassiz, BC, Canada

Abstract

Omnivory involves numerous feeding relationships and a complex web of interactions. When using omnivores in biocontrol, these interactions need to be understood to maximize feeding on the target species and minimize non-target interactions. *Dicyphus hesperus* is used along with *Encarsia formosa* for biocontrol of whiteflies in greenhouse tomato crops. *Dicyphus hesperus* is a generalist omnivore which feeds on all components of the system. To quantify these interactions, stable isotope analysis was used to identify trophic position with nitrogen isotopes ($\delta^{15}N$) and plant sources with carbon isotopes ($\delta^{13}C$). Feeding trials were used to establish baseline isotopic data for *D. hesperus* and their diet, including *Verbascum thapsus*, an alternative plant food. Cage trials were used to monitor population abundances and the isotopic signature of *D. hesperus*. In feeding trials, *D. hesperus* were enriched relative to their food, suggesting an elevated trophic position. However, large amounts of isotopic variation were found within all diet components, with only *V. thapsus* exhibiting a distinct signature. In cage trials, the average $\delta^{15}N$ and $\delta^{13}C$ of the omnivore declined over time, coinciding with declines in total available prey, though it may be confounded by changes in temperature. The range of $\delta^{13}C$, but not the range of $\delta^{15}N$, also declined over time. This suggests a change in the plant source within the diet, but also some unquantified variability within the population. We suggest that diet variability exists within *D. hesperus* populations, declining as prey become less abundant.

Keywords: stable isotope, omnivory, *Dicyphus hesperus*, whitefly, tomato, diet switching

(Accepted 17 July 2008)

Introduction

Omnivory, defined as feeding at more than one trophic level, is widespread in nature, increasing the complexity and influencing the stability of food webs (McCann & Hastings, 1997; Coll & Guershon, 2002; Arim & Marquet, 2004). Omnivores are increasingly used in biological control though their effectiveness can vary with available dietary sources (Agrawal *et al*., 1999; Eubanks & Denno, 1999; Coll & Guershon, 2002). Therefore, it is important to isolate the mechanisms influencing variation in the occurrence and strength of the interactions surrounding an omnivore to maximize the efficiency of these biocontrol systems. Variation in omnivory may arise from age-related differences among individuals (Polis *et al*., 1989; Branstrator *et al*., 2000), differences in food quality (Diehl, 2003; Janssen *et al*., 2003; Singer & Bernays, 2003; Magalhaes *et al*., 2005), risk

*Author for correspondence
Fax: +1-519-9713609
E-mail: vanlaerh@uwindsor.ca
†Current address: Department of Biological Sciences, University of Alberta, Edmonton, AB, Canada, T6G 2E9

associated with foraging (Singer & Bernays, 2003), nutritional needs (Gadd & Raubenheimer, 2000) or food availability (Mooney & Tillberg, 2005). However, omnivory is not a simple interaction between two species and may involve numerous indirect interactions (Diehl, 1995; Bruno & O'Connor, 2005). This complexity suggests that more advanced techniques may be necessary to infer the degree of omnivory.

Diet composition is one way to infer the relative strength of trophic interactions for an omnivore, though classic methods are not always feasible. Stable isotope analysis is a technique using the ratios of naturally occurring isotopes within organisms to determine both resource and habitat use (Newsome *et al*., 2007). The ratio of heavy to light nitrogen isotopes ($\delta^{15}N$) is generally used as an indicator of trophic position as the lighter isotope tends to be preferentially excreted, leading to an accumulation of the heavier isotope at higher trophic levels (Deniro & Epstein, 1981). Carbon isotope ratios ($\delta^{13}C$) are used as an indicator of the plant source, as this isotopic ratio is determined during photosynthesis and shows little change between trophic levels (Deniro & Epstein, 1978). This technique is often used to determine diets and has been used to measure the degree of omnivory (Bluthgen *et al*., 2003; Mooney & Tillberg, 2005). Most commonly, the interpretation of omnivory using stable isotopes involves the determination of trophic position within a dietary mixing model (Newsome *et al*., 2007). These models quantify the proportion of each source in the diet using a series of linear equations incorporating the isotopic signatures of the diet components and the subject. Alternatively, variation within isotopic signatures has also been proposed as an indicator of diet breadth and omnivory (Bearhop *et al*., 2004) although there are some issues with this approach (Newsome *et al*., 2007). When coupled with abundance estimates, a concurrent measure of dietary change provided by stable isotope analysis should elucidate the relationships between omnivory and community dynamics.

Dicyphus hesperus Knight (Heteroptera: Miridae), is an omnivorous insect which requires both plant and prey in its diet (McGregor *et al*., 1999). It is currently used as a biological control agent for greenhouse whitefly (*Trialeurodes vaporariorum* Westwood [Homoptera: Aleyrodidae]) and on greenhouse tomato, (*Lycopersicon esculentum* Mill [Solanaceae]), in Ontario and British Columbia, Canada. Also within this system, the parasitoid *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) is routinely used for biological control of whitefly (Hoddle *et al*., 1998) and mullein (*Verbascum thapsus* L. [Scrophulariaceae]) is included as a supplemental high quality plant food for *D. hesperus* (Sanchez *et al*., 2003). Intraguild predation, a special case of omnivory where a predator eats its competitors, also occurs, with *D. hesperus* feeding on parasitized whitefly (McGregor & Gillespie, 2005). These feeding relationships lead to a complicated food web (fig. 1). It is important to understand the dynamics of these interactions to maximize feeding upon whiteflies and to minimize plant feeding and interference with the parasitoid.

To investigate the factors affecting changes in diet choice for *D. hesperus*, we established a series of experiments. To determine baseline isotopic data, feeding trials were used to compare *D. hesperus* with their diet sources and to compare among diet sources. We hypothesized that *D. hesperus* would be enriched in ^{15}N relative to their diet, with the prey being slightly enriched relative to the plant component of the diet. Whiteflies parasitized by *E. formosa* were expected to show an increase in $\delta^{15}N$ relative to healthy pupae, with $\delta^{13}C$ being used to differentiate among plant sources. To determine the factors influencing the degree of omnivory, we constructed cages where the omnivore would be subjected to varying populations of whitefly and their parasitoid and multiple plant species. Dietary changes were tracked using stable isotope analysis and then compared with known changes in prey abundance. Plant feeding was expected to increase with declining prey populations, and intraguild predation was expected to vary according to the ratio between healthy and parasitized pupae.

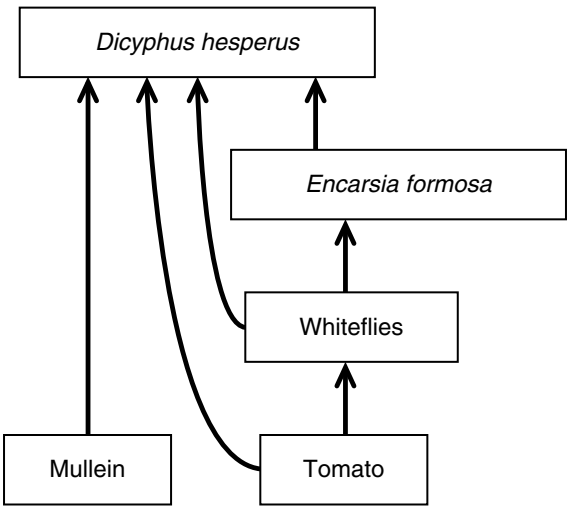

Fig. 1. The food web used within this study. Arrows represent the direction of biomass transfer.

Materials and methods

Plant and insect sources

For greenhouse trials, tomato plants var. Rhapsody were grown hydroponically in rockwool growing medium in a greenhouse at the Agriculture and Agri-Food Canada Greenhouse and Processing Crops Research Centre (GPCRC) in Harrow, Ontario, Canada. Plants were watered and fertilized through a fertigation system. The nutrient solution contained 230 ppm NO_3, 10 ppm NH4, 60 ppm P, 460 ppm K, 200 ppm Ca, 80 ppm Mg, 3 ppm Fe and 0.5 ppm Mn with an EC of 2800 μScm^{-1} and a pH of 5.5. Tomato plants for the feeding trials were cultivated in BM2 germinating mix soil (Berger Peat Moss Co.) at the University of Windsor greenhouse in Windsor, Ontario, Canada. Plants were grown under natural light supplemented with high pressure sodium lights (16 h light photoperiod), flooded daily and fertilized with 4 ml of all purpose 20-20-20 fertilizer (Plant-Prod$^{(R)}$) mixed in one litre of water once per week. Mullein plants were grown from seeds originally collected in Summerland, British Columbia, Canada. They were cultivated from seed at the University of Windsor greenhouse and then transferred to the GPCRC for use in the greenhouse cage trials. The mullein at both locations were maintained using the same methods as used for tomato.

https://doi.org/10.1017/S0007485308006378 Published online by Cambridge University Press

Colonies of *D. hesperus* were established from individuals originally collected on white hedge nettle (*Stachys albens* Gray [Lamiaceae]) at 500 m elevation in the Sierra Nevada Mountains in California, at 25° 42′ N, 118° 50′ W. Colonies were kept at 24°C and 16 h light photoperiod. Insects were reared on tobacco (*Nicotiana tabacum* L. [Solanaceae]) and fed a diet of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs. *Encarsia formosa* were obtained from Koppert Biological Systems (EN-STRIP) as parasitized whitefly pupae. It should be noted that the term pupae is used to refer to the final juvenile instar of whitefly. These are not true pupae but are commonly referred to as such and will be for the remainder of this manuscript. Adult parasitoids were allowed to emerge before use in the greenhouse trials, but were presented to *D. hesperus* as parasitized whitefly pupae on the card for the feeding trials. Whiteflies for the greenhouse cage trials were obtained from a colony maintained on tomato at the GPCRC. The whiteflies for the colony were collected from commercial greenhouses in southwestern Ontario, Canada. Whiteflies for the feeding trials were collected from a colony grown on tomato in the University of Windsor greenhouse. The whiteflies for this colony were originally collected from commercial greenhouses in the region.

Feeding trials

To determine changes in feeding by monitoring isotopic signatures, baseline isotopic data needs to be established. This data gives the average fractionation rate between trophic transfers. Fractionation rates can be defined as the difference between the isotopic composition of the resource and the consumer resulting from metabolic processes within the consumer, usually given in parts per thousand (‰). To this end, a series of feeding trials were used to determine fractionation rates between *D. hesperus* and their possible diets, including whitefly and parasitized whitefly. Comparisons were also made among plant materials grown under common conditions and between parasitized and non-parasitized whiteflies taken from a single colony.

For the feeding trials, adult *D. hesperus* were taken from the rearing colony and placed in 500-ml foam cups with one insect per cup. Age was standardized among individuals by selecting only insects which had reached sexual maturity within one week of the experiment. Within each cup there was a covered water reservoir and a tomato leaflet with the petiole inserted into the reservoir. Depending on the treatment, the leaflet was either clean or infested with whitefly pupae. Into each of the cups with a clean leaf, a single card containing approximately 80 parasitized pupae was inserted. Cards were used instead of infested leaves due to difficulties encountered in establishing colonies of *E. formosa*. Prey and plant material were replaced in both treatments every 2–3 days to ensure the availability of prey and the consistency of the plant resource. After 30 days, *D. hesperus* individuals were collected for isotopic analysis. This experiment was completed a second time.

To determine isotopic differences among diet components, representative diet samples of whitefly pupae, parasitized pupae and tomato leaves were collected throughout the feeding trials for isotopic analysis. Samples of parasitized pupae from the whitefly colony were also collected to compare against healthy whitefly pupae and tomato to determine fractionation rates among these diet components.

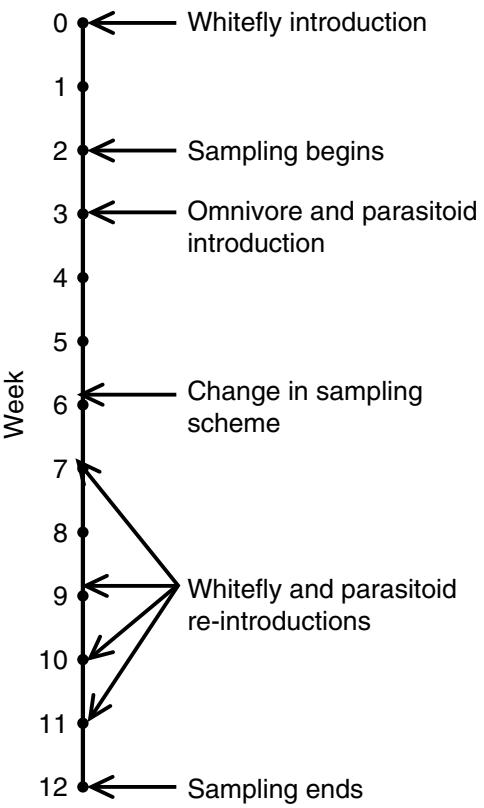

Fig. 2. Timeline of events within the greenhouse cage trials.

For each prey collection, prey items were collected from a single tomato leaf and contained a minimum of twenty pupae. To compare between plant sources available within the greenhouse cage trials, mullein and tomato which were grown under common conditions were used. Tobacco leaves and *E. kuehniella* eggs were also collected for isotopic analysis to represent the rearing diet of *D. hesperus*. All samples were frozen at −10°C until analysis.

Greenhouse cage trials

Cage trials were conducted to correlate changes in the isotopic signature of *D. hesperus* with changes in abundances of prey types within a greenhouse at the GPCRC. Cages were constructed using 3.8 cm ABS plastic pipe and covered with polypropylene non-woven fabric (Agryl-P17), a commonly used insect barrier due to its small mesh size. All cages were 1.44 m^2 at the base and 2 m in height. Each cage enclosed six tomato plants with a single potted mullein plant suspended in the center of the cage near the top. Mullein was included to facilitate the establishment and survival of the omnivorous *D. hesperus* (Sanchez *et al*., 2003). Whiteflies were introduced once the tomato plants had reached a minimum height of 1 m. Into all cages, 30 adult whiteflies were introduced and allowed three weeks to establish within the cage before omnivore and parasitoid introduction. The introduction of whiteflies is marked as week zero according to the timeline used within this manuscript (fig. 2). Thirty-six *D. hesperus* individuals were introduced into each cage, with the introduction split evenly among adults (at an even sex

https://doi.org/10.1017/S0007485308006378 Published online by Cambridge University Press

ratio), late instar juveniles and early instar juveniles. In order to create a variable parasitism environment, five cages received 15 adult female parasitoids (high release rate) and five cages received five adult females (low release rate). Both whitefly and *E. formosa* were re-released into the cages at their initial release rates at weeks 7, 9, 10 and 11 due to declining whitefly populations.

In weeks 2, 4 and 5, nine leaves were removed with three leaves randomly selected from the top, middle and bottom of three plants within each cage to monitor whitefly and *E. formosa* populations. It was found that the random removal of a leaf from the top section often included no visible whiteflies. Thus, this sampling scheme was altered beginning the sixth week to minimize the impacts on whitefly populations and especially the removal of whitefly eggs and 1st instar nymphs which were difficult to detect. Leaf sampling was changed from a random sampling scheme to a directed sampling scheme designed to target specific stages in the whitefly populations. This targeting was possible due to the aggregated and structured nature of whitefly populations (van Lenteren & Noldus, 1990). Three leaves were removed from two plants each week, according to a schedule such that each plant was sampled every 3rd week. Six leaves were now removed from each cage weekly for prey population surveys. The 4th, 8th and 12th leaves from the top of the plant were removed to target nymphs, pupae and exuviae, respectively. Whitefly nymphs, pupae and parasitized pupae were counted on each leaf by visual scans aided by the use of a stereomicroscope.

Beginning in week 6, up to five adult *D. hesperus* individuals per cage were removed every two weeks for isotopic analysis. The number of *D. hesperus* removed was determined by the number of individuals caught within ten minutes of searching the cage. Other losses of prey and natural enemies may have occurred during weekly crop maintenance, as senescing basal leaves and secondary shoots were removed from the plant following commercial propagation practices. All removed leaves were placed on the floor of the cage for a period of one week to minimize any losses of natural enemies and prey. The experiment was conducted for 12 weeks from the introduction of whiteflies into the cages. This allowed for at least two generations of all insects. Temperature readings were recorded every 15 min throughout the experiment. At the completion of the experiment, tomato leaf tissue was collected from six random plants for isotopic analysis.

Isotope analyses

All samples were dried at 70°C for 72 h prior to analysis. Plant tissues were analyzed as individual leaves. Each individual leaf was homogenized using a mortar and pestle and then weighed to approximately 450 μg. Healthy and parasitized whitefly pupae were grouped according to their source, whether it was a specific leaf or card. Whitefly and *E. formosa* samples were weighed to approximately 250 μg for analysis. Each sample contained a minimum of ten whitefly or *E. formosa* pupae. Each sample type was repeated between three and six times, with the majority of sample types having five replicates. *Dicyphus hesperus* were analyzed individually as whole insects. All *D. hesperus* were starved for 24 h prior to being frozen to minimize contamination by gut contents. In cases where the weight of the insect exceeded 600 μg, the insect was homogenized and a sample of 300 μg was taken. *Ephestia kuehnella* eggs were grouped into a single sample and weighed to approximately 300 μg for analysis.

All samples were placed into tin capsules and analyzed for $\delta^{13}C$ and ^{15}N at the University of Windsor stable isotope laboratory using a Thermo Finnigan Flash 1112 elemental analyzer coupled to a Thermo Finnigan Delta Plus mass spectrometer, operating in continuous flow mode. The samples collected from the cage trials and the first feeding trial were analyzed on a separate run of the elemental analyzer than the samples collected during the second feeding trial. Samples are expressed in delta notation as: $\delta X = [(R\text{sample}/R\text{standard})-1] \times 1000$ with X representing either ^{15}N or ^{13}C and Rsample and Rstandard representing the ratio of the heavy to light isotope ($^{15}N/^{14}N$ or $^{13}C/^{12}C$) of the sample or the standard, respectively. For nitrogen, the standard is atmospheric nitrogen and for carbon the standard is PeeDee belemnite. The precision was better than 0.1‰ for ^{13}C and 0.3‰ for ^{15}N for the initial run and 0.2‰ for ^{13}C and 0.3‰ for ^{15}N for the second run.

Statistical analysis

To determine the significance of fractionation rates between *D. hesperus* and their diet, the isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$) of *D. hesperus* from the initial feeding trial were compared to their diet components using multivariate analysis of variance (MANOVA). Feeding trial data for *D. hesperus* from the second trial were discarded due to sample contamination. MANOVA allows the comparison of means of multiple dependent variables given the same set of independent variables, while retaining the results of the univariate ANOVAs (Zar, 1999). Thus, both $\delta^{13}C$ and $\delta^{15}N$ were set as dependent variables with the levels of the independent variable being the different dietary components, including immature whitefly, parasitized immature whitefly and the plant components. Both the multivariate and univariate results are reported. In this analysis, trials with *D. hesperus* fed whitefly were analyzed independently from those where *D. hesperus* was fed *E. Formosa*, as the commercial origin of *E. formosa* used in this part of the experiment makes a direct comparison invalid.

Other analyses were conducted to determine differences between diet components. To determine the significance of fractionation rates between tomato, whitefly and *E.* formosa, samples were taken for all three organisms from a colony maintained at the University of Windsor. Both $\delta^{13}C$ and $\delta^{15}N$ of all three organisms were compared independently using a general linear model with trial number as a random factor. MANOVA was not used for this analysis, as random factors cannot be included in this statistical test. For this analysis, carbon data were rank transformed and nitrogen data were log transformed prior to analysis. To compare the signatures of possible diet contributors within the greenhouse trials, mullein, tomato, whitefly and parasitized whitefly were grown under common conditions at the University of Windsor. Results from these samples were then compared using MANOVA for both $\delta^{13}C$ and $\delta^{15}N$ following rank transformation for carbon and log transformation for nitrogen. For all analyses, *post hoc* analysis was done using Tukey's test when appropriate.

Within the greenhouse cage trials, insect counts were taken on roughly a weekly basis. These counts were then summed per cage to give a value for total prey within that cage and then log transformed. The total prey count data

https://doi.org/10.1017/S0007485308006378 Published online by Cambridge University Press

were split into two phases. These phases represent the two different sampling schemes employed and are roughly correlated to the population growth and decline of whitefly populations. The initial phase ran from week 2 until week 5 and coincided with population growth. The second sampling phase began in week 6 until week 12 and coincided with population decline. These two phases were analyzed independently using linear regression. We were unable to statistically determine weekly differences in the abundances of parasitized pupae or parasitism rates. A low frequency of observed parasitism within the cages prevented statistical analysis.

The isotopic signatures of *D. hesperus* were analyzed in two ways. They were analyzed as weekly averages for each cage to determine the mean change over time and as the range of values within each cage to look at variability within the population. Both the mean of the $\delta^{13}C$ and $\delta^{15}N$ measurements and the range of values for each cage were regressed against time individually using linear regression. Cages for which only one individual was captured were excluded from the analysis of the range of isotopic values. Values for the range of $\delta^{13}C$ were log transformed prior to analysis. To document environmental conditions, daily mean, minimum and maximum temperature data were also regressed vs. time. All statistical analyses were conducted using SPSS 15.0 (SPSS, 2006).

Results

Feeding trials

Dicyphus hesperus remained isotopically distinct from their diet, when fed either whitefly (Wilks' $\lambda_{4,14} = 0.04$, $P < 0.001$; fig. 3a) or parasitized pupae (Wilks' $\lambda_{4,18} = 0.043$, $P < 0.001$; fig. 3b). These differences held for both $\delta^{15}N$ (whitefly, $F_{2,8} = 40.14$, $P < 0.001$; *E. formosa*, $F_{2,10} = 55.70$, $P < 0.001$) and $\delta^{13}C$ (whitefly $F_{2,8} = 53.18$, $P < 0.001$); *E. formosa*, $F_{2,10} = 21.85$, $P < 0.001$). *Dicyphus hesperus* fed whitefly pupae and tomato were enriched in $\delta^{13}C$ by an average of 5.52‰ from tomato ($P < 0.001$) and 5.65‰ from whitefly ($P < 0.001$) with a mean enrichment of 5.59‰ from the entire diet. Nitrogen enrichment for these same insects was 5.30‰ from tomato ($P = 0.001$) and 7.09‰ from whitefly ($P < 0.001$) with a mean of 6.20‰. *Dicyphus hesperus* fed parasitized whitefly differed from their diet by 5.68‰ for $\delta^{13}C$ on average, being enriched from tomato by 5.20‰ ($P = 0.002$) and 6.15‰ from the parasitized whitefly ($P < 0.001$). Average enrichment for $\delta^{15}N$ was 5.59‰, being 6.25‰ more enriched than parasitized pupae ($P < 0.001$) and 4.92‰ more enriched than tomato ($P < 0.001$). Visual inspection shows that the isotopic signature of these insects is midway between the rearing diet and the experimental diet (fig. 3).

No significant differences were found between tomato leaves, whitefly and parasitized whitefly taken from the whitefly colony (fig. 4) in $\delta^{13}C$ ($F_{2,2} = 1.27$, $P = 0.441$) and $\delta^{15}N$ ($F_{2,2} = 2.69$, $P = 0.271$). No significant effects of trial number ($\delta^{13}C$, $F_{1,2} = 13.93$, $P = 0.065$; $\delta^{15}N$, $F_{1,2} = 0.05$, $P = 0.838$) or interactions between trial number and food type were found ($\delta^{13}C$, $F_{2,14} = 1.80$, $P = 0.201$; $\delta^{15}N$, $F_{2,14} = 1.96$, $P = 0.178$), but the differences between trials in $\delta^{13}C$ are nearly significant. Mullein, tomato, whitefly and parasitized whitefly cultivated within the same greenhouse showed significantly different isotopic signatures (Wilks' $\lambda_{6,26} = 4.04$, $P = 0.050$; fig. 4). Univariate analysis showed that these differences were related to $\delta^{13}C$ ($F_{3,14} = 7.58$, $P = 0.030$) and not $\delta^{15}N$ ($F_{3,14} = 2.02$, $P = 0.158$). Mullein was enriched in ^{13}C by 4.24‰ relative to whitefly ($P = 0.002$) and 2.07‰ relative to parasitized pupae ($P = 0.032$), thus showing the utility of carbon to differentiate between plant sources.

Greenhouse cage trials

Average prey populations grew initially until week 5, two weeks after the addition of the natural enemies to the cages (fig. 5; $R^2 = 0.314$, $P = 0.001$). Average prey populations began to decline after this week and continued to decline until the experiment was terminated (fig. 5; $R^2 = 0.064$, $P = 0.04$). This decline in available prey coincided with declines in both $\Delta^{13}C$ (fig. 6; $R^2 = 0.323$, $P < 0.001$) and $\delta^{15}N$ (fig. 7; $R^2 = 0.338$, $P < 0.001$). The range of values found within each cage declined over time for $\delta^{13}C$ (fig. 8; $R^2 = 0.178$, $P = 0.015$), but there was no change in the range of $\delta^{15}N$ ($P = 0.833$). Final values for $\delta^{13}C$ and $\delta^{15}N$ show that, in week 12, *D. hesperus* was enriched relative to the tomato leaves by 2.24‰ for carbon and 1.01‰ for nitrogen. Average ($R^2 = 0.069$, $P = 0.009$) and maximum ($R^2 = 0.219$, $P < 0.001$) temperatures declined over time (fig. 9), but minimum temperatures did not statistically vary ($P = 0.801$).

Discussion

Feeding trials

The diet of an omnivore is usually complex and stable isotope analysis has been suggested as a means to isolate the relative proportions of different diet components (Bluthgen *et al*., 2003; Mooney & Tillberg, 2005). Our attempts to apply this technique to the diet of *D. hesperus* met with mixed success. In general, the feeding trials showed the isotopic signature of *D. hesperus* to be influenced by the food that they eat. In accordance with our hypothesis, we found *D. hesperus* to be enriched relative to their diet. However, they were unexpectedly enriched in both ^{15}N and ^{13}C (fig. 3). We had expected *D. hesperus* to be enriched in ^{15}N and perhaps mildly in ^{13}C, but found their $\delta^{13}C$ values to be much greater than is often reported (Post, 2002; Spence & Rosenheim, 2005). When looking at the results graphically, it becomes apparent that the isotopic signatures for *D. hesperus* were influenced by their rearing diet. The *E. kuehniella* eggs fed to the insects during rearing were greatly enriched in both isotopes, which likely had residual effects on the isotopic signatures of these insects following the feeding trials. We had expected the length of the trial to be sufficient to determine fractionation rates as the trial length of 30 days was longer than previous studies of tissue turnover time in insects (Ostrom *et al*., 1997). However, this residual effect may be due to the lack of turnover in tissues such as the exoskeleton and tracheae following the final molting. As we are unable to quantify the influence of the rearing diet, our estimates of fractionation rates may be biased.

We were also unable to statistically differentiate between whitefly, parasitized whitefly and tomato (fig. 4). The differences were expected to be minimal between these components, as phloem feeding insects are not highly enriched relative to their diet (Spence & Rosenheim, 2005; Sagers & Goggin, 2007) though we had expected them to be distinct. Differences between parasitized and healthy whitefly were expected to be more pronounced (Langellotto *et al*., 2005,

https://doi.org/10.1017/S0007485308006378 Published online by Cambridge University Press

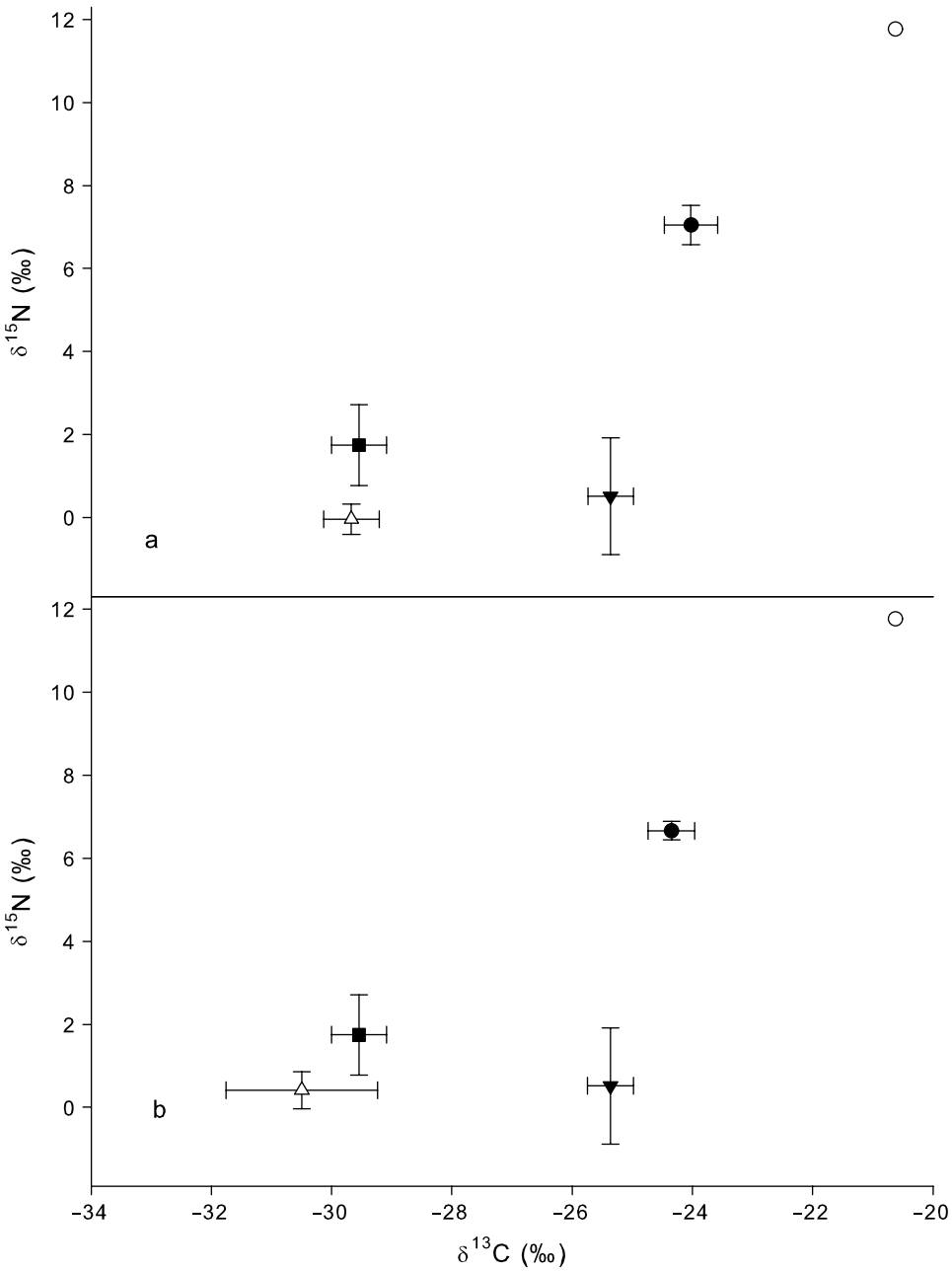

Fig. 3. Average stable isotope signatures of *D. hesperus* and diet components. *Ephestia kuehniella* eggs and tobacco leaves represent the rearing diet. (a) *Dicyphus hesperus* fed whitefly and tomato leaves. (b) *Dicyphus hesperus* fed whitefly parasitized by *E. formosa* and tomato leaves. Error bars represent one standard error (●, D. Hesperus; ○, E. kuehniella eggs; ▼, tobacco; △, whitefly; ■, tomato).

2006), but these studies used adult parasitoids and hosts for isotopic analysis. In the current study, immature whiteflies and parasitoids were used because they are the predominant stages where predation by *D. hesperus* occurs. Our inability to distinguish between healthy and parasitized whitefly is not without precedence (Tooker & Hanks, 2004) and is likely due to how fractionation occurs in these insects. Fractionation occurs due to discrimination against the heavy isotope during metabolic processes (Deniro & Epstein, 1978, 1981), but wastes are not excreted by the parasitoid until just prior to emergence (Hoddle *et al.*, 1998). If wastes are not excreted, then there can be no enrichment of the heavier isotopes. As we were not able to differentiate between these diet components, we cannot directly infer the degree of omnivory. These results make the use of stable isotopes to measure omnivory in any biocontrol system containing phloem feeding insects and parasitoids unviable without further knowledge.

Mullein plants were enriched in $\delta^{13}C$ relative to both whitefly and parasitized whitefly, but not tomato leaves

https://doi.org/10.1017/S0007485308006378 Published online by Cambridge University Press

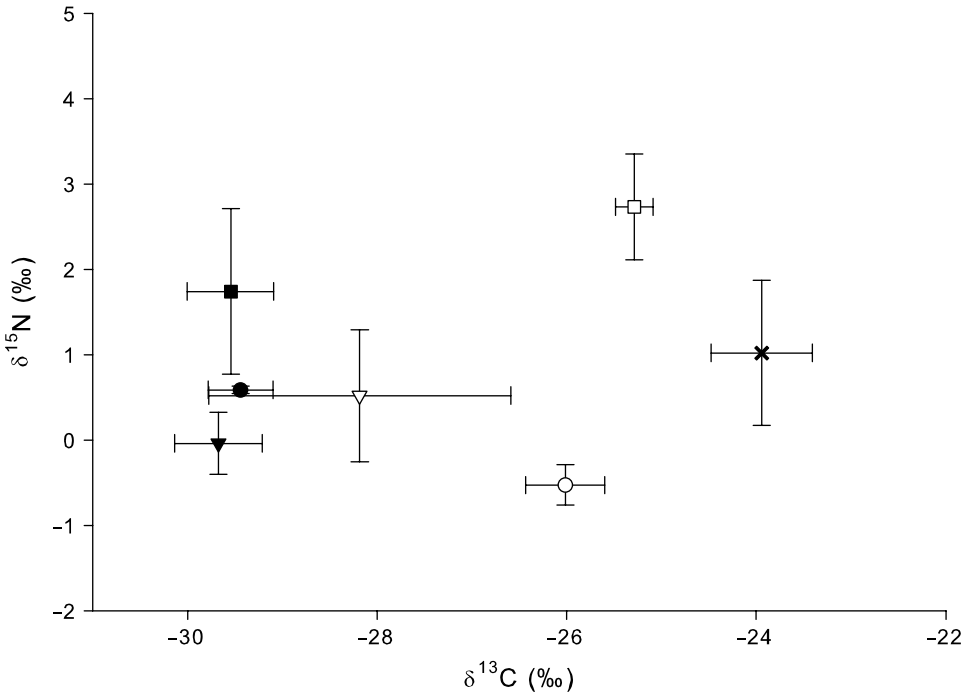

Fig. 4. Average stable isotope signatures for diet components for *D. hesperus* from feeding trials 1 (filled symbols) and 2 (open symbols). Mullein signatures are from plants grown concurrent with samples analyzed during trial 2. Error bars represent one standard error (●, Encarsia; ▼, whitefly; ■, tomato; X, mullein).

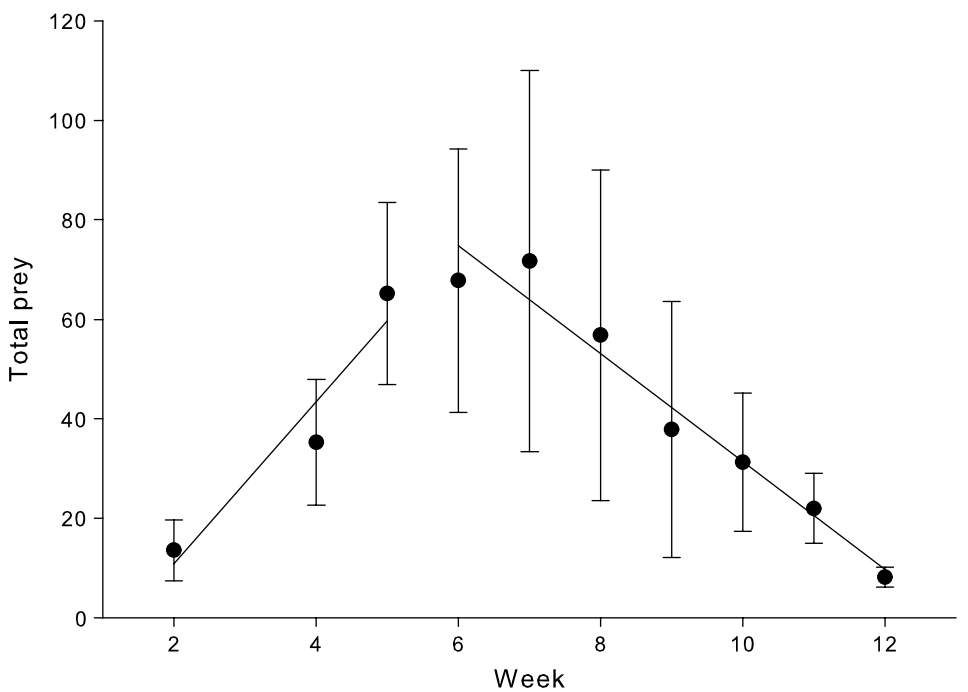

Fig. 5. Prey numbers within cages over time. Regression lines represent population growth phase ($R^2 = 0.314$) and population decline phase ($R^2 = 0.110$). Transition occurs at change in sampling protocol. Regression analyses were conducted on log transformed data. Error bars represent one standard error.

(fig. 4). It is common for phloem feeding insects to be slightly depleted in $\delta^{13}C$ relative to their host plant (Spence & Rosenheim, 2005). This depletion was likely large enough for the insects to be statistically distinct, but not tomato. We also found a large amount of isotopic variability found within each of the diet components, which overshadowed

https://doi.org/10.1017/S0007485308006378 Published online by Cambridge University Press

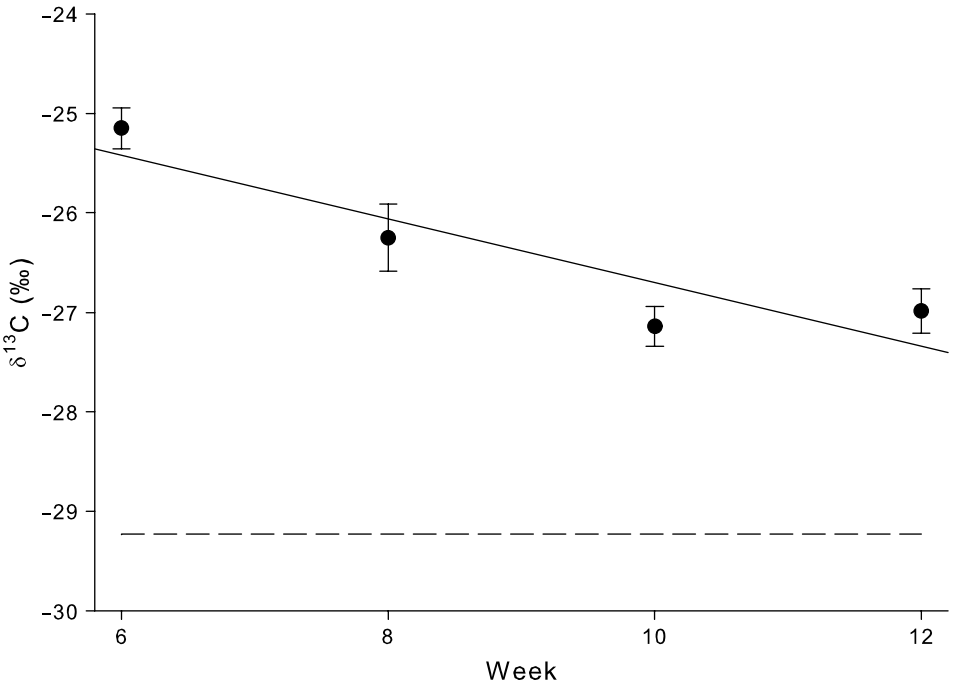

Fig. 6. $\delta^{13}C$ signatures of *D. hesperus* during greenhouse cage trials. Averages were taken for all individuals within each mesocosm. Data points represent the mean of the averages. Regression line represents decline over time ($R^2 = 0.323$). Dashed line shows the average value of tomato leaves samples from the cages in week 12. Error bars represent one standard error.

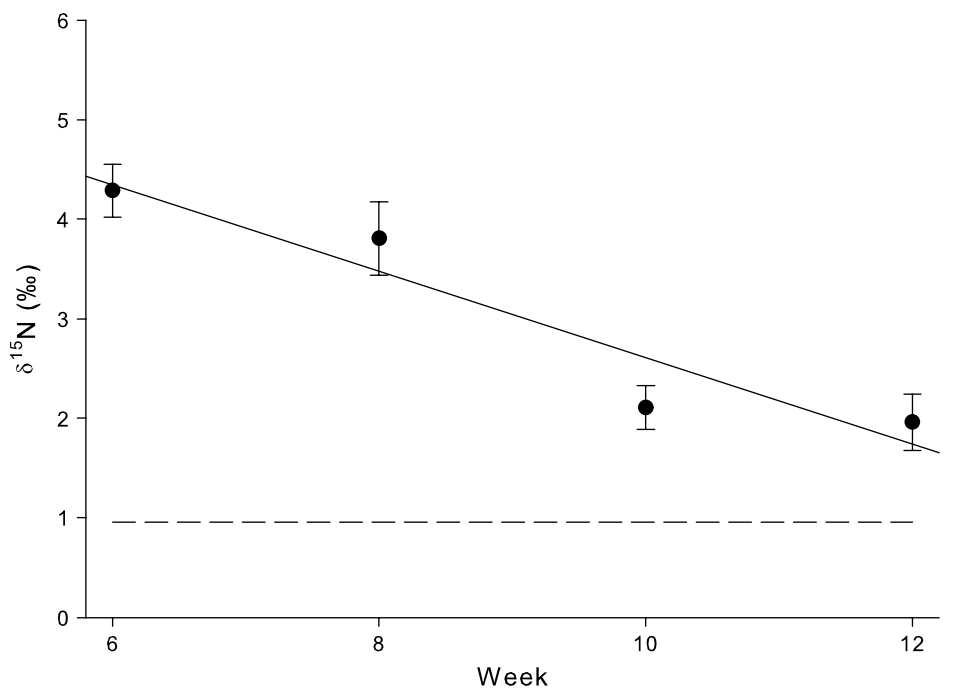

Fig. 7. $\delta^{15}N$ signatures of *D. hesperus* during greenhouse cage trials. Averages were taken for all individuals within each mesocosm. Data points represent the mean of the averages. Regression line represents decline over time ($R^2 = 0.338$). Dashed line shows the average value of tomato leaves samples from the cages in week 12. Error bars represent one standard error.

differences among diet components. Variation in $\delta^{15}N$ and $\delta^{13}C$ were quite high within the diet components with an average range of 3.56‰ for $\delta^{15}N$ and 2.90‰ for $\delta^{13}C$. Recent meta-analyses have found a mean trophic enrichment of −0.53‰ for $\delta^{13}C$ and 1.88‰ for $\delta^{15}N$ (Spence & Rosenheim, 2005). Using these values, the average range of

https://doi.org/10.1017/S0007485308006378 Published online by Cambridge University Press

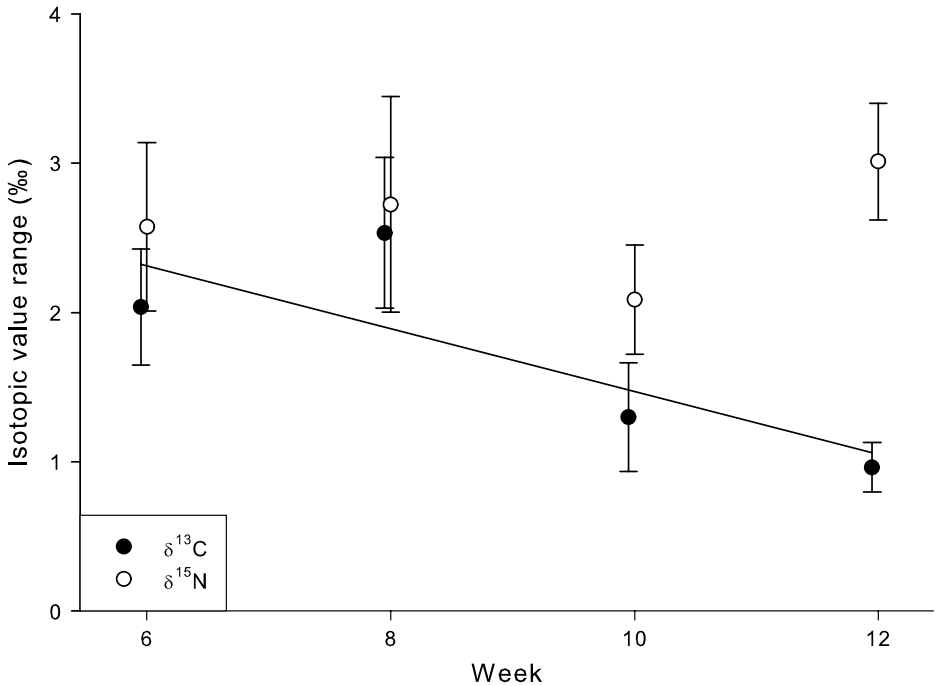

Fig. 8. Range of isotopic values (max–min) found within each cage for both $\delta^{13}C$ and $\delta^{15}N$. Regression line shows the decline in the range of carbon values over time ($R^2 = 0.178$). No trend was found for nitrogen values. Error bars represent one standard error (●, $\delta^{13}C$; ○, $\delta^{15}N$).

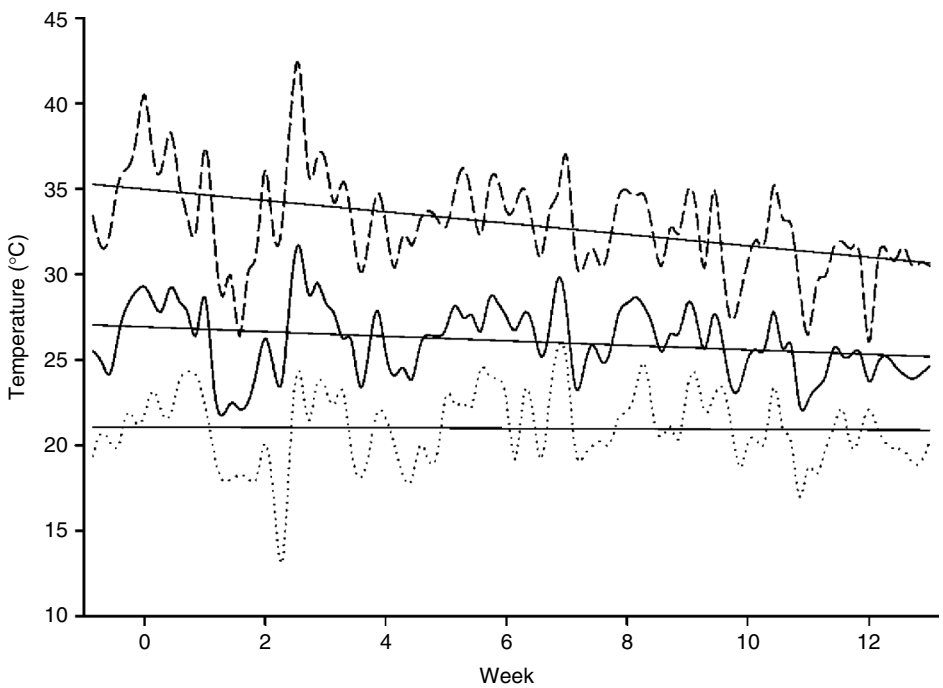

Fig. 9. Temperature readings over the course of the greenhouse cage trials. Data use daily mean, minimum, and maximum temperatures. Both maximum ($R^2 = 0.219$) and mean ($R^2 = 0.069$) values declined over time. Minimum temperatures fluctuated but showed no overall trend (——, mean; . . .., minimum; – – –, maximum).

isotopic signatures found within diet components equaled approximately 5.5 trophic levels for carbon and two trophic levels for nitrogen. Variation in whitefly and parasitoid samples likely just reflect variation in the leaves from which they were collected, as each insect sample was taken from a distinct leaf and plant. Thus, all variation within diet

https://doi.org/10.1017/S0007485308006378 Published online by Cambridge University Press

components can be attributed to variation within plant groupings.

Numerous factors can influence the isotopic signature of plants. Plant $\delta^{15}N$ can vary with the age of the plant (Handley & Scrimgeour, 1997), between plant organs (Evans, 2001) and leaves of different ages (Zanne *et al*., 2006), as well as with small differences in microhabitat (Handley & Scrimgeour, 1997; Dawson *et al*., 2002). Carbon signatures can also be affected by genetic variation and many types of environmental variation (Farquhar *et al*., 1989; Smedley *et al*., 1991; Dawson *et al*., 2002). Given the variety of factors influencing isotopic discrimination, it is difficult to isolate a single factor influencing the variability of plant samples. Many of the environmental factors which influence the attractiveness of different plant parts to herbivores (Orians & Jones, 2001; Orians *et al*., 2002) also influence isotopic heterogeneity. For example, the shading of individual leaves can alter $\delta^{13}C$ (Le Roux *et al*., 2001) and can also alter nitrogen content and the amount of secondary plant compounds, making them more appealing to herbivorous insects (Nichols-Orians, 1991; Crone & Jones, 1999). Future studies should take into account isotopic variability both within plants and within populations, as they may be related to which plant parts are actually consumed. The investigation of such possibilities may reduce the variability of results often found in stable isotope ecology.

Greenhouse cage trials

The greenhouse cage trials showed some success in showing changes in diet composition, though the methods were not as direct as initially intended. Our inability to distinguish between diet sources makes the use of a diet mixing model problematic (Newsome *et al*., 2007) and thus requires alternative evidence for the determination of diet changes. Though it may be inappropriate for the determination of niche breadth (Matthews & Mazumder, 2004), the use of variation in isotopic signature for determining the degree of omnivory may still be applicable in a very qualitative sense (Bearhop *et al*., 2004). The negative correlation of both $\delta^{13}C$ and $\delta^{15}N$ with time suggest that some change was occurring within the cages (figs 6 and 7). One interpretation of these data is that the diet of *D. hesperus* was changing over time. This is supported by declining prey populations over this period (fig. 5). However, the signatures of prey were found to be indistinct from plant samples during feeding trials. Thus, these changes in isotopic signature cannot be definitively related to changes in the amount of prey in the diet.

It is also possible that the isotopic signatures of the plants were declining over time due to other factors, including changes in environmental conditions. Temperature and plant age can affect plant physiology and thus isotopic composition (Handley & Scrimgeour, 1997; Dawson *et al*., 2002). Temperatures within the greenhouse varied over the summer, with both the mean temperature and the maximum daily temperature declining over the course of the experiment (fig. 9), potentially affecting the $\delta^{13}C$ and $\delta^{15}N$ of the plants. Also, the plants matured over the course of the experiment, which may be another source of isotopic variation. Given the variability of the isotopic signature found within plant tissues, we are unable to attribute the decline in isotopic enrichment with any one factor.

Though it is likely that the physiological condition of the plants contributed to the decrease of $\delta^{13}C$ and $\delta^{15}N$ over time for *D. hesperus*, the concurrent decline in the range of $\delta^{13}C$ values suggests the occurrence of some other process (fig. 8). This decrease in $\delta^{13}C$ variation over time may be related to changes in the proportion of individuals feeding upon different plant types. As prey decline, *D. hesperus* is expected to increase feeding on mullein, a preferred plant within the diet (Sanchez *et al*., 2003). At week 12, *D. hesperus* was only slightly enriched relative to the tomato leaves when considering $\delta^{15}N$, but showed greater enrichment in ^{13}C. Though not significant, mullein leaves were enriched relative to tomato leaves during feeding trials. This ^{13}C enrichment relative to the tomato leaves at the end of the trial may be due to *D. hesperus* feeding on mullein leaves. The decline in prey availability, coupled with an increased proportion of *D. hesperus* individuals feeding on mullein, suggests that omnivory did decline as prey became less available. It is still possible that the carbon enrichment seen in *D. hesperus* is due to the incorporation of tomato leaves from earlier in the trial, but previous research suggests that carbon shifts are relatively rapid (Ostrom *et al*., 1997). However, due to an absence of isotopic data for mullein leaves from the cage trials, this remains conjecture.

The variability in signatures within each cage at any given sampling date should reflect variability in diet choice within the population. Variability in $\delta^{15}N$ did not change over time, suggesting that there are differences in diet choice within the population that are independent of the amount of prey available. These differences may be due to individual preferences, age structure, size structure or sex, though we cannot differentiate among them. Age-related differences should be minimal as all individuals sampled were reproductive adults, and size explained a very small proportion of the variability (unpublished data). However, differences related to sex and individual preference were unaccounted for.

Summary

We were unable to detect changes in the degree of omnivory for *D. hesperus*. This was due to both the variability within sources and the isotopic similarity of these sources. However, we were able to detect shifts in plant feeding from tomato to mullein. Our results also suggest that there is some component of variation in the diet content of *D. hesperus* individuals that remains independent of prey abundance. The variability found within diet sources underscores the idea that the variability of diet sources needs to be quantified to use stable isotope analysis in a quantitative fashion (Newsome *et al*., 2007). This variation likely reflects differences in the physiological condition of plant tissues that is not explicitly considered in insect isotope studies (e.g. Spence & Rosenheim, 2005) but may warrant future consideration. Thus, given our current understanding of stable isotope ecology, we cannot directly detect changes in omnivory or intraguild predation for biological control agents feeding on phloem feeding insects and their parasitoids. This precludes the application of this technique to the evaluation of numerous biological systems, though it may be applicable in cases where the pests feed upon leaf tissue.

Other diet quantification techniques may be more appropriate, but we were unable to use them in this particular

https://doi.org/10.1017/S0007485308006378 Published online by Cambridge University Press

instance. *Dicyphus hesperus* uses extra-oral digestion, which makes classic gut content analysis intractable. However, molecular approaches may be more feasible, though it is unknown what effects extra-oral digestion has on DNA degradation. If this technique is to be used, these effects need to be quantified first, as DNA quality can have a significant effect on the utility of molecular gut content analysis (King *et al.*, 2008). The applicability of any of these techniques will require the researcher to first understand the requirements and assumptions of the technique prior to application.

Acknowledgements

We thank the greenhouse and entomology staff at the Greenhouse and Processing Crops Research Centre for logistic support, especially M. Pachal, as well as the staff at the University of Windsor stable isotope lab for their assistance. This research was funded through the NSERC network grant 'Biocontrol Network', with support from Agriculture and Agri-Food Canada, the British Columbia Greenhouse Vegetable Growers Association, Koppert Biological Systems and the University of Windsor. J.A. Bennett was supported by an Ontario Graduate Scholarship.

References

Agrawal, A.A., Kobayashi, C. & Thaler, J.S. (1999) Influence of prey availability and induced host-plant resistance on omnivory by western flower thrips. *Ecology* **80**, 518–523.

Arim, M. & Marquet, P.A. (2004) Intraguild predation: a widespread interaction related to species biology. *Ecology Letters* **7**, 557–564.

Bearhop, S., Adams, C.E., Waldron, S., Fuller, R.A. & Macleod, H. (2004) Determining trophic niche width: a novel approach using stable isotope analysis. *Journal of Animal Ecology* **73**, 1007–1012.

Bluthgen, N., Gebauer, G. & Fiedler, K. (2003) Disentangling a rainforest food web using stable isotopes: dietary diversity in a species-rich ant community. *Oecologia* **137**, 426–435.

Branstrator, D.K., Cabana, G., Mazumder, A. & Rasmussen, J.B. (2000) Measuring life-history omnivory in the opossum shrimp, *Mysis relicta*, with stable nitrogen isotopes. *Limnology and Oceanography* **45**, 463–467.

Bruno, J.F. & O'Connor, M.I. (2005) Cascading effects of predator diversity and omnivory in a marine food web. *Ecology Letters* **8**, 1048–1056.

Coll, M. & Guershon, M. (2002) Omnivory in terrestrial arthropods: Mixing plant and prey diets. *Annual Review of Entomology* **47**, 267–297.

Crone, E.E. & Jones, C.G. (1999) The dynamics of carbon-nutrient balance: Effects of cottonwood acclimation to short-and long-term shade on beetle feeding preferences. *Journal of Chemical Ecology* **25**, 635–656.

Dawson, T.E., Mambelli, S., Plamboeck, A.H., Templer, P.H. & Tu, K.P. (2002) Stable isotopes in plant ecology. *Annual Review of Ecology and Systematics* **33**, 507–559.

Deniro, M.J. & Epstein, S. (1978) Influence of diet on distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta* **42**, 495–506.

Deniro, M.J. & Epstein, S. (1981) Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica et Cosmochimica Acta* **45**, 341–351.

Diehl, S. (1995) Direct and indirect effects of omnivory in a litoral lake community. *Ecology* **76**, 1727–1740.

Diehl, S. (2003) The evolution and maintenance of omnivory: Dynamic constraints and the role of food quality. *Ecology* **84**, 2557–2567.

Eubanks, M.D. & Denno, R.F. (1999) The ecological consequences of variation of plants and prey for an omnivorous insect. *Ecology* **80**, 1253–1266.

Evans, R.D. (2001) Physiological mechanisms influencing plant nitrogen isotope composition. *Trends in Plant Science* **6**, 121–126.

Farquhar, G.D., Ehleringer, J.R. & Hubick, K.T. (1989) Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* **40**, 503–537.

Gadd, C.A. & Raubenheimer, D. (2000) Nutrient-specific learning in an omnivorous insect: The American cockroach *Periplaneta Americana* L. learns to associate dietary protein with the odors citral and carvone. *Journal of Insect Behavior* **13**, 851–864.

Handley, L.L. & Scrimgeour, C.M. (1997) Terrestrial plant ecology and N-15 natural abundance: The present limits to interpretation for uncultivated systems with original data from a Scottish old field. *Advances in Ecological Research* **27**, 133–212.

Hoddle, M.S., van Driesche, R.G. & Sanderson, J.P. (1998) Biology and use of the whitefly parasitoid *Encarsia formosa*. *Annual Review of Entomology* **43**, 645–669.

Janssen, A., Willemse, E. & van der Hammen, T. (2003) Poor host plant quality causes omnivore to consume predator eggs. *Journal of Animal Ecology* **72**, 478–483.

King, R.A., Read, D.S., Traugott, M. & Symondson, W.O.C. (2008) Molecular analysis of predation a review of best practice for DNA-based approaches. *Molecular Ecology* **17**, 947–963.

Langellotto, G.A., Rosenheim, J.A. & Williams, M.R. (2005) Enhanced carbon enrichment in parasitoids (hymenoptera): A stable isotope study. *Annals of the Entomological Society of America* **98**, 205–213.

Langellotto, G.A., Rosenheim, J.A. & Williams, M.R. (2006) Assessing trophic interactions in a guild of primary parasitoids and facultative hyperparasitoids: stable isotope analysis. *Oecologia* **150**, 291–299.

Le Roux, X., Bariac, T., Sinoquet, H., Genty, B., Piel, C., Mariotti, A., Girardin, C. & Richard, P. (2001) Spatial distribution of leaf water-use efficiency and carbon isotope discrimination within an isolated tree crown. *Plant, Cell and Environment* **24**, 1021–1032.

Magalhaes, S., Janssen, A., Montserrat, M. & Sabelis, M.W. (2005) Host-plant species modifies the diet of an omnivore feeding on three trophic levels. *Oikos* **111**, 47–56.

Matthews, B. & Mazumder, A. (2004) A critical evaluation of intrapopulation variation of delta C-13 and isotopic evidence of individual specialization. *Oecologia* **140**, 361–371.

McCann, K. & Hastings, A. (1997) Re-evaluating the omnivory-stability relationship in food webs. *Proceedings of the Royal Society of London Series B: Biological Sciences* **264**, 1249–1254.

McGregor, R.R. & Gillespie, D.R. (2005) Intraguild predation by the generalist predator *Dicyphus hesperus* on the

parasitoid *Encarsia formosa*. *Biocontrol Science and Technology* **15**, 219–227.

McGregor, R.R., Gillespie, D.R., Quiring, D.M.J. & Foisy, M.R.J. (1999) Potential use of *Dicyphus hesperus* Knight (Heteroptera: Miridae) for biological control of pests of greenhouse tomatoes. *Biological Control* **16**, 104–110.

Mooney, K.A. & Tillberg, C.V. (2005) Temporal and spatial variation to ant omnivory in pine forests. *Ecology* **86**, 1225–1235.

Newsome, S.D., Martinez del Rio, C., Bearhop, S. & Phillips, D.L. (2007) A niche for stable isotope ecology. *Frontiers in Ecology and the Environment* **5**, 429–436.

Nichols-Orians, C.M. (1991) Environmentally induced differences in plant traits consequences for susceptibility to a leaf-cutter ant. *Ecology* **72**, 1609–1623.

Orians, C.M. & Jones, C.G. (2001) Plants as resource mosaics: a functional model for predicting patterns of within-plant resource heterogeneity to consumers based on vascular architecture and local environmental variability. *Oikos* **94**, 493–504.

Orians, C.M., Ardon, M. & Mohammad, B.A. (2002) Vascular architecture and patchy nutrient availability generate within-plant heterogeneity in plant traits important to herbivores. *American Journal of Botany* **89**, 270–278.

Ostrom, P.H., ColungaGarcia, M. & Gage, S.H. (1997) Establishing pathways of energy flow for insect predators using stable isotope ratios: Field and laboratory evidence. *Oecologia* **109**, 108–113.

Polis, G.A., Myers, 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.

Post, D.M. (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* **83**, 703–718.

Sagers, C.L. & Goggin, F.L. (2007) Isotopic enrichment in a phloem-feeding insect: influences of nutrient and water availability. *Oecologia* **151**, 464–472.

Sanchez, J.A., Gillespie, D.R. & McGregor, R.R. (2003) The effects of mullein plants (*Verbascum thapsus*) on the population dynamics of *Dicyphus hesperus* (Heteroptera: Miridae) in tomato greenhouses. *Biological Control* **28**, 313–319.

Singer, M.S. & Bernays, E.A. (2003) Understanding omnivory needs a behavioral perspective. *Ecology* **84**, 2532–2537.

Smedley, M.P., Dawson, T.E., Comstock, J.P., Donovan, L.A., Sherrill, D.E., Cook, C.S. & Ehleringer, J.R. (1991) Seasonal carbon isotope discrimination in a grassland community. *Oecologia* **85**, 314–320.

Spence, K.O. & Rosenheim, J.A. (2005) Isotopic enrichment in herbivorous insects: a comparative field-based study of variation. *Oecologia* **146**, 89–97.

Tooker, J.F. & Hanks, L.M. (2004) Trophic position of the endophytic beetle, *Mordellistena aethiops* Smith (Coleoptera: Mordellidae). *Environmental Entomology* **33**, 291–296.

van Lenteren, J.C. & Noldus, L.P.J.J. (1990) Whitefly-plant relationships: behavioural and ecological aspects. pp 47–90. *in* Gerling, D. (*Ed.*) *Whiteflies: Their Bionomics, Pest Status and Management*. Andover, Intercept.

Zanne, A.E., Lower, S.S., Cardon, Z.G. & Orians, C.M. (2006) ^{15}N partitioning in tomato: vascular constraints versus tissue demand. *Functional Plant Biology* **33**, 457–464.

Zar, J.H. (1999) *Biostatistical analysis*. 929 pp. New Jersey, Prentice Hall.