

15N-enrichment of plant tissue to mark phytophagous insects, associated parasitoids, and flower-visiting entomophaga

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Abstract

New techniques are presented on the use of 15N to mark insects. 15N, a stable isotope of nitrogen, was enriched above natural abundance in plant and insect tissues. Two laboratory studies demonstrated that enriched 15N-concentrations could be tracked from plant to insect using mass spectrometry. In the first study, adult Cotesia plutellae (Kurdjimov) (Hymenoptera: Braconidae) and Hippodamia convergens Guérin-Méneville (Coleoptera: Coccinellidae) were allowed to feed at the flowers of rapid-cycling Chinese cabbage plants that had been fertilized with 15N-enriched potassium nitrate (KNO3-15NO3). Both insect groups were found to have significantly elevated 15N levels after visiting the flowers of the 15N-enriched plants for 48 hours. In the second study, 15N-enriched bean plant (Phaseolus vulgaris L.) tissue was incorporated into an insect diet and fed to navel orangeworms, Amyelois transitella (Walker) (Lepidoptera: Pyralidae). When the navel orangeworm larvae were 4th instars, they were removed from the diet and exposed to the parasitoid, Goniozus legneri Gordh (Hymenoptera: Bethylidae). Results indicated that the enriched 15N-concentration of the bean plants was transferred to the navel orangeworms and, subsequently, to the parasitoids. This work may provide useful techniques to help establish whether agriculturally important entomophaga visiting 15N-enriched flowers or parasitizing enriched sentinel larvae in the field can be effectively marked with 15N.

Introduction

Insects are often marked to better follow their dispersal, colonization patterns, growth, longevity, density, or behavior (see reviews by Southwood, 1978; Akey, 1991). As described by Akey (1991), useful markers should be easy to apply, easy to recognize, require little manipulation, maintain a consistent signal, and have relatively innocuous biological effects on marked individuals. Recent studies have shown stable isotope analysis to be a safe and useful method for tracking energy and material flow in insect food webs (Hershey et al., 1993; Ostrom et al., 1997). The manipulation of stable isotope ratios within insects may be a sound means of reducing unanticipated effects on the fitness of marked individuals.
(Stimmann, 1991), and vary widely (in background concentration) in field systems (Van Steenwyk, 1991).

The ideal marking technique will often be context-dependent. As a marker for flower-visiting insects, 15N should be particularly well-suited because assimilated N remains within the plant for long periods, and plant N is often preferentially allocated to reproductive structures (Ledgard & Smith, 1992). Ledgard & Smith (1992) monitored 15N levels in perennial plant structures over the course of three years after applying the 15N-enriched fertilizer only in the first year. The fact that elevated 15N levels could be monitored over such an extended period is an indication of the relative persistence of N in an organism and the degree to which plants conserve N. Comparisons of the 15N-concentrations in new and older tissues showed that plants can allocate a greater proportion of their available N to new growth, as indicated by increased 15N concentrations in shoots, leaves, and reproductive structures (Ledgard et al., 1994). These studies are based on the premise that the rare isotope gets absorbed and metabolized by organisms just as the common isotope does; therefore, an organism synthesizing nitrogenous compounds from 15N-enriched resources should reflect the elevated 15N availability.

Nitrogen availability is often the central limiting factor to the growth, development, and fecundity of insects (Hagen et al., 1984), so it follows that certain 15N-enriched compounds are likely to be assimilated and remain within an insect once consumed. Predaceous and parasitic insects, in particular, have high food conversion rates (weight gain relative to weight ingested) (Hagen et al., 1984). The primary factors affecting the 15N-enrichment of an organism are the 15N concentration of the food source, the quantity consumed, the metabolism of the organism, the degree of assimilation, and the size of the nitrogen pool in the organism (Ostrom et al., 1997).

Living cells do not readily differentiate between the stable isotopes of an element (Francis, 1954). Almost all known elements occur as mixtures of two or more isotopes, and the isotopes of an element exist at fairly constant ratios relative to one another (Francis, 1954). Enrichment or depletion of the natural abundance of a stable (non-decaying) isotope within an organism will change its isotopic ratio. The marker, therefore, is the altered isotopic ratio. This ratio can be manipulated within a given system, population, individual, or subunit thereof. Studies have documented that insects fed 15N-enriched food sources closely reflected the 15N concentration of their food or deviated by a consistent and very small degree (Ostrom et al., 1997). 15N has been useful as a marker for aquatic insects (Hershey et al., 1993), as a physiological tracer to determine metabolic pathways (Hirayama et al., 1997), as a tracer for energy flow through trophic levels (Ostrom et al., 1997), and as an aid in phylogenetic analyses (Tayasu et al., 1998).

Given that many entomophagous insect species require supplemental nourishment from flowers (Jervis et al., 1993) and that plant N gets concentrated in reproductive structures, we hypothesized that propagating flowering plants with 15N-enriched fertilizer would produce pollen and nectar sufficiently enriched with 15N to label floral feeding adult entomophagous insects. Additionally, we tested the hypothesis that 15N, when presented in an assimilable form for a particular herbivorous insect, will produce a discernable marker that will be passed on to parasitoids developing on the herbivore. Delivering 15N to insects in this manner allows for the natural uptake of a rare isotope, which should minimize effects on behavior and/or fitness.

Materials and methods

Floral feeding trials

All floral feeding trials were conducted with Chinese cabbage plants (Brassica rapa L. subsp. pekinensis [Lour.]) that had been bred to flower 16 days after sowing (Wisconsin Fast Plants™). These plants allowed for rapid replication of experiments as well as reliable coordination of flowering and insect availability. Seeds were sown in Jiffy Mix™ and vermiculite in 32-plug foam trays (~ 10 × 16 × 6 cm). The plants were grown under continuous fluorescent lighting and relatively constant temperature (24 ± 2 °C), as described by Williams & Hill (1986).

Each foam tray rested on a mat of felt, supported by a thin plastic base. Ribbons of the felt mat extended through a hole in the plastic base and down into a water reservoir, allowing the seedlings to obtain water as needed. Additionally, plants were misted with de-ionized (DI) water every 2–3 days.

15N-fertilization. All plants received the same N-P-K provisions (N-P-K::13:5:37). The fertilizer was a liquid solution of potassium nitrate (KNO3) and sodium phosphate monobasic (NaH2PO4) dissolved in DI water at 17.8 g l−1 and 1.25 g l−1, respectively. For
the purpose of $^{15}$N-enrichment, KNO$_3$-$^{15}$NO$_3$ (10% $^{15}$NO$_3$) was purchased from Isotec, Inc. (Miamisburg, OH). This was mixed with common KNO$_3$ to achieve a KNO$_3$-$^{15}$NO$_3$ (5% $^{15}$NO$_3$) solution.

Soil fertilization of all plants was conducted 3, 7, 14, and 21 days after sowing. To each plug of a given tray, 5 ml of fertilizer was added. Plug trays with control-plants received unenriched KNO$_3$ fertilizer, and trays with $^{15}$N-enriched plants received the KNO$_3$-$^{15}$NO$_3$ (5% $^{15}$NO$_3$) fertilizer. Enriched and control plants differed only in the proportion of $^{15}$N in their total N provision. Two trays each (64 plants total) of the $^{15}$N-enriched and control plants were produced for each of three replicates.

Floral feeding. Two species of adult entomophaga, *Cotesia plutellae* (Kurdjimov) (Hymenoptera: Braconidae) and *Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae), were purchased from a commercial insectary (Biofac, Inc., Mathis, TX). These insect species were chosen because they are important natural enemies of common pests of the Brassicaceae. To ensure that the shipments of insects were not arriving with elevated or depleted $^{15}$N levels relative to the local background level, samples of both species (approximately 40 specimens) were removed for subsequent $^{15}$N analysis.

The remaining *C. plutellae* and *H. convergens* were each divided into two groups: (1) exposure to flowers of $^{15}$N-enriched plants and (2) exposure to flowers of unenriched plants. To isolate the insect groups on the respective plants, the plants were placed inside 30 x 40 x 46 cm mesh cages, under the same lighting conditions used for plant propagation. Within each cage, a single tray of 32 plants was provided. Two cages contained the enriched plants, and two other cages had the unenriched plants. At least three days were allowed to elapse between the last fertilization of the plants and the introduction of the insects into the cages. Two groups of 20-40 *C. plutellae* wasps and two groups of 8-10 *H. convergens* beetles were caged with the plants for 48 h, and then collected. Contamination of samples was avoided by collecting control specimens first and by thoroughly cleaning the sampling materials (aspirators, forceps). *C. plutellae* wasps were collected in groups of 10 wasps; a single sample represented 10 living individuals, and *H. convergens* beetles were collected as samples of 2–3 living individuals. Living individuals were chosen for analysis based on the assumption that they spent more time feeding at the flowers. The grouping of individuals was necessary to accommodate the biomass requirements of the sample analysis.

Immediately following the collection of insects after the 48-h period, a second 48-h feeding period was initiated (the plants in the trays were caged continuously for 96 h). Each insect group was again caged separately, and whichever species had been in a given cage for the first 48 h, the other insect species was placed in the cage for the latter 48 h. Therefore, both insect species had the opportunity to feed at a given tray of plants. The opportunity to feed first at a tray was randomized between the two insect species. This order was inversed in the latter 48 h period in case the first insect group had limited or somehow altered the availability of nectar or pollen for the second group. At the end of the 96-h period, 10 flowers (corollae, carpels, stamens, and sepals) were clipped from the plants in each cage to measure their $^{15}$N-enrichment. This series of experiments was replicated three times; for each replication, a new group of plants was propagated, and new insects were ordered from the same commercial insectary.

A related study was conducted to ascertain whether the insects could pick up $^{15}$N from merely crawling over plant surfaces of $^{15}$N-enriched plants. $^{15}$N-enriched plant material was placed in petri dishes (100 x 20 mm) with four *H. convergens* individuals per dish for 48 h. There were two petri dishes set up for each assay, and the assay was replicated twice. Therefore, a total of 16 coccinellid adults, comprising 8 samples, were assayed for their ability to accumulate $^{15}$N in the absence of flowers. Beetles from the insectary shipments and those caged on unenriched plants served as controls. The coccinellids were chosen for this assay because they were more active than the wasps and tended to crawl rather than fly.

**Artificial diet trials**

An artificial diet was prepared to test whether an $^{15}$N-enrichment signal could be introduced into an herbivorous host and subsequently passed to an insect parasitoid of that host. The navel orangeworm, *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae), served as the host, and the parasitoid was a gregarious ectoparasitic wasp, *Goniozus legneri* Gordh (Hymenoptera: Bethylidae). These species were chosen because they are easily maintained in laboratory cultures.

For the diet, bean plants (*Phaseolus vulgaris* L.) were propagated in a greenhouse using potting soil
mixed at a 3:1 ratio of peat to vermiculite. The bean plants were propagated in small pots (diam. 20 cm × 15 cm) and fertilized solely with enriched potassium nitrate (10% 15NO3) and sodium phosphate monobasic, NaH2PO4. The respective quantities of KNO3 and NaH2PO4 added to 1 liter DI water were 9.0 g and 2.0 g. Plants were fertilized semi-weekly at a rate of 40-50 ml per occasion and were watered as needed. When the plants had fruited, the leaves, petioles, stems, and pods were plucked and dried for use in the diet. Samples of the dried bean material were set aside for isotopic analysis.

Two separate diets were prepared for the A. transitella larvae, both containing the following ingredients and relative quantities: red flakey wheat bran (3,450 ml), honey (850 ml), glycerin (850 ml), DI water (600 ml), Vanderzant modification vitamin mixture for insects (10 ml), and brewer’s yeast (100 g). This mix was the standard recipe and served to rear G. legneri colony (control). Into a small volume of the standard diet (~800 ml), the 15 N-enriched bean plant material was added and mixed at a bean:diet ratio of approximately 2:3. The enriched material had been finely chopped and sterilized in an autoclave before being added to the diet.

The diets were placed into petri dishes (150 × 15 mm) and compressed such that the dishes were approximately half-filled. Sections of paper towels with 15 mm) and compressed such that the dishes were approximately half-filled. More diet was being added to the diet. Samples to be submitted for 15 N isotopic analysis were frozen, oven-dried, and ground to a small grain-size (< 1 mm). A. transitella samples consisted of a single dried, ground larva; G. legneri samples were 1–2 mg samples of dried, ground adults (4–5 adults per sample). Analyses were conducted using a Tracermass ANCA-MS (Automated 15N13C Analyzer-Mass Spectrometry) stable isotope analyzer (Europa Scientific, Crewe, UK). Results from analyses are expressed herein as the atomic percentage (atom%), which is the fraction of the nitrogen pool that is 15N.

To ensure that the mass spectrometer is calibrated to a high degree of precision, various materials of known 15N concentration are analyzed along with the experimental samples. ANOVA tests were used for most of the statistical analyses. Where data did not conform to assumptions of normality and/or homogeneous variances, Mann-Whitney rank sum tests were performed (MINITAB, 1995); such analyses are noted in the text.

Weighing of wasp eggs and adults. Eggs of G. legneri individuals were removed from A. transitella larvae using a fine brush and placed in glass vials. G. legneri adults were killed by freezing and placed in vials. The vials of eggs and adults were put in a drying oven for one week; each egg and adult was weighed individually. The egg and adult dry-weights were sought to determine the ratio of adult mass to egg mass. This ratio may explain the degree to which the 15N signal is diluted between generations.

Results

Floral feeding trial. The mean 15N concentration of flowers collected from 15N-enriched plants (4.90561 ± 0.04733 atom%, n = 6) was significantly higher than that of control flowers (0.36912 ± 0.00186 atom%, n = 6) (Mann-Whitney rank sum test, P < 0.01). The mean 15N concentration of enriched flowers was close...
to that of the treatment fertilizer (5.0 atom%), while control flowers had $^{15}$N concentrations within the typical range for terrestrial plants (0.365–0.370 atom%), as reported by Europa Scientific, ANCA-MS product literature (Crew, U.K.).

Caged *Cotesia plutellae* wasps and *Hippodamia convergens* beetles were observed foraging at the Wisconsin Fast Plants™ flowers. Within ~5 min of release within a cage, several wasps could be seen foraging within the corolla, presumably taking nectar and/or pollen. The beetles tended to crawl about the cage for 10–20 min before finding and foraging among the flowers. Both insect groups, however, spent most of the time resting either on the plants (flowers, leaves, stems) or clinging to the mesh of the cages.

The mean $^{15}$N concentrations of the *C. plutellae* and *H. convergens* samples in the $^{15}$N-enriched treatment were significantly higher than those of their respective controls (Table 1; ANOVA for *C. plutellae*, *P* < 0.001; Mann–Whitney rank sum test for *H. convergens*: *P* < 0.01). There were no significant differences between the mean $^{15}$N concentration of specimens taken directly from insectary shipments (not allowed to visit flowers) and those taken from control cages (Table 1; for *C. plutellae*: *P* > 0.05; for *H. convergens*: *P* > 0.05). *C. plutellae* and *H. convergens* samples taken directly from insectary shipments had mean $^{15}$N concentrations (Table 1) consistent with typical concentrations of $^{15}$N in terrestrial animals (0.366–0.372 atom%, Europa Scientific, ANCA-MS product literature, U.K.).

The petri dish assays with *H. convergens* beetles showed there was no significant difference in $^{15}$N concentration between the beetles walking on $^{15}$N-enriched plant material (0.3697 ± 0.0011 atom%) and those confined to control plant material (0.3691 ± 0.0006 atom%; Table 1) (*P* > 0.05). When compared with the $^{15}$N concentrations of beetles allowed to visit $^{15}$N-enriched flowers (0.3948 ± 0.0384 atom%), the petri dish beetles $^{15}$N concentration was significantly lower (Mann–Whitney rank sum test: *P* < 0.05).

**Table 1.** $^{15}$N atomic percentages of insects allowed to visit flowers of enriched and unenriched Wisconsin Fast Plants™. Significant statistical differences (ANOVA or Mann–Whitney test, *P* < 0.05) between column means are denoted by differing letters ( ) Numbers in parentheses are sample sizes.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean $^{15}$N atomic percentages ± s.d.</th>
<th><em>Cotesia plutellae</em></th>
<th><em>Hippodamia convergens</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Insectary shipments</td>
<td>0.37008 ± 0.00132 ± (5)</td>
<td>0.36890 ± 0.00158 ± (6)</td>
<td></td>
</tr>
<tr>
<td>Unenriched plants</td>
<td>0.36851 ± 0.00019 ± (5)</td>
<td>0.36907 ± 0.00060 ± (6)</td>
<td></td>
</tr>
<tr>
<td>Enriched plants</td>
<td>0.38604 ± 0.00744 ± (5)</td>
<td>0.39480 ± 0.03840 ± (6)</td>
<td></td>
</tr>
</tbody>
</table>

*Enriched diet trials.* The $^{15}$N concentration of the bean plant material was significantly enriched, compared to the standard diet (Mann-Whitney rank sum test: *P* < 0.0001), and *A. transitella* larvae feeding on the $^{15}$N-enriched diet had significantly greater $^{15}$N concentrations than the larvae feeding on the standard diet (Mann–Whitney rank sum test: *P* < 0.0001; Table 2). The *G. legneri* control-samples had a mean $^{15}$N concentration similar to that of the *A. transitella* controls (*P* > 0.05), but when the parasitoids were allowed to develop on $^{15}$N-enriched *A. transitella* larvae, the wasp progeny had a mean $^{15}$N concentration that was much higher than that of parasitoids reared from unenriched hosts (Mann–Whitney rank sum test: *P* < 0.0001; Table 2). The substantial difference indicates that the $^{15}$N signal was successfully passed from host to parasitoid.

While the targeted wasps were significantly enriched above the background of $^{15}$N in the unenriched wasps, the level of $^{15}$N in the enriched wasps was significantly lower than their hosts, the enriched *A. transitella* larvae (Mann–Whitney rank sum test: *P* < 0.001; Table 2). Furthermore, the $^{15}$N concentration of the wasps declined to control levels by the next generation (*P* > 0.05).

The mean dry-weight of the *G. legneri* eggs was 0.0041 ± 0.0005 mg (*n* = 20); the mean adult mass was 0.3460 ± 0.0191 mg (*n* = 22). The ratio of adult mass to egg mass was approximately 84:1.
Table 2. $^{15}$N atomic percentages of insect specimens and plant tissues from the enriched diet trials. ‘Unenriched material’ represents the natural background abundance of $^{15}$N in plant and insect tissues; ‘target material’ is the group of plant and insect specimens enriched with $^{15}$N. Significant statistical differences (ANOVA or Mann–Whitney test, $P < 0.05$) between column means are denoted by differing letters.

<table>
<thead>
<tr>
<th>Sample material</th>
<th>Mean $^{15}$N atom% ± s.d.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unenriched material</td>
<td></td>
</tr>
<tr>
<td>Standard diet$^1$</td>
<td>0.36832 ± 0.00276 a (11)</td>
</tr>
<tr>
<td>Amyelois transitella$^2$</td>
<td>0.36920 ± 0.00044 a (11)</td>
</tr>
<tr>
<td>Goniozus legneri$^3$</td>
<td>0.36919 ± 0.00039 a (11)</td>
</tr>
<tr>
<td>Target material</td>
<td></td>
</tr>
<tr>
<td>Bean tissues$^1$</td>
<td>8.1850 ± 1.1690 b (12)</td>
</tr>
<tr>
<td>Amyelois transitella$^2$</td>
<td>0.66950 ± 0.13700 c (15)</td>
</tr>
<tr>
<td>Goniozus legneri$^3$</td>
<td>0.48651 ± 0.02307 d (8)</td>
</tr>
<tr>
<td>G. legneri (progeny)$^3$</td>
<td>0.36931 ± 0.00044 a (11)</td>
</tr>
</tbody>
</table>

1 Individual sample consists of 5 mg aliquot, dry-weight.
2 Individual sample consists of a single larva.
3 Individual sample consists of 4–5 wasps.

( ) Numbers in parentheses are sample sizes.

Discussion

In both the floral feeding and enriched diet studies, the $^{15}$N concentration of enriched plant material was almost equal to that of the fertilizer, which is likely the result of the enriched fertilizer being the plants’ primary nitrogen source. Both C. plutellae and H. convergens, when allowed to visit the flowers of $^{15}$N-enriched plants, effectively marked themselves with $^{15}$N. The degree of enrichment of these insects was quite small compared to the $^{15}$N level of the enriched plants, and the probable reason is that the mass of enriched amino acids consumed at the flowers had to have been exceedingly small relative to the insects’ biomass. It is also possible that the wasps and beetles visiting the flowers were ‘dusted’ with enriched pollen and did not actually ingest enriched material; however, Hagen et al. (1984) and Jervis et al. (1993) detail the degree to which adult entomophagous insects (H. convergens, in particular) are known to supplement their diet with floral resources. Our observations of the insects foraging in the corollae of the flowers is a strong indication that these insects were feeding and marked themselves with enriched material.

The samples of C. plutellae and H. convergens taken directly from insectary shipments produced essentially the same $^{15}$N levels as the insects feeding at unenriched plants. To generate the $^{15}$N signal in unenriched adult insects, the insects must feed/visit flowers that are substantially enriched with $^{15}$N.

The petri dish assay showed that when confined to a relatively small volume with highly enriched plant material, the adult insects did not take on $^{15}$N above background levels. Given that a) the insects did not take on enriched $^{15}$N levels after crawling over highly enriched vegetative plant structures, and that b) only the insects allowed to feed at $^{15}$N-enriched flowers had elevated $^{15}$N levels, we conclude that the insects were marked with $^{15}$N only from their visits to flowers of enriched plants. This transfer from plant to insect represents a detectible signal and thus, a functional intratrophic marker.

The herbivorous A. transitella larvae in the second study assimilated much more $^{15}$N from their $^{15}$N-enriched diet, compared to the floral feeding adult insects. The likely reason for the relative strength of the marker’s signal in the A. transitella larvae is that the mass of enriched material consumed by the larvae was a larger proportion of their total biomass. However, these larvae did appear to avoid the bean material to a degree. Using the 2:3 ratio of bean material to non-bean material in the enriched diet, and assuming random feeding by the larvae, the level of $^{15}$N in the enriched diet (and the larvae feeding on it) would have been approximately 3.20 atom%. Considering that the bean material was added to a pre-made diet, the beans may not have been thoroughly coated with other critical ingredients, such as honey and yeast. As a result, the beans may have been much less palatable to the developing larvae. Since the larvae apparently fed preferentially, the mean $^{15}$N concentration for the enriched diet as a whole was not assessed. It is possible that the larvae did feed on the bean components but did not readily assimilate the constituent amino acids; however, this scenario seems much less probable because current investigations have shown that when the larvae are reared on enriched beans alone, the $^{15}$N atom% of the larvae is virtually equal to the bean $^{15}$N atom% (S.A. Steffan & K.M. Daane, unpubl.).

The signal generated in the A. transitella larvae was readily passed on to the parasitoids feeding on them. The $^{15}$N concentrations in the targeted G. legneri specimens were substantially elevated above the background levels of $^{15}$N in the unenriched G. legneri group. Despite the significant enrichment of the wasps, their $^{15}$N atom% was slightly lower than their hosts. These findings do not entirely agree with those of Ostrom et al. (1997) which demonstrated that the
isotopic ratio of an insect will be a close reflection of the isotopic ratio of its food source. The discrepancy may have resulted from a situation in which the A. transitella hosts had consumed but not yet assimilated enriched food sources. As the parasitoid larvae developed, the host tissues available for consumption were enriched with $^{15}$N to a certain level, but the parasitoid larvae may not have been able to feed on the undigested gut contents of the host. As a result, highly enriched material in the gut of the host may have been included in the isotopic analysis of the host, but not in the analysis of the parasitoids.

As a marker for field populations of G. legneri, highly enriched sentinel hosts should provide a signal strong enough to effectively mark such populations, and the signal should be sustained throughout the lifetime of the parasitoid. The reason for this is that nitrogen is a major constituent of fibrin (the protein accounting for a large part of muscular tissue), chitin (nitrogenous polysaccharides), and the multitude of amino acids necessary for insect life (Borror et al., 1981). It is improbable that $^{15}$N atoms in the insect exoskeleton and musculature would be supplanted by other isotopes of nitrogen as an adult insect sought to reproduce before dying. It is possible, though, that with synovigenic species, the level of $^{15}$N-enrichment might be decreased (diluted) if the insect gained substantial biomass upon feeding on unenriched food sources to develop their reproductive apparatus.

The progeny of the marked parasitoids did not carry a discernable $^{15}$N signal. The lack of a signal in the progeny is explained by our finding that the adult:egg ratio is approximately 84:1. When an enriched egg hatches and the larva begins synthesizing proteins from an unenriched host, the larva’s $^{15}$N concentration is likely to be diluted down toward the natural background abundance of $^{15}$N.

For the purpose of multitrophic or inter-generational insect marking, $^{15}$N-enrichment appears to hold promise. However, there are several significant drawbacks to using $^{15}$N-enrichment for insect marking, the foremost of which appear to be the relatively large sample mass required for analysis, the substantial expense of the enrichment material, and the expense of isotopic analysis.

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References


