

The use of C₃ and C₄ plants to study natural enemy movement and ecology, and its application to pest management

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Abstract. Plants using either the C₃ or the C₄ photosynthetic pathway show a number of characteristic differences, including distinct ratios of the natural carbon isotopes, ¹³C and ¹²C, in their tissues. The carbon isotope ratios are transferred with little distortion to herbivores feeding on plants of either photosynthetic type; this is also reflected at higher trophic levels. This knowledge has been successfully applied to investigations of the ecology of both vertebrate and invertebrate animals, but there has been little application to pest management. However, with both C₃ and C₄ plants being widely grown, carbon isotopes allow for safe, natural, and efficacious marking of arthropods in many agricultural systems. The rationale for, and a brief synopsis of, the use of carbon isotopes is presented for animal ecology in general, followed by a research framework for using C₃ and C₄ plants to study the movement of predators and parasitoids in multiple-crop systems. Lastly, we present recent examples of entomological research on natural enemy movement using carbon isotope ratios.

1. C₃ and C₄ photosynthetic types and carbon isotopes

Carbon fixation is the process by which plants convert light, water and carbon dioxide into organic molecules that store solar energy. Most terrestrial plants are classified as C₃ plants because their photosynthetic pathway, the Calvin cycle, produces three-carbon molecules as initial products in carbon fixation (Calvin, 1962). An alternate pathway of photosynthesis, the Hatch-Slack pathway, has evolved independently on several occasions (Hatch, 1982; Ehleringer and Monson, 1993). Plants using the latter method of photosynthesis initially produce four-carbon molecules, and are consequently referred to as C₄ plants (Hatch and Slack, 1966). These two types of plant show many additional differences including leaf anatomy, water use efficiency, and distribution (Laetsch, 1974; Ehleringer *et al.*, 1997). As a generalization, most C₃ plants are dicotyledons that are most successful in temperate environments, while C₄ plants are monocotyledons with predominantly tropical or subtropical distributions.

Another scientifically useful difference between C₃ and C₄ plants is the relative abundances of two isotopes of carbon, ¹³C and ¹²C, in the tissues of each plant type. During photosynthesis, all plants discriminate among carbon isotopes in favour of ¹²C, which results in plant tissues containing less ¹³C than the atmosphere. However, the ¹³C levels of C₄ plant tissues are distinctly higher than those of C₃ plants. Carbon isotope ratios

(¹³C/¹²C) are expressed as the parts per thousand (‰) change ($\delta^{13}\text{C}$) relative to an analytical standard; values of C₃ plants typically range from –9 to –19‰, while C₄ plants show ratios between –22 and –35‰ (O’Leary, 1988). The bodies of herbivores feeding on C₃ plants, C₄ plants, or a mix of both types, reflect the overall isotopic composition of their food, and the bodies of predators or parasitoids consuming these herbivores will also reflect the isotopic concentration of their prey’s diet (DeNiro and Epstein, 1978; Petelle *et al.*, 1979; Ostrom *et al.*, 1997; Oelbermann and Scheu, 2002). The transfer of carbon isotope values is not exact: in general there is a small enrichment of ¹³C ($\cong 0.8\%$) in an animal relative to its diet, whether the diet is plant or animal-based. This increase in the concentration of ¹³C in body tissues is balanced by a slight decrease in levels of ¹³C in carbon dioxide respired by an animal (DeNiro and Epstein, 1978). When the food source of an animal changes from C₃ to C₄-based (or vice versa), the isotopic composition of its body will gradually move towards the value associated with the new food source (Ostrom *et al.*, 1997; Prasifka *et al.*, 2003). However, for tissues that grow slowly or not at all (e.g., the exoskeleton of an adult insect), carbon isotope values may not change with recent diet, but reflect the more distant feeding history of an individual (see Tallamy and Pesek, 1996).

2. Uses of carbon isotopes in animal ecology

The consistent relationship between plants of a given photosynthetic type (C₃ or C₄) and the animals dependent upon them has proven to be a valuable tool for biologists. Carbon isotopes, either alone or in combination with other tools, have been used to investigate a broad array of topics including dietary specialization, community ecology and movement. The simplest studies have examined dietary preferences of herbivores. Fry *et al.* (1978a,b) used the stable isotope composition of plants and insects to investigate insect feeding on plants of C₃ or C₄ type, showing that some herbivore species fed within a photosynthetic type, while a few consumed a mixture of C₃ and C₄ plants. Work by Boutton *et al.* (1983) compared the utilization of plant photosynthetic types by termites at two different sites in African grasslands. Termites were shown to concentrate on a vegetation type within a colony, but the use of plant resources varied

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between locations. The mutualism between *Azteca* ants and *Cercropia* trees was explored using carbon isotopes by Sagers *et al.* (2000), who showed that food bodies produced by *Cercropia* provide only 18% of the worker ant resources. Surprisingly, the consumption of other insects appears to account for the majority of food utilized by *Azteca* workers.

The resource utilization or dietary preference of predators can be similarly investigated using carbon isotopes. A study by Des Marais *et al.* (1980) investigated the carbon isotope composition of insectivorous bats in an agricultural area. Based on the isotopic ratios of non-agricultural biomass, crop plants, and bat guano, it was concluded that approximately 50% of the diet of the bats comprised pests of alfalfa and cotton. Magnusson *et al.* (1999) used the isotope ratios of frogs, lizards and birds in an Amazonian savanna determine the contributions of C₃ and C₄ plants to their diets. Results showed intermediate $\delta^{13}\text{C}$ values for the omnivorous and insectivorous vertebrates, suggesting predators utilized several different foods, while most of the herbivorous prey species appeared to concentrate on a single plant type.

As an extension of studies on diet, carbon isotopes have also been used to examine animal movements. In general, research has focused on the movements of vertebrate animals over relatively large scales (Chisholm *et al.*, 1986; Alisauskas *et al.*, 1998; Marra *et al.*, 1998). In these cases, individuals with carbon isotope ratios distinct from those of local plant species are likely to be immigrants. Known habitats within the migratory range of a species are considered as possible sources of these individuals. Among these habitats, only locations where dietary resources have isotope ratios similar to those of immigrant animals are considered likely sources. However, data collected using other techniques are often used to support $\delta^{13}\text{C}$ evidence of movement (see Hobson, 1999). As one exception, Hobson *et al.*, (1999) showed that the origin of monarch butterflies (*Danaus plexippus* [L.]) could be broadly determined using $\delta^{13}\text{C}$ values. While all monarchs fed on the same food source (milkweed, *Asclepias* sp.), temperature and atmospheric pressure differences across North America produced detectable differences in carbon isotope ratios of monarch butterfly adults.

3. Using C₃ and C₄ plants to study natural enemy movement

Although the shifts in carbon isotope ratios transferred between plants, herbivores and natural enemies are consistent, small changes in plant $\delta^{13}\text{C}$ values occur based upon the growing conditions or age. As a result of this inherent variability, carbon isotope ratios should be used to assess movement only in settings where distinct sources of ^{13}C are available (i.e., systems in which both C₃ and C₄ plants are present). Most crops are C₃ plants, but several important C₄ crops are grown for human and animal consumption (table 1), resulting in C₄ crops being grown with extensive non-tropical ranges.

Crop rotations between C₃ (cotton, wheat, soybeans) and C₄ (corn, sorghum) plants provide ideal systems for studying movement and ecology of natural enemies. Where crops of both photosynthetic types are grown concurrently, predator or parasitoid movement between C₃ and C₄ crops is indicated by the $\delta^{13}\text{C}$ values of collected natural enemies. In a system with a corn-soybean rotation, a predator collected in soybeans with very negative (C₄-range) carbon isotope values has most likely

Table 1. Common and scientific names of some important cultivated plants grouped by C₃ or C₄ photosynthetic type

Photosynthesis	Common name	Scientific name
C ₃	Wheat, common	<i>Triticum aestivum</i> L.
	Wheat, rivet	<i>Triticum turgidum</i> L.
	Rice	<i>Oryza sativa</i> L.
	Cotton	<i>Gossypium hirsutum</i> L.
	Potato	<i>Solanum tuberosum</i> L.
	Sunflower	<i>Helianthus annuus</i> L.
	Soybean	<i>Glycine max</i> (L.) Merr.
	Cassava	<i>Manihot esculenta</i> Crantz
	Barley	<i>Hordeum vulgare</i> L.
	C ₄	Corn (maize)
Sorghum		<i>Sorghum bicolor</i> (L.) Moench
Millet, finger		<i>Eleusine coracana</i> (L.) Gaertn.
Millet, pearl		<i>Pennisetum americanum</i> (L.) R. Br.
Sugarcane		<i>Saccharum officinarum</i> L.

Photosynthetic types as indicated by Downton (1971) and Smith (1982).

moved from nearby corn to the point of collection in soybeans. By extension, the analysis of many individuals provides information about the overall population movement in such a system. Other systems that include C₃ crops and one of several C₄ forage or hay species (see Smith, 1982) could similarly be used. However, because isotope values are more accurately indicators of feeding than movement *per se*, this method is most powerful when combined with supporting data. For example, measuring the changes in predator densities over time can buttress the argument that natural enemies are entering or leaving a particular habitat; overall densities should decrease as predators depart from one area, but increase concurrently at locations into which they are immigrating.

There is no single 'best' technique for studying movement in all instances; each has its own unique set of limitations and advantages. The most obvious limitation of using carbon isotopes to study movement is that not all systems of interest involve both C₃ and C₄ habitats. Additionally, the expertise and equipment needed to analyse carbon isotope ratios may make it an unattractive technique for some researchers. However, many research laboratories can perform isotope analyses on a contract basis. Carbon isotopes also have at least two major advantages as an investigative tool on movement. First, the isotope ratio or 'mark' is obtained naturally by feeding. This is contrasted with mark-release-recapture methods, which may alter the behaviour of marked individuals (e.g., agitation dispersal; see Turchin, 1998). Also, some applied marks may not label a sufficient proportion of a population to allow for robust, interpretable recapture data (e.g., Kieckhefer and Olson, 1974), whereas the carbon isotope ratios created by feeding in C₃ and C₄ habitats can mark entire communities over large areas. In this respect, carbon isotopes may perform better than marks that must be applied directly onto predator or parasitoid populations.

4. Research examples with beneficial insects

The theoretical basis for using carbon isotopes ratios of C₃ and C₄ plants as tools in pest management is relatively simple, but this technique has rarely been applied to study movement by either herbivores (but see Gould *et al.*, 2002) or natural enemies. However, recent studies are showing the utility of this method to

study the movement and feeding behaviour of natural enemies in agroecosystems. Work by Ostrom *et al.* (1997) examined the resources used by the ladybird beetle *Coleomegilla maculata lengi* (Timberlake) in a system containing corn (C₄), wheat and alfalfa (C₃). Isotope ratios of beetles leaving crop fields were used to assess the relative contributions of these crops to the beetle's overall diets. Interestingly, beetles collected while leaving alfalfa and wheat fields were found to have obtained most of their previous dietary carbon from corn, although at the time of collection (May) no beetles were collected from corn. This study was not designed to explicitly study movement, but the results clearly indicated movement by *C. maculata*; because these beetles overwinter as adults, the results suggest that ladybird beetles produced in corn the previous year were the colonists for wheat and alfalfa in the subsequent spring.

In another agricultural system with both C₃ (cotton) and C₄ (corn, sorghum) crops, we used carbon isotopes to investigate movement and reproduction of the convergent lady beetle (*Hippodamia convergens* Guérin-Ménéville). Objectives were to determine: (1) the time period over which adult beetles colonize cotton from nearby corn or sorghum fields, and (2) whether corn and sorghum contributed directly to the production of lady beetle larvae in cotton. Adults and egg masses of *H. convergens* were collected from four cotton fields near Taylor, TX (USA) from the first appearance of lady beetle adults to their departure from all cotton fields (10 weeks). All cotton fields shared one border with an adjacent grain sorghum field. Several corn fields were also located near each cotton plot, but corn and selected cotton fields did not always share a common border. Samples of plant leaf material were also collected from all crops.

All samples were processed and analysed according to the methods outlined in Prasifka *et al.* (2003). Adult beetles collected in cotton with isotope ratios closer to the C₄ crops corn and sorghum were interpreted as being recent immigrants into cotton. Since eggs are produced as a result of recent feeding by female *H. convergens*, eggs with isotope values nearer to those of corn and sorghum are assumed to have been produced by recently immigrated females from resources consumed in either corn or sorghum. Small shifts in $\delta^{13}\text{C}$ values occur between trophic levels (i.e., from plant to herbivore, from herbivore to predator), so the threshold isotope ratio denoting a sample as having originated in a C₄ crop would not be the midpoint between the isotope ratios of plant samples, but the midpoint of the expected $\delta^{13}\text{C}$ values of beetles feeding only in cotton or only in sorghum and corn. That is, using the estimated isotope shifts from Prasifka *et al.* (2003), the expected isotope ratio of a beetle feeding solely in cotton should be adjusted by -0.2‰ , while the value for those feeding in either C₄ crop would be increased by 2.2‰ . The midpoint between these expected $\delta^{13}\text{C}$ values was accordingly used as a threshold level to detect recently immigrated adult beetles or the production of eggs by immigrating female *H. convergens*. The use of such a threshold is conservative (i.e., individuals just below the threshold may have moved from corn or sorghum, but are not assessed as immigrants), but this technique acknowledges that uncultivated plants in the area may contribute to cotton predator populations. In particular, other C₃ plants with isotope ratios more positive than the $\delta^{13}\text{C}$ of cotton may act as sources of *H. convergens*. As an alternative method, mass-balance equations may be used to give more precise estimates of the contributions of C₃ and C₄

plants when only two or three plant species may act as sources of the predator or parasitoid species of interest (see Ostrom *et al.*, 1997). For each sample date, the proportion of all adult beetles or eggs attributed to nearby C₄ crops was calculated to indicate overall population-level trends. To support carbon isotope results, data on the field densities of adults and eggs of *H. convergens* in cotton were also collected.

The results indicated that mean isotope ratios ($n=4$ fields per crop; \pm SE) of corn ($-12.38 \pm 0.38\text{‰}$) and sorghum ($-12.71 \pm 0.27\text{‰}$) were relatively indistinct, thus emigration from either C₄ crop and into cotton ($\delta^{13}\text{C} = -27.74 \pm 0.66\text{‰}$) could not be effectively differentiated between corn and sorghum. Based on the isotope ratios of the crop samples and expected shifts between trophic levels (from Prasifka *et al.*, 2003), *H. convergens* adults were considered to have been recent immigrants into cotton from nearby C₄ crops if their $\delta^{13}\text{C}$ values were above (i.e., less negative than) -19.23‰ . The isotope ratios of adult beetles ($n=511$ individuals) suggested a period of intense immigration into cotton by *H. convergens* that coincided with overall increases in the density of adult beetles (figure 1), with 23% of all beetles collected above the threshold level. While nearby corn and sorghum apparently provided some of the very first colonists to area cotton fields, the movement of beetles from corn or grain sorghum fields appeared to continue for 5–6 weeks, suggesting a relatively long period of adult *H. convergens* movement from the nearby C₄ crops into area cotton fields.

H. convergens egg masses ($n=41$) were found only over a 6 week period that started with the first appearance of *H. convergens* adults in cotton. Using the same threshold level (-19.23‰), egg masses produced from feeding in C₄ crops were found on half of the dates on which eggs were collected (figure 2). Overall 24% of egg masses collected were assessed as produced by resources (consumed by the female parent) in surrounding corn and sorghum fields. While this amount is only quarter of the total egg production in cotton, the contributions of nearby corn and sorghum fields clearly extended beyond immigration of *H. convergens* adults, directly to the production of the subsequent generation of ladybird beetles.

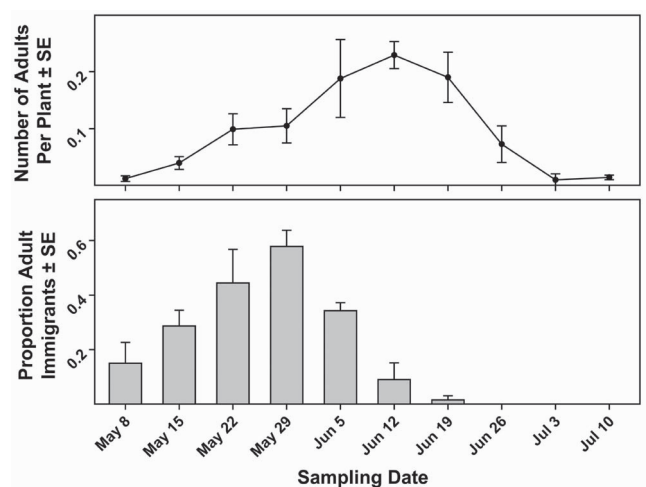


Figure 1. Mean proportion (\pm SE) of recent immigrants and mean density of *H. convergens* adults in cotton. Errors are based on each of four individual fields as one replicate (i.e., error bars represent between-site variation).

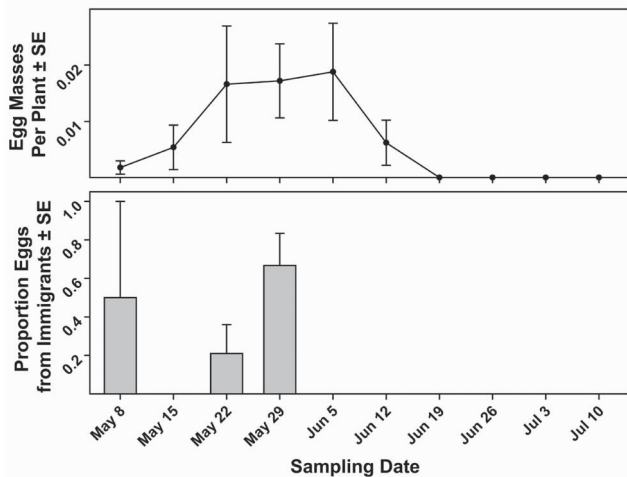


Figure 2. Mean proportion (\pm SE) of egg masses produced by immigrating *H. convergens* females and mean egg mass density in cotton. Errors are based on each of four individual fields as one replicate (i.e., error bars represent between-site variation).

5. Conclusions

The carbon isotope ratios of plants with distinct photosynthetic mechanisms have been used to study movement and behaviour of animals for decades, but little use of this tool has been applied to pest management. As a marking mechanism, carbon isotopes allow for safe, natural, and efficacious marking of arthropods in many agricultural systems. Stable isotopes have been used to determine the larval host-use by polyphagous agricultural pests (Tallamy and Pesek, 1996; Gould *et al.*, 2002), and this application may be equally useful in helping to reveal unknown parasitoid-host associations. Knowing the habitats that are most valuable as sources of predators and parasitoids could also be important in efforts to increase natural enemy numbers through conservation biological control. A relatively small number of studies indicates the usefulness of this technique to track insect movements and study natural enemy ecology, but as use of carbon isotopes in answering applied questions becomes more common, perhaps the unique advantages of this method will become clearer.

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