glands can serve as important morphological markers, indicating thereby the relative extent of hypericin present in a strain, without the need of chemical estimation. These dark-coloured glands can also serve as important breeding tools, as strains with more number of glands per petal/leaf can be isolated with consequent higher hypericin content. Strains developed on the basis of higher number and size of glands can be easily segregated from the rest, especially in any cultivation or breeding programme.

As is known in the literature[4,12–14] and also confirmed by the authors, flowers of *H. perforatum* contain much higher hypericin content (approximately 1%) compared to leaves (approximately 0.2%). The higher hypericin content in flowers is probably due to the presence of much larger number of glands in petals compared to leaves.

1. Newall, C. A., Anderson, L. A. and Phillipson, J. D., In *Herbal Medicine Guide for Health Care Professionals*, Pharmaceutical Press, London, 1996, pp. 250–252.
2. Dewick, P. M., In *Medicinal Natural Products; Biosynthetic Approach*, John Wiley, Nottingham, 2001, 2nd edn, pp. 65–67.
3. Grieve, M., In *Modern Herbal: Medicinal, Culinary and Cosmetic Properties*, Tiger Book International, London, 1992, pp. 707–708.
4. Laakman, G., Schule, C., Bagahi, T. and Kieser, M., *Pharmacopsychiat*, 1998, **31**, 54.
5. Fernie, W. T., In *Herbal Samples*, John Wright and Company, Bristol, 1897, vol. 20, pp. 50–52.
6. Vickery, A. R., *Econ. Bot.*, 1981, **35**, 289–295.
7. Sirvent, T. M., Walker, L., Vance, N. and Gibson, D. M., *Econ. Bot.*, 2002, **56**, 48–58.
8. Collett, H., In *Flora Simlensis: Handbook of Flowering Plants of Shimla and the Neighbourhood*, W. Thacker and Co. London, 1971, 3rd edn, p. 56.
9. Grunwald, J., *Nutraceuticals World*, 1999, 22–25.
10. Hooker, J. D., In *Flora of British India*, L. Reeve and Co Ltd, Kent, 1875, vol. I, pp. 252–257.
11. Kirtikar, K. R. and Basu, B. D., In *Indian Medicinal Plants*, M/s Periodical Experts, Delhi 1935, vol. I, 2nd edn, pp. 255–257.
12. Seidler, I., Ozykowaska, K., Dabrowska, J. and Zygmint, R., *Herba Pol.*, 1999, **45**, 169–172.
13. Kitanov, G., *Acta Pharm.*, 2000, **50**, 65–68.
14. Kartnig, T., Heydel, B. and Lasser, I., *Agrarforschung*, 1997, **4**, 299–302.

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Identifying the dietary source of polyphagous *Helicoverpa armigera* (Hübner) using carbon isotope signatures

An animal with unlimited choice of food has an advantage over the one feeding on scarce resource, which is a logical benefit accruing to generalists. Evolutionarily, an insect that can feed on many host plants – polyphagous – enjoys better chance of survival in nature. On the other hand, monophagous insects have evolved superior abilities to search for the host plants of their preference. *Helicoverpa armigera* (Hübner), a highly dreaded pest of several agricultural crops, is highly polyphagous, feeding on more than 170 species of plants belonging to 41 families[1]. However, the fitness of *H. armigera* population depends upon the host plant. This means though the insect is polyphagous, it may develop preference to a particular host, which however is not rigid[2]. Thus, it is believed that *H. armigera* feeding on different hosts would develop into specific biotypes. Biotypes are more commonly distinguished by survival and development on a specific host or by developing feeding preference, oviposition[3].

There seems to be no equivocal agreement on the concept of the development of host biotypes in *H. armigera* populations. 'Mark–release–recapture' technique is widely used for demonstration of feeding behaviour of an insect[4]. This technique involves marking the adult moths, releasing them in a particular crop patch, subsequently tracking and capturing of them on different hosts as an indication for their host acceptance. The marked moths from crop-A, if captured on crop-B might suggest host shift to crop-B. However, this settlement of the insect on crop-B could simply be an accidental landing of the insect. Further, mere landing of the moths on crop-B need not be followed by settlement and host utilization. On the other hand, observing for the oviposition of the marked moths on a different host plant could clearly indicate the development of biotypes. This would be impossible unless an accurate signature of the moths and their eggs is available.

In order to precisely understand the feeding behaviour of an insect, determining what the insect has actually eaten will be the best evidence for host utilization. Because of the similarity of the organic molecules of any host plant, this would initially seem like an impossibility. Stable isotope signatures of animal tissues can be used to study the trophic ecology[5], nutritional status[6] and geographic origin of animals[7]. This has led to an explosive burst of research in a new frontier in animal ecology. The carbon isotopic composition of an animal has been shown to accurately reflect its diet[8,9] and hence can provide useful leads to the identification of dietary sources[10,11].

During the photosynthetic conversion of carbon from inorganic to the organic form, plants prefer the lighter isotope of

carbon (^{12}C) to the heavier one (^{13}C). This isotope fractionation ($\delta^{13}C$) renders the organic molecules significantly depleted in ^{13}C compared to the atmospheric air. Several physical and biochemical processes of carbon assimilation, including diffusion[12,13], dissolution[14] and carboxylation[15] result in this isotope discrimination. Following the discovery of C_4 photosynthesis, several workers discovered that C_4 plants are isotopically distinct from C_3 plants[16,17]. C_3 plants have a $\delta^{13}C$ value around –28‰, while C_4 plants have a value of approximately –14‰. In subsequent years, a number of laboratories around the world made similar measurements on thousands of plant species and established a clear distinction between C_3 and C_4 species[18]. Therefore, $\delta^{13}C$ has become a standard test for the identification of biochemical origin of the organic compounds. The carbon isotope signatures have been successfully used to infer on the feeding behaviour of animals[10,11,19,20]. Such studies however, have not been attempted for determining the feeding habits of insects.

In this study, we provide a carbon isotope-based evidence for determination of probable hosts on which *H. armigera* could have fed as a larva. Initially a controlled experiment was conducted under laboratory conditions where the neonates were fed till pupation on different hosts such as tomato, bendi, chickpea, maize and a chickpea-based artificial diet. A set of pupae was collected from a nearby bendi field along with fruit samples from the same field. In another experiment, insects were reared on bendi till the end of the second instar and subsequently shifted to chickpea-based artificial diet till pupation. The isotopic signatures of the pupae and food material on which the insect had fed were analysed using the Isotope Ratio Mass Spectrometer (Delta plus, Thermo-Finnigan, Bremen, Germany) interfaced with an elemental analyser (CN1112, Carlo Erba, Italy) through a continuous flow device (ConfloIII, Thermo-Finnigan Bremen, Germany). Isotopic analysis was carried out with an analytical uncertainty of less than 0.1‰ and was measured at the Department of Crop Physiology, University of Agricultural Sciences, Bangalore.

The $\delta^{13}C$ differed significantly among the several host plant species with maize, a C_4 species having the lowest $\delta^{13}C$. Among C_3 host species, bendi had the lowest $\delta^{13}C$ compared to others (Table 1). These differences would hence form a good reference point for determination of feeding behaviour of insects. Accordingly, a significant positive relationship between pupal $\delta^{13}C$ and host plant $\delta^{13}C$ was noticed (Figure 1), suggesting that the insects acquire isotopic signatures from what they feed on.

To more conclusively assess this aspect, *H. armigera* larvae were fed on bendi up to the end of 2nd instar and then shifted to a chickpea-based diet till pupation with agar as one of the important components. Agar, a seaweed, acquires its carbon isotopic signature from the dissolved inorganic carbon in its aquatic conditions and hence had a highly enriched $\delta^{13}C$ and the chickpea-based diet was less negative (–18.43‰). The organic matter of the pupae should consist of the carbon the larvae acquired from bendi as well as from the diet. The results illustrated in Figure 2 indicate that $\delta^{13}C$ of the pupae was intermediate between that of bendi and the diet.

Table 1. Differences in carbon isotope composition ($\delta^{13}C$) in host and pupae of *H. armigera*

Host/diet	$\delta^{13}C$ of host	$\delta^{13}C$ of pupa
Bendi – lab experiment	–23.43 ± 0.19	–22.75 ± 0.09
Maize	–15.58 ± 0.10	–15.52 ± 0.10
Chickpea	–26.83 ± 0.15	–26.89 ± 0.09
Tomato	–27.56 ± 0.12	–27.93 ± 0.15
Bendi – field collection*	–23.30 ± 0.17	–22.36 ± 0.16

*Pupae collected from a bendi field were analysed *vis-à-vis* the host carbon isotope values.

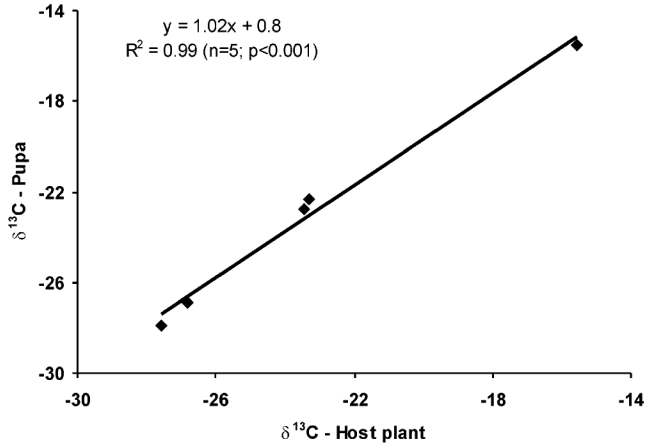

Figure 1. Relationship between isotopic composition ($\delta^{13}C$, ‰) of host and pupae of *H. armigera*.

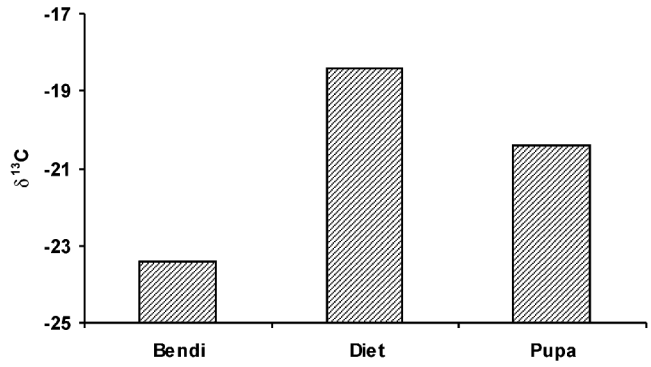

Figure 2. Variation in carbon isotope composition (‰) of host and pupa of *H. armigera* fed on bendi and shifted to the diet after 2nd instar.

Further, $\delta^{13}C$ of the pupae of *H. armigera* collected from a bendi field was determined *vis-à-vis* host plant samples. We noticed that the insect $\delta^{13}C$ closely matched that of the host plant (Table 1), reiterating that the carbon isotope signatures indicate the feeding habits of insects.

The results suggest that the carbon isotopic signature of the insect accurately reflects the host organic carbon source and thus would provide vital clues on host plant on which the moth has fed as a larva. Though this technique would be extremely powerful in identifying the insects that fed on a C_3 or a C_4 host[21], it may not be as sensitive to differentiate among C_3 host species. To resolve the feeding behaviour of the insects on various C_3 hosts plants, we propose that nitrogen isotope ratios could provide the required distinction. The dietary nitrogen isotope signatures are also known to be preserved among the animals feeding on plants[22]. Symbiotically, nitrogen fixing C_3 leguminous species are known to have significant differences in nitrogen isotope ratios compared to that of non-leguminous species[23]. Hence nitrogen isotope composition ($\delta^{15}N$) can be used to distinguish the nitrogen fixing C_3 species from the non-N-fixing C_3 plants. The leguminous plant that fixes atmospheric N_2 would have a $\delta^{15}N$ close to that of atmospheric nitrogen, while a non-leguminous C_3 plant acquiring its nitrogen nutrition from inorganic fertilizers, would significantly differ from that of the nitrogen-fixing plants[24].

Furthermore, since isotope signatures of the larva (and hence the moth) should also be reflected in their eggs, determining the isotopic composition of the eggs can be used to assess if there has been any host shift among insects. Variations in the isotopic signatures of the eggs and the host plant on which they have been laid can conclusively prove the existence or otherwise, of the host biotypes in *H. armigera* populations.

In view of the highly encouraging trends shown by our initial experiments, more closely controlled experiments are in progress to validate this concept.

1. Manjunath, T. M., Bhatnagar, V. S., Pawar, C. S. and Sithanantham, S., Proceedings of the Workshop on Biological control of *Heliothes* sp., 1985, pp. 11–15.
2. Tulsi Jyothi, B., M Sc (Agric.) thesis, Univ. Agric. Sci. Bangalore, 1991.
3. Diehl, S. R. and Bush, G. L., *Annu. Rev. Entomol.*, 1984, **29**, 471–504.
4. Charles-Krebs, J., *Ecological Methodology*, Harper Collins, New York, USA, 1989.
5. Romanek, C. S., Gaines, K. J., Bryan Jr A. N. and Brisbin Jr I. L., *Oecologia*, 2000, **125**, 584–594.
6. Kurle, K. M and Worthy, G. A. J., *Oecologia*, 2001, **126**, 254–265.
7. Rubenstein, D. R., Chamberlain, C. P., Holmes, R. T., Ayers, M. P., Waldbauer, J. R., Graves, G. R. and Tuross, N. C., *Science*, 2002, **295**, 1062–1065.
8. DeNiro, M. J. and Epstein, S., *Geol. Soc. Am. Abstr. Prog.*, 1976, **8**, 834–835.
9. DeNiro, M. J. and Epstein, S., *Geochim. Cosmochim. Acta*, 1978, **42**, 495–506.
10. Sukumar, R. and Ramesh, R., *Oecologia*, 1992, **91**, 536–539.
11. Sukumar, R., Bhattacharya, S. K. and Krishnamurthy, R. V., *Curr. Sci.*, 1987, **56**, 11–14.
12. O'Leary, M. H., *Phytochemistry*, 1981, **20**, 553–567.
13. O'Leary, M. H., *J. Physiol. Chem.*, 1984, **88**, 823–825.
14. Mook, W. G., Bommerson, J. C. and Staverman, W. H., *Earth Planet. Sci. Lett.*, 1974, **22**, 169–175.
15. Roeske, C. A. and O'Leary, M. H., *Biochemistry*, 1984, **23**, 6275–6284.
16. Bender, M. M., *Radiocarbon*, 1968, **10**, 468–472.
17. Smith, B. N. and Epstein, S., *Plant Physiol.*, 1971, **47**, 380–384.
18. O'Leary, M. H., *BioScience*, 1988, **38**, 328–336.
19. Odendaal, T. A. F., Thorp, J. A. L. and Anasuya, C., *Paleobiology*, 2002, **28**, 378–388.999.
20. Pate, F. D., *J. Archaeol. Method Theory*, 1994, **1**, 161–209.
21. Gould, F., Blair, N., Reid, M, Rennie, T. L., Lopez, J. and Micinski, S., *Proc. Natl. Acad. Sci. USA*, 2002, **99**, 16581–16586.
22. DeNiro, M. J. and Epstein, S., *Geochim. Cosmochim. Acta*, 1981, **45**, 341–351.
23. Wanek, W. and Arndt, S. K., *J. Expt. Bot.*, 2002, **53**, 1109–1118.
24. Shearer, G. B., Kohl, D. H. and Commoner, B., *Soil Sci.*, 1974, **118**, 308–316.

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