Gut sugar analysis in field-caught parasitoids: adapting methods originally developed for biting flies

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Abstract. The ability to determine the presence and identity of sugars in the guts of adult parasitoids in the field would aid researchers in addressing long-standing problems in parasitoid ecology. Until very recently, however, gut sugar analyses have not been carried out on parasitoids. This is despite the development and use of methodologies for gut sugar analyses in biting flies (mosquitoes, sand-flies, black-flies, horse- and deer-flies, and biting midges) for decades. Methods used have been the cold anthrone test for the detection of gut sugars, and various forms of chromatography for the identification of gut sugars. We review the use of these methods in biting fly research and then describe the nascent field of gut sugar analyses in parasitoids. Both cold anthrone and chromatography tests have begun to be used on field-caught parasitoids, and we describe progress from our own work. We used cold anthrone on the aphid parasitoid Aphelinus albipodus (Hymenoptera: Aphelinidae), and results from one field study show that approximately one-fifth of individuals tested were positive for gut sugars. The characteristics of the field site point to the primary source of these gut sugars as being aphid honeydew. We also analysed the gut contents of Diadegma insulare (Hymenoptera: Ichneumonidae), a parasitoid of diamondback moth. In this case, HPLC analyses showed that over 85% of field-captured individuals had fed upon sugars. These same analyses suggested that honeydew may have been a major source of the gut sugars in this case also, but the sugar profiles suggest some nectar feeding. Understanding the importance of various sugar sources on parasitoid activity and effectiveness will facilitate the incorporation of sugar sources in habitat manipulation programmes as a part of IPM.

1. Introduction

Jervis et al. (1992) reviewed methods by which sugar meals could in principle be detected and identified within insect parasitoids. At that time, these methods had only been applied to biting flies and (to a much lesser extent) ants (Skinner, 1980) and true fruit-flies (Chang et al., 1977). Within the last few years, however, researchers have begun adapting the methods originally pioneered by van Handel (1972, 1985) for mosquitoes and Lewis and Domoney (1966) for sand-flies and black-flies, to parasitoid wasps. These techniques hold great promise for addressing long-standing questions in parasitoid field ecology, including the role of sugar-feeding in the suppression of pest insects by parasitoids (Heimpel and Jervis, 2004) and effects on the realized lifetime reproductive success of parasitoids in the field (Heimpel et al., 1998; Casas, 2000). We begin this article by reviewing the use of gut sugar detection in flies (Diptera), focusing on the methods used as well as the insights gained from applying these methodologies to field-caught flies. Next, we discuss the transfer of these technologies to parasitoids, focusing on examples from our own work.

2. Pioneering work with haematophagous flies

The pioneer of gut and hemolymph sugar detection in insects was Emile van Handel, who developed a series of simple biochemical assays that relied on the color sensitivity of an organic reagent, anthrone (9(10H)-Anthracenone; C_{14}H_{12}O), in the presence of various sugars. It had been known to chemists for decades that solutions of anthrone and sulfuric acid turn from yellow to blue/green when they come into contact with most sugars (Morris, 1948; Seifter et al., 1950; Scott and Melvin, 1953). Importantly, the specific sugars that induced the colour change varied with temperature. In particular, the monosaccharide fructose (either alone or as a moiety of other sugars including sucrose) reacts with anthrone at room temperature within 1 h, whereas a number of other sugars require either a brief incubation at 90 °C or incubation at room temperature of at least 12 h (van Handel, 1967). Van Handel recognized the importance of this difference for studies of insect sugar feeding: fructose and sucrose were not known from insect hemolymph, so it follows that a positive reaction from an insect using the ‘cold anthrone test’ (i.e., the anthrone test run at room temperature for less than 12 h) is a demonstration that one or both of these sugars are present in the insect gut. He easily showed that newly-emerged or starved mosquitoes yield negative cold anthrone tests, and that sugar-fed mosquitoes test positive (van Handel, 1972; see also Smith and Kurtz, 1994). With these laboratory tests, he showed that it should be possible to use the cold anthrone test to determine whether field-caught mosquitoes had fed upon sugar. This was a large step forward despite the fact that the cold anthrone test cannot distinguish between the source of sugar (i.e., floral nectar, extrafloral nectar, homopteran honeydew, fruit juices).

Since van Handel’s laboratory studies in the 1960s and 70s, the cold anthrone test has been used to assess sugar-feeding...

For females of some species of biting flies, the prevalence of sugar feeding changes as the reproductive cycle progresses. For instance, in one black-fly species, females carrying few mature eggs were more likely to contain gut sugars than females with many mature eggs, possibly because reproductively mature females have a reduced tendency to forage for food (Walsh and Garms, 1980). In some mosquitoes and biting midges, on the other hand, females with mature eggs were more likely to contain gut sugars than females without mature eggs (Magnarelli, 1980; Mullens, 1985). In this case, the explanation may be that females with fully developed eggs have a higher requirement for energy inputs (and therefore engage in more sugar feeding) due to oviposition, or oocyte production, of reserves carried over from the larval stage. In some tabanids, there is evidence that sugar feeding is especially prevalent after oviposition (Leprince and Lewis, 1986; Leprince, 1989). In most of the haematophagous fly species studied, however, ovarian condition in the field has no bearing on the presence of gut sugars (Magnarelli, 1978, 1981; Cupp and Collins, 1979; Brenner and Cupp, 1980; Magnarelli and Anderson, 1981; Nasci and Edman, 1984; Reisen et al., 1986; Andersson and Jaenson, 1987). This is consistent with the frequent taking of sugar meals throughout the course of the life of the insect (Yuval, 1992). Andersson and Jaenson (1987) provided data suggesting that Culex pipiens females engaged in sugar feeding just before seeking blood meals.

Strong diurnal patterns in the amount of gut sugars have been detected in the males of two mosquito species. In Culex tarsalis and Anopheles freeborni, gut sugar levels are very low during swarming at dusk, with sugar feeding occurring primarily at night, after swarming (Reisen et al., 1986; Yuval et al., 1994). Andersson and Jaenson (1987) also noted nectar feeding by males of C. pipiens throughout the night. In the mosquito Psorophora ferox, however, 90% of swarming males tested positive for gut sugars (Magnarelli, 1980).

Since fructose is present in most, if not all, natural sugar sources (floral and extraloral nectar, homopteran honeydew, fruit juices), the cold anthrone test cannot be used to identify the source of gut sugars (Jervis et al., 1992; Heimpel and Jervis, 2003). Various forms of chromatography, however, can be brought to bear on this problem. Honeydews contain a wide variety of signature sugars that are not present in nectars and that are, in many cases, specific to particular groups of homopterans (Wäckers, 2001; Heimpel and Jervis, 2004). Signature sugars can be detected using HPLC (high performance liquid chromatography), GC (gas chromatography) and TLC (thin layer chromatography). Once again, hematophagous flies have been at the forefront of applications of these methods to characterize sugar-feeding habits of insects in the field. Chromatography has been used to identify gut sugars other than simple nectar sugars (sucrose, fructose and glucose) in field-caught sand-flies (Lewis and Domoney, 1966; Moore et al., 1987; MacVicker et al., 1990; Wallbanks et al., 1991), mosquitoes (Scheafer and Miura, 1972; Burkett et al., 1998, 1999; Russell and Hunter, 2002), black flies (Lewis and Domoney, 1966; Burgin and Hunter, 1997a,b,c), and tabanids (Magnarelli and Anderson, 1981; Hunter and Ossowski, 1999; Janzen and Hunter, 1998; Ossowski and Hunter, 2000). Collectively, these studies have demonstrated a great deal of honeydew-feeding, with substantial fractions of all species testing positive for honeydew sugars such as melezitose, erlose, stachyose, or maltose (in many cases, numerous honeydew sugars are found). Nectar feeding cannot be ruled out in many of these cases, however, because fructose and sucrose, which are found in both nectar and honeydew and are breakdown products of honeydew sugars, are invariably found in field-caught flies also. The most common pattern is that some flies test positive only for the nectar sugars fructose, sucrose and glucose, and some test positive for these sugars in addition to honeydew sugars. The most likely explanation for this pattern is that the former group of flies has fed only upon nectar and the latter group has fed upon honeydew, and possibly nectar also. In one study of sand-flies collected in various regions of Italy however, each individual tested positive for melezitose, maltose and an unidentified sugar that may have been erlose (MacVicker et al., 1990). In this case, honeydew may have been the primary (or even sole) source of gut sugars. Conversely, in some mosquito species, less than 10% of individuals testing positive for gut sugars contained honeydew sugars (Burkett et al., 1999; Russell and Hunter, 2002).

3. Technology transfer to parasitoids

3.1. Anthrone tests

The use of sugar assays to analyse parasitoid gut contents began very recently with a laboratory study of the braconid wasp Macrocentrus grandii (Olson et al., 2000). The usefulness of van Handel’s cold anthrone test as a means of identifying the presence of fructose in the gut was confirmed for M. grandii, as have laboratory studies on Diadegma insulare (Ichneumonidae), Cotesia glomerata (Braconidae), Pteromalus puparum (Pteromalidae) (Lee et al., 2004; Lee and Heimpel, in press), Trichogramma ostriniae (Heimpel, unpublished) and Aphelinus alipodus (Aphelinidae) (see below). In an extension of the work done by Olson et al. (2000) Fadamiro and Heimpel (2001) demonstrated that sucrose meals are detectable for up to 3 days post-feeding in M. grandii adults in the laboratory. The first published accounts of cold anthrone tests being used on field-caught parasitoid wasps can be found in Casas et al. (2003) and Lee and Heimpel (2003). Lee and Heimpel’s (2003) study was done in cabbage fields supplemented with flowering buckwheat (Fagopyrum esculentum) and of over 400 (unidentified) parasitoid wasps captured, more than half tested positive for gut sugars. Heimpel and Jervis (2004) review as-yet unpublished results of cold anthrone tests done on eight species.
of parasitoids. The prevalence of field captured individuals that were cold-anthrone-positive ranged widely between less than 20% for *M. grandii*, *T. ostriniae* and *Aphytis aonidiae* (Aphelinidae) to greater than 70% for *C. glomerata* and *C. rubecula*.

We recently conducted cold anthrone tests on the aphid parasitoid *Aphelinus albipodus*, following a release of this species against the soybean aphid, *Aphis glycines*, in Minnesota (Heimpel et al., 2004). Like other aphelinids, *Aphelinus albipodus* is very small, measuring approximately 1 mm in length. We modified van Handel’s (1972) cold anthrone test by placing an individual *A. albipodus* into a 5 µl droplet of anthrone solution on a microscope slide and gently squashing it with a cover slip to rupture the gut and expose the gut contents to the anthrone solution. After 1 h at room temperature, preparations of *A. albipodus* that had fed upon either honey or soybean aphid honeydew produced a green halo around the parasitoid body, while control (starved) wasps did not produce such a halo (table 1). We conducted the same anthrone squash on 378 field-caught *A. albipodus* after a mass-release into a soybean field, and the overall proportion of parasitoids that were cold-anthrone positive was 21%. Parasitoids not testing positive for gut sugars could either have never fed, or they may have digested a sugar meal.

The prevalence of gut sugars increased over the course of the day (table 2), suggesting that gut sugars may have been digested overnight. There were no flowering plants within the soybean field and all recaptures of *A. albipodus* were made within 20 m of the release site, far from any flowering plants at the edge of the field. We therefore assume that the primary source of the gut sugars in the recaptured parasitoids must have been soybean aphid honeydew. Surprisingly though, there was no relationship between aphid density and the proportion of *A. albipodus* that tested positive for gut sugars (regression of fraction testing positive on aphids/plant; $r^2 < 0.03; p > 0.15$; range of aphid densities: 3 – 207 per plant).

### 3.2. Chromatography

HPLC methods to identify parasitoid gut sugars have been developed by Wäckers and Steppuhn (2003) for *C. glomerata* and *Microplitis mediator*, both of which attack lepidopteran cabbage pests. Over 80% of field-caught *C. glomerata* tested positive for gut sugars, and a higher fraction of individuals collected from a field with an adjacent flowering margin tested positive for gut sugars than from a control field without a flowering margin. Despite this, a majority of the captured parasitoids (both *C. glomerata* and *M. mediator*) contained honeydew sugars. A minority of parasitoids contained only nectar sugars (fructose, glucose and sucrose) and these were only found in the field with the flowering margin. In addition, Wäckers and Steppuhn found that honeydew from the cabbage aphid, *Brevicoryne brassicae* and the cabbage whitefly, *Aleyrodes proletella* had distinct sugar profiles which can be useful for determining the specific honeydew sources the parasitoids had fed on.

We recently characterized the sugar profile of soybean aphid honeydew using HPLC. Soybean aphids are becoming common in the midwestern USA, and numerous parasitoid species may encounter their honeydew in the field (Heimpel et al., 2004). Soybean aphid honeydew contained ‘signature’ sugars such as maltose and erlose (table 3; figure 1A). We used HPLC to determine sugar profiles of 15 *D. insulare* wasps collected from cabbage plots surrounded by soybean that either had borders of flowering buckwheat or not. Four classes of sugar profiles were evident among the field-collected *D. insulare* (table 4; figure 1B). Two wasps had only trace amounts of the gut-specific sugars fructose and sucrose (less than 0.6 ppm) indicating that they probably had not fed recently. Thirteen had fructose, from 2.6 to 55 ppm, and sometimes sucrose, and were considered to have fed relatively recently. Of these fed wasps, three had substantial levels of maltose (maltose is present in newly emerged *D. insulare*) and five had maltose and erlose, which suggests the possibility that they had fed on either soybean aphid honeydew or honeydew produced by a different homopteran species. This method does not distinguish between wasps that only fed on honeydew and wasps that fed on honeydew and nectar. Five wasps contained only fructose and sucrose, which suggests that they fed upon floral nectar only. Four of the five nectar-fed wasps were found in plots with flowering buckwheat. Further analyses are being conducted to determine how quickly *D. insulare* degrades honeydew and nectar sugars since this affects our estimation of nectar- and honeydew-fed wasps. These preliminary results are similar to those of Wäckers and Steppuhn (2003), in that feeding on honeydew appeared to be common despite the availability of floral nectar. With additional analyses, we will determine if wasps near flowering plants feed more in general and how often they feed only on nectar sugars.

The methods outlined here can help determine what sugar sources parasitoid wasps have fed on. Furthermore, the methods outlined here can help determine what sugar sources parasitoid wasps have fed on.
Table 3. Sugar profile of soybean aphid honeydew, as determined by HPLC

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Percent of samples testing positive</th>
<th>Mean ± S.E. of samples testing positive (ppm)</th>
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<tbody>
<tr>
<td>Sucrose</td>
<td>100% 5/5</td>
<td>22.6 ± 3.04</td>
</tr>
<tr>
<td>Glucose</td>
<td>100% 5/5</td>
<td>22.4 ± 4.5</td>
</tr>
<tr>
<td>Fructose</td>
<td>100% 5/5</td>
<td>19.9 ± 2</td>
</tr>
<tr>
<td>Erlose</td>
<td>100% 5/5</td>
<td>20.5 ± 4.77</td>
</tr>
<tr>
<td>Maltose</td>
<td>100% 5/5</td>
<td>5.2 ± 1.05</td>
</tr>
<tr>
<td>Trehalose</td>
<td>100% 5/5</td>
<td>2.6 ± 2.14</td>
</tr>
<tr>
<td>Manitol</td>
<td>100% 5/5</td>
<td>0.39 ± 0.03</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>100% 5/5</td>
<td>0.05 ± 0.01</td>
</tr>
<tr>
<td>Melizitose</td>
<td>20% 1/5</td>
<td>0.02</td>
</tr>
<tr>
<td>Melibiose</td>
<td>0% 0/5</td>
<td></td>
</tr>
<tr>
<td>Raffinose</td>
<td>0% 0/5</td>
<td></td>
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</tbody>
</table>

Table 4. Numbers of field-caught D. insulare exhibiting various HPLC sugar profiles

<table>
<thead>
<tr>
<th>Sugar profile</th>
<th>Plots with buckwheat</th>
<th>Plots without buckwheat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trace fructose and sucrose</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Fructose, sometimes sucrose (F, ~S)</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>(F, ~S) + maltose</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>(F, ~S) + maltose + erlose</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

Figure 1. Representative HPLC chromatograms from samples of soybean aphid honeydew (A) and an individual field-captured D. insulare (B).
with field experiments, we can determine the frequency with which parasitoids use these sugar sources, and whether the sugar improves their fecundity, foraging behaviour and attack rate on pests. Understanding the relevance of various sugar sources and the effects they have on parasitoid behaviour will allow IPM practitioners to design more beneficial habitats for parasitoids. Currently, the challenge remains to develop methods that differentiate between exclusive honeydew feeding and feeding from both honeydew and nectar sources, and to identify feeding from specific flowering plant species.

Acknowledgements

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