SHORT COMMUNICATION

A simple gravimetric technique for estimating honeydew or nectar production

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Abstract: We describe a simple gravimetric technique for measuring the standing crop or production of carbohydrate-rich solutions such as honeydew or nectar. Simulated honeydew was sampled by absorbing droplets of solutions of known concentration and volume with dried and weighed pieces of filter paper. The change in mass of the paper after redrying provides an estimate of the total solution carbohydrates. This method was compared with a widely-used technique, whereby the volume and concentration of droplets is measured with microcapillary tubes and a sugar refractometer. A factor was derived to convert gravimetric refractometer readings (g sucrose 100 g^{-1} solution) to volumetric carbohydrate concentration (g carbohydrate 100 ml^{-1} solution) for the simulated honeydew solutions. There was no difference in the ratio of measured-to-expected carbohydrate mass between the two techniques, showing that the quick, easy, and accurate filter-paper method is appropriate for measuring carbohydrate-rich solutions.

Keywords: honeydew; nectar; sampling method.

Introduction

Measurement of the sugar contained in honeydew and other carbohydrate-rich exudates is important for quantifying a variety of ecological processes. For example, measurements of exuded sugars have been used to quantify nectar reward for visits to flowers by pollinating animals (e.g. Corbet and Delfosse, 1984; Hodges, 1995; Biernaskie *et al*., 2002), to investigate interactions between honeydew-producing aphids and the social ants that tend them (e.g. Yao and Akimoto, 2001; Fischer *et al*., 2002), and to investigate the effect of honeydew on the quality of farmed cotton (Henneberry *et al*., 2000). In New Zealand, measurements of honeydew produced by scale insects (*Ultracoelostoma* spp.) feeding on the phloem sap of beech (*Nothofagus* spp.) trees have been used to implicate competition for honeydew by introduced social wasps in declines in the breeding success of the endangered forest parrot *Nestor meridionalis* (Beggs and Wilson, 1991).

Several methods are commonly used to sample honeydew or nectar, and have been reviewed most recently by Corbet (2003) (see also Dafni, 1992; Kearns and Inouye, 1993). Corbet (2003) recommends a widely used method, whereby drops of honeydew or nectar are sampled with glass microcapillary tubes of known capacity, and drop volume determined by measuring the length of the tube occupied by the drop. Total carbohydrate content can be estimated by measuring the concentration of sucrose in solution with a sugar refractometer. While this technique is generally fast, simple to use, and reliable, it is problematic in two situations. First, when sampling very small volumes (<1 µl), only refractometers with very closely set prisms will allow accurate concentration measurements. McKenna and Thomson (1988) reported a useful method for sampling small volumes, where small (<20 mm^2) pieces of filter paper are used as wicks to absorb nectar droplets. Once the wicks are dry, the sugars are redissolved and the total carbohydrate content assessed using the anthrone method as described by Umbreit *et al*. (1972). Second, when sugar solutions are very concentrated, such as when nectar or honeydew

droplets have been exposed to dry air, drops are too viscous to be drawn into capillary tubes. Several techniques have been used in such situations, but these are either not useable in the field (e.g. Nunez, 1977), require diluting of droplets (Dafni, 1992), or involve washing of nectar from harvested flowers (Mallick, 2000) making them unsuited to repeat measurements.

When sampling honeydew produced by scale insects in New Zealand beech forest, we have found neither the microcapillary-and-refractometer (MR) or McKenna and Thomson (MT) methods particularly useful. While Murphy and Kelly (2003) used the MT method to sample honeydew, it can be problematic when using filter paper wicks, as paper fibres can inadvertently be added to sample solutions when washing carbohydrate from the filter papers in boiling water (McKenna and Thomson, 1998; p. 1306). When the sample solutions are boiled in anthrone reagent, containing 95% sulphuric acid (Umbreit *et al*., 1972; p. 261), any paper fibres dissolve completely, adding erroneously to the estimated carbohydrate. The MR method has been most commonly used to sample honeydew in New Zealand beech forests (e.g. Moller and Tilley, 1989; Kelly *et al*., 1992; Dungan and Kelly, 2003). We have found it to be very time consuming in the field, as individual drops must be collected into very small glass tubes. The method tends to be impractical as honeydew droplets are often too viscous to be sampled with capillary tubes (Kelly *et al*., 1992; Dungan and Kelly, 2003). We have also found it beneficial to calculate the mass of sugar present in the standing crop of honeydew, as this can be used to estimate the energy available to foraging animals from this food source. This requires an additional conversion factor to adjust the gravimetric sucrose concentration values derived from the refractometer to volumetric concentration values so sugar mass can be estimated from the microcapillary tube volume measurements.

Here we propose and validate a simple gravimetric technique that largely overcomes these limitations. Pre-dried and weighed pieces of standard filter paper are used as wicks to sample honeydew in the field. The change in mass of the paper after redrying provides an estimate of the mass of carbohydrate in the honeydew. Although we have used the method to sample honeydew, it will be equally applicable to sample nectar and other carbohydrate solutions. Our validation took three steps. First, we made stock solutions of mock honeydew using concentrations of honeydew carbohydrates determined by Grant and Beggs (1989). Dilutions of these solutions were used to create a factor to convert gravimetric sucrose refractometer readings (i.e. % w/w; g sucrose 100 g^{-1} solution) to volumetric total carbohydrate concentrations (i.e. % w/v; g carbohydrate 100 ml^{-1} solution). We then applied known volumes of solution of known concentration to filter papers to compare expected carbohydrate mass with that observed after drying the filter papers at two temperatures. Finally, we compared the filter paper (FP) and MR techniques by collecting drops of simulated honeydew from a fine plastic tube, designed to mimic a scale insect's waxy anal tube. This required the refractometer conversion factor calculated in the first step. Our aim was not to compare all available sampling techniques. Rather, we compared a widely used method that we have found problematic (MR), with a rapid, easy to use, but as-yet untested technique (FP).

Methods

Solution preparation and refractometer conversion

Stock solutions of simulated honeydew were prepared by dissolving carbohydrates in distilled water. Simple sugars in the carbohydrate solution were sucrose, glucose, and fructose (Sigma-Aldrich, St. Louis, MO, U.S.A.), and the more complex oligosaccharides were provided by a low-dextrose-equivalent hydrolysed corn starch, commercially available as a food ingredient (Dridex 30®, Penford Foods, Auckland, N.Z.; typical carbohydrate composition listed in Table 1). Powdered carbohydrates were dried to constant mass in a forced-draught oven at 50°C and mixed to the proportions determined by Grant and Beggs (1989), listed in Table 1. Stock solutions of 50% and 80% w/v (50% w/v = 50 g total carbohydrate 100 ml^{-1} solution) were made, and then stored at 4°C to limit microbial growth. From each of these, three replicate dilutions were made in 10% decrements, such that the 80% solution was diluted to 70%, 60%, ... 10%, and the 50% solution diluted to 40%, 30%, 20% and 10%. The

Table 1. Carbohydrate composition of simulated honeydew. The proportional contribution of each carbohydrate to the total carbohydrate mass was determined by Grant and Beggs (1989), and data on the carbohydrate composition of Dridex 30® were obtained from the supplier (Penford Foods, Auckland, N.Z.).

Carbohydrate	Proportion of total carbohydrate mass	Typical composition
Glucose	0.02	
Sucrose	0.23	
Fructose	0.42	
Dridex 30®	0.33	
Glucose		0.03
Maltose		0.11
Maltotriose		0.17
High oligosaccharides		0.69

sugar concentration of each of the diluted solutions was estimated with a sucrose refractometer (HSR-500, Atago, Japan), and the resulting estimates used to derive a relationship to convert gravimetric sucrose concentration (% w/w) to total volumetric carbohydrate concentration (% w/v). The relationship was derived by fitting a quadratic equation to the means of the refractometer readings of the various solutions, using non-linear least-squares regression (SigmaPlot 7.0, SPSS Inc., Chicago, IL, U.S.A.) For comparison, equivalent values for the conversion applied by Dungan and Kelly (2003), and for a solution of pure sucrose obtained by recalculating values from published standards (CRC Press, 2003), were calculated.

FP method validation

Labelled pieces of 90-mm-diameter laboratory filter paper (Watman No.1, Whatman International, Maidstone, U.K.) were dried in a forced-draught oven for 48 hours at 70°C, then cooled to room temperature in an air-tight desiccator. Filter paper is quite hygroscopic, and will absorb moisture from the air (up to 0.2 mg min^{-1} for the 90-mm discs) on removal from a desiccator, even in a dry laboratory. When weighed on a microbalance, the mass of the papers would increase during the time taken to weigh them, but only by <0.2 mg. To standardise the error associated with this increase the dried filter papers were weighed on a balance with an animal-weighing function (MP225D, Sartorius AG, Goettingen, Germany). This function determines an average value for the weighed mass over 20 seconds once the balance reading has stabilised to nearest 0.1 mg.

Precise volumes of the two stock solutions were applied to the pre-dried papers, which were then dried in the forced-draught oven for 48 hours. The expected mass of carbohydrate on each filter paper, determined from the volumes of stock solutions applied, was compared with the mass of carbohydrates measured with the balance. To test the effect of drying temperature on the dry weight of the honeydew solutions, four replicates of each mass were weighed after drying papers at 70°C, and another set of replicates weighed after drying at 40°C.

Comparison of FP and MR methods

A simulated honeydew tube was created by stripping the PVC insulation from a short length of fine electronics wire so as to yield a tube with internal diameter of 0.25 mm. The tube was taped to the side of a plastic container so as to mimic a scale insect's tube. A drop of honeydew solution was able to be deposited directly on the end of the tube with a micropipette, in a realistic simulation of a honeydew droplet. A sample from each of the two stock honeydew solutions was diluted by half, to give solutions of 80%, 50%, 40% and 25% w/v. An additional solution of approximately 100 % w/v was also made. A 10-µl drop of honeydew was deposited on the tube, then drawn into a 50-µl capillary tube and the length of capillary tube occupied was measured with vernier callipers. This was repeated until 50 µl had been deposited, and the combined sample was then evacuated onto the measuring prism of the refractometer. Five replicates were measured for each of the four solution concentrations. The process was repeated using the filter paper technique described above to wick the droplets from the simulated honeydew tube.

Results and discussion

Refractometer conversion factor

The equation for converting gravimetric sucrose concentration values to volumetric total carbohydrate concentration values was virtually identical to that estimated for a pure sucrose solution (Fig. 1). The concentration of pure sucrose in our simulated honeydew solution was less than 25% of the total carbohydrate content. The similarity between the pure sucrose and honeydew-derived conversion equations suggests that refractometer-derived measures of total carbohydrate content are relatively insensitive to changes in carbohydrate composition. The conversion equation we derived is markedly different from the correction applied by Dungan and Kelly (2003), which would have underestimated the true carbohydrate concentration in the simulated honeydew solutions by about 20%. This difference is attributable to the correction factor recommended by Grant and Beggs (1989), whereby refractometer concentration values are divided by 1.145 to estimate total carbohydrate content. Dungan and Kelly (2003) estimated the proportion of net primary production released as honeydew in a high altitude *Nothofagus solandri* forest to be 1.8%. Applying our revised correction to their calculations shows that proportion of annual carbon uptake converted to honeydew is better estimated as 2.2%.

FP method validation

The mass of carbohydrate measured after drying the filter papers almost exactly matched the mass expected, based on the volume and concentration of the solutions applied to the paper (Figure 2). There was a weakly significant effect of drying temperature on the ratio of observed to expected carbohydrate (one-way ANOVA, $F_{1,72} = 5.52$, $P = 0.02$). Samples dried at 40°C slightly overestimated the total honeydew carbohydrate ($r^2 = 0.99$, $P < 0.001$), whereas the reverse was true for samples dried at 70°C ($r^2 = 0.99$, $P < 0.001$). In each

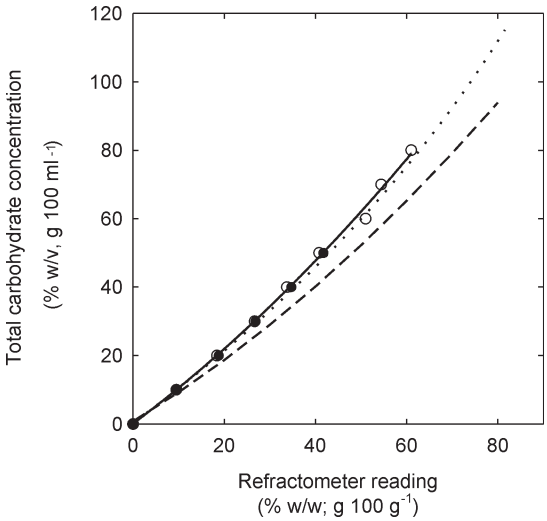

Figure 1. Comparison of gravimetric carbohydrate concentrations measured with the sugar refractometer, and volumetric carbohydrate concentrations determined by dilutions of stock solutions of known concentration. There was no difference in concentrations determined from solutions of 80% w/v (○) and 50% w/v (●). A quadratic relationship fitted to the pooled data (solid line, $y = 0.123 + 0.994x + 0.0049x^2$, $r^2 = 0.99$, $P < 0.001$) was indistinguishable from a relationship derived for pure sucrose solutions (dotted line), recalculated from published standards (CRC Press, 2003).The correction factor employed by Dungan and Kelly (2003) is also shown (dashed line). Error bars (obscured) are ± 1 S.E. of the mean of three replicate dilutions.

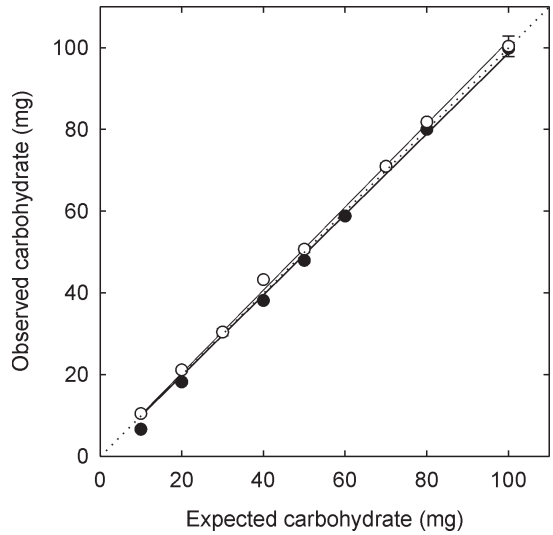

Figure 2. Relationship between the mass of total carbohydrate deposited on filter papers from solutions of known concentration, and the actual mass of carbohydrate weighed after subsequent drying at 40°C (○) and 70°C (●). Error bars (partially obscured) are ± 1 S.E. of the mean of four replicate dilutions. Dotted line denotes 1:1 relationship.

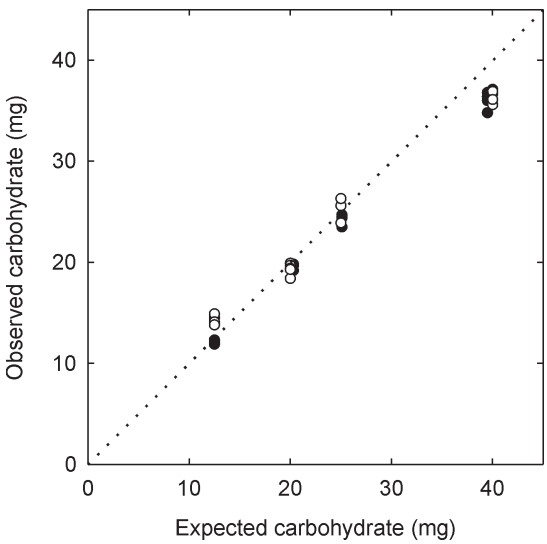

Figure 3. Comparison of the mass of carbohydrate measured on sampling droplets of solution deposited on a simulated honeydew tube using the filter paper (○) and microcapillary- and refractometer (●) methods. Dotted line denotes 1:1 relationship.

case the estimation error is less that 1% of the total carbohydrate mass. We recommend drying at 70°C to reduce possible carbohydrate loss due to microbial activity. It is unlikely that this temperature will change the mass of carbohydrate present on the paper (by either volatilisation or hydrolysis, for example), although it may increase the volatilisation of other organic compounds that are present in small quantities in honeydew and nectar (Dafni, 1992; Grant and Beggs, 1989).

Comparison of FP and MR methods

Our results show that the FP technique is an appropriate method for sampling sugar-rich carbohydrate solutions. There was no evidence that the ratio of observed to expected carbohydrate differed between the FP and MR techniques (one-way ANOVA $F_{1,40} = 2.64$, $P = 0.11$; Figure 3) when sampling honeydew from a simulated scale insect tube. The filter paper method slightly overestimated the carbohydrate mass when 50 µl of 25% w/v solution was sampled. Both methods underestimated the carbohydrate mass when 80% w/v solution was sampled, but by a very similar amount. It is possible that the more concentrated solution was adsorbed more strongly onto the simulated scale insect tube, and we are not able to say whether this differs from an actual insect's tube.

Attention must be paid to appropriate laboratory techniques when using this method. Although we observed a marginally significant effect of drying temperature on the accuracy of the method, this was less than the potential error associated with improper drying before honeydew sampling. A sample of 20 filter papers of 90 mm diameter stored in a relatively warm, dry office contained on average 9.2 ± 0.2 mg of water, equivalent to 1.8% of their average mass. It is important that papers are labelled first, then thoroughly dried before the pre-sampling weighing. From field experience, the greatest errors in this method arise from inadequate drying before papers are taken into the field. As an additional check extra labelled and dried papers can be prepared and taken into the field. These are not used for sampling, but are subjected to the same storage and transport conditions. They are redried and reweighed with the papers used for sampling, and can be used to test that all papers were dried correctly before field sampling, and used to derive a correction factor if they were not. Papers should be weighed individually, as we have found that the mass of individual 90-mm-diameter papers can differ from the mean mass by up to 2.5%; at times this is greater than the mass of carbohydrate that has been sampled.

The filter papers can develop large static charges as they cool in the desiccator. Static charge can create inaccurate balance readings. Care should be taken to discharge any charge, using an inexpensive computer anti-static mat, before samples are transferred to the microbalance. Care should also be taken when using the FP method in the field. Filter papers should be folded carefully, to avoid accidentally wiping off the sampled carbohydrate solution, and stored in individual plastic bags. If there is likely to be a long delay between sampling and drying, papers should be kept as cool as possible (ideally <4°C), or stored with a desiccant (e.g. coarse-grade silica gel in fine mesh bags) to reduce microbial activity. We have had acceptable results even when papers have been stored in less-than ideal conditions (high temperature and humidity) for several days.

While the FP method will be most useful in situations where solutions are very concentrated, or where large numbers of samples need to be taken relatively quickly, we have found it possible to sample very small volumes of solution with the FP method with acceptable results. We applied 2-µl droplets of 25% w/v honeydew solution to a sample of 20 8×2 mm rectangles of filter paper, and measured total carbohydrates (mean ± 1 S.E.) of 0.55 ± 0.03 mg (*cf.* 50 mm expected from droplet concentration and volume). This small over-estimation may be due more to errors in applying small volumes with a pipette, rather than errors in the FP method *per se*. The FP method is not appropriate where the concentration or volume of a carbohydrate solution is of interest (e.g. Potts *et al.*, 2003). Where these data are required they could be estimated from the relationship between drop volume and the size of the spot that the drop makes on the filter paper (Dafni, 1992; p. 140). This approach should be used with caution, as the relationship between paper-spot size and droplet volume is intimately dependent on droplet concentration.

The FP method offers advantages over the MR method because it is quick and easy to use in the field, can be used to accurately measure total carbohydrates produced over large areas (e.g. nectar produced by large compound inflorescences; *cf.* Lloyd *et al.*, 2002), and is able to be used to sample very concentrated carbohydrate solutions. Very viscous solutions, >100% w/v, can still be collected simply by pre-moistening the filter paper so that the carbohydrate solution dissolves directly onto the filter paper. We have found that the speed with which honeydew can be sampled with the FP method is particularly useful when determining rates of honeydew production. It is relatively easy for one person to sample all the honeydew from 50-cm-tall bark bands on 20 trees within an hour (~2000 droplets) for example, whereas only 200 droplets might be sampled using microcapillary tubes (assuming a sampling rate of between three and four drops per minute). Of course, the filter paper method takes considerably longer once

the drying and weighing requirements are accounted for, but its speed in the field, accuracy, and ability to easily sample highly viscous droplets more than make up for this additional time requirement.

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