Ecology, 69(4), 1988, pp. 1306–1307

A TECHNIQUE FOR SAMPLING AND MEASURING SMALL AMOUNTS OF FLORAL NECTAR[1]

Mary A. McKenna[2] and James D. Thomson[3]

Since the work of Hocking (1953), capillary tubes and refractometers have become standard equipment for measuring floral nectar volumes and sugar concentrations, from which the energy value of nectar can be determined (see Bolten et al. 1979), for studies of plant–pollinator energetics. Although the technique is rapid and easy, it is poorly suited for small (<1 μL) nectar volumes. It is difficult to ensure that the tube has taken up all the available nectar; this potential sampling error becomes proportionally larger as nectar volumes decrease. Viscous nectars compound the problem by resisting capillary uptake. Furthermore, only refractometers with extremely closely set prisms will yield clear concentration readings with small volumes of solution.

The capillary-and-refractometer technique presents additional complications for experiments involving repeated sampling from a single flower (to determine the secretion rate of nectar following an initial draining, for example). Thorough probing with a glass capillary tube may damage the nectary tissue, which could (1) introduce cell contents to the nectar (Willmer 1980), and (2) alter or destroy the function of the nectary.

To avoid these problems encountered in the study of nectar secretion in *Aralia hispida* Vent. (Araliaceae) (J. D. Thomson and M. A. McKenna, *personal observation*), we developed an alternative procedure that uses small filter paper strips as wicks to collect nectar samples for subsequent colorimetric analysis. This analysis requires greater time per sample than the refractometer method, but the extra time is spent in the laboratory, at one's convenience. Obtaining nectar samples using the wick sampling procedure is probably quicker than using capillary tubes and adapts itself very well to organizing complicated experimental designs.

We cut our wicks from Whatman Number 1 filter paper, using a punch designed for cutting paper "points" for mounting insects. These wicks are narrowly ovoid (2 × 8 mm) and taper to a point. The wicks are held with forceps for soaking up the nectar. We have found it convenient to impale the wick on an insect pin first. Pinned wicks can be prepared in advance and are easily manipulated by hand without the hazard of skin contaminants. It may be advantageous to use the rounded end of the wick rather than the point for certain floral and nectary morphologies. If there is sufficient nectar in one flower to saturate a wick, additional wicks can be used as required. We find it convenient to outline a sampling scheme in advance on a piece of ruled paper clipped to a block of plastic foam. After each sample, the wick is simply pinned in its appropriate place on the sheet and allowed to air dry for subsequent analysis in the laboratory up to several months later.

We have used the anthrone method (Umbreit et al. 1972:261) to determine the total carbohydrate content of a nectar sample, as follows. Redissolve sugars from the dried wicks by vortexing the wicks in 5 mL of boiling distilled water for 1 min. Place 2-mL samples of the resulting solution, reagent blanks, and a series of sugar standards (below), in screw cap test tubes in an ice bath. Add 4 mL of fresh anthrone reagent (0.4 g anthrone [Sigma Chemical Company, St. Louis, Missouri] in 200 mL concentrated H_2SO_4), cap and vortex the tubes, and place them in a boiling water bath for 10 min. Let them cool to room temperature, then read absorbances on a spectrophotometer.

We prepared a calibrating series of sugar standards to range from 10 to 300 μg of total sugar per 2 mL of standard with equal amounts of fructose and glucose. The choice of a fructose/glucose standard was based on Percival's (1961) report of fructose/glucose nectar in *Hedera* (also Araliaceae). Experimenting with various proportions of fructose:glucose indicated that the anthrone analysis is relatively insensitive to the proportions in the mixture. Standard sugar solutions refrigerated for <14 d continue to give excellent replication of fresh standard curve absorbance values. It may be necessary to dilute the redissolved nectar samples to ensure that the final absorbances fall within the range of the standard series.

By estimating total carbohydrate, rather than obtaining separate measurements of volume and refractive index, the wick method provides less information than conventional sampling. This is hardly a sacrifice in circumstances where small volumes or other circumstances make the conventional approach unreliable. It would also be possible to identify and quantify the eluted sugars from the wicks if the sample is free from contamination from pollen or stigmatic exudate. In addition we suspect that the wick method may be preferable to capillary extraction even for larger volumes, if it is important to extract all floral nectar. Our impression is that careful mopping with a wick can pick up residual nectar that remains behind after care-

ful extraction by capillary tube, and that wick-sampling more closely approximates a bee's tongue in picking up nectar.

Acknowledgments: Research supported by NSF DEB 8206959 to J. D. Thomson. We thank M. B. Cruzan for advice and J. Knapp, L. Neuberger, and T. Matteo for technical assistance.

Literature Cited

Bolten, A. B., P. Feinsinger, H. G. Baker, and I. Baker. 1979. On the calculation of sugar concentration in flower nectar. Oecologia (Berlin) **41**:301–304.

Hocking, B. 1953. The intrinsic range and speed of flight of insects. Transactions of the Royal Entomological Society of London **104**:223–345.

Percival, M. S. 1961. Types of nectar in angiosperms. New Phytologist **60**:235–281.

Umbreit, W. W., R. H. Burris, and J. F. Stauffer. 1972. Manometric and biochemical techniques. Burgess, Minneapolis, Minnesota, USA.

Willmer, P. G. 1980. The effects of insect visitors on nectar constituents in temperate plants. Oecologia (Berlin) **47**:270–277.

[1] *Manuscript received 23 February 1987; revised 13 November 1987; accepted 19 November 1987.*

[2] *Department of Botany, Howard University, Washington, D.C. 20059 USA.*

[3] *Ecology and Evolution Department, State University of New York, Stony Brook, New York 11794 USA.*

ERRATA

In an article by Philip Dixon et al. ("Bootstrapping the Gini coefficient of inequality," *Ecology* **68**(5):1548–1551) there was an error in the equation on page 1549. The correct equation should be:

$$G = \frac{1}{\bar{X}n(n-1)} \sum_{i=1}^{n} (2i - n - 1) X_i.$$

The numerical results are correct.

In an article by J. Lovett Doust and L. Lovett Doust ("Modules of production and reproduction in a dioecious clonal shrub, *Rhus typhina*," *Ecology* **69**(3):741–750) the text on pages 746 and 747 should appear after that on pages 748 and 749.