**Debinski Lab Nectar Sampling Protocol**

Last updated August 3, 2016, Audrey McCombs

**Equipment**

2 Bellingham and Stanley sugar refractometers modified for nectar

* 1 covers the Brix range of 0-50 and the other covers 45-80

Fixed bore microcapillary pipets (various sizes)

* Smaller flowers require smaller diameter microcapillary pipets, and flowers with large amounts of nectar may require tubes that can hold larger volumes

Millimeter ruler

KimWipes

Distilled water

Weather meter that measures wind speed, temperature and humidity

**Background**

Pilgrim Creek warming plots in Grand Teton National Park. Control and heat treatment, six plots each. See maps for plot assignments and locations. (Note: previously we had four treatments: heat, snow removal, heat plus snow removal, and control; three replicates per treatment. But in 2015 and 2016 there was no snow removal, so for those years we only have the heat treatment.) Two species sampled: arrowleaf balsamroot (*Balsamorhiza sagittata*) and sulfurflower buckwheat (*Eriogonum umbellatum*). *B. sagittata* flowers first, then as it’s senescing, *E. umbellatum* flowers. *E. umbellatum* presents as flower stalks on a groundcover mat—no individually identifiable plants. B. sagittata individually labeled plants (see map). Phenology of flowers as per vegetation monitoring protocol.

**Weather and timing**

Sample between the hours of 7am and 9am, when no water droplets (dew or rain) are visible on plants. Record time, wind, temp, humidity, and sun condition (sun or shade) frequently during sampling. We are not bagging flowers (assuming that nectar consumers are not active this early in the morning.)

**Choosing samples**

*Balsamorhiza sagittata*

Sample 8 flowers per plot, or as many flowers as are in the plot if there are fewer than 8. For each plant that has at least one flower fully-flowering (FF, not in bloom or senescing/senesced) with at least 5 open disc florets, we chose the flower that is most likely to have nectar. (Disc florets most likely to have nectar: disc floret is yellow instead of orange and dried up, the stigmas have emerged and are not curled up, and the disc floret is not floppy with a bulge at the bottom.) If the plant only has 1 FF flower, we sample it (as long as it has 5 open disc florets and even if it doesn't look great.) We sample from the best flower of the first plant, then move on to the next plant with at least one FF flower and sample from the best flower of that plant. We continue until the best flower on all plants with FF flowers have been sampled. If there are more flowers and we haven't yet taken 8 samples from the plot, then we return to the first plant and sample from the next-best flower. Continue until 8 samples have been taken. If there are fewer than 8 FF flowers in the plot, we sample all FF flowers that have at least 5 open disc florets and move to the next plot.

*Eriogonum umbellatum*

Similar to *B. sagittata*, except: 1) no individual plants, so sampling is by plot quadrant, and 2) sample 3 florets with one microcap. (This is because many florets don’t produce nectar. Using a new microcap for every floret, even when it’s empty, would be time-consuming and would blow through our microcap supply. So one recorded sample is an aggregate of nectar samples from three florets.) Chose the two best flowers in the first quadrant, then the two best flowers in the second quadrant, etc., until all four quadrants have been sampled.

**Extracting Nectar**

Nectary in both these species is at the bottom of the flower above the sepal. Gently insert microcaplliary pipette into flower, being careful not to damage the nectary tissue. Once contact with the nectar is made capillary action will cause the tube to fill. Wait until the nectar stops flowing and if necessary carefully reposition the pipet within the flower to capture any remaining nectar.

Record the size of the microcapillary pipette the length (mm) of nectar within the tube.

Deposit nectar onto the refractometer. Close the flap, point the instrument at the sun, and record the value. You’re making a guess about whether the nectar will be above BRIX 45 or below BRIX 50. You can use previous measurements as a guide, but you still might guess wrong, in which case the BRIX reading will be off the scale. Record these as <45 or >50 on the data sheet, and try to guess better next time. With larger volumes (e.g. >10-15mm) you might try and split the sample by placing only half on one refractometer while retaining the other half in the microcap. If the reading is off the scale you can try the other half on the other instrument. This takes practice, though, controlling how much of the sample you place on the refractometer.

**Calculating nectar volume**

Nectar volume is calculated using the following equation:

**Determining sugar concentration**

The BRIX reading is g of sugar to 100 g of solution, or % w/w. Nectar concentrations in the literature (e.g. Willmer 2011 p. 206 and Nicolson et al 2007 p. 303) are given in these same units: % w/w. Sugar concentration therefore can be analyzed as straight BRIX values, without conversion. The hand-held refractometer is calibrated to read BRIX at 20° C, and readings taken at different temperatures should theoretically be corrected. Temperature correction tables can be found in the distributer’s handbook for the Bellingham & Stanley refractometers (Appendix 5; also Appendix 2 from *The Soft Drinks Companion*), but in practice the correction is very small—less than 1 BRIX for even a 10° C reading at BRIX 70.

**Determining sugar mass**

First you will need to convert the percent sucrose (your BRIX value, g/100g or % w/w) to concentration by volume (mg/mL). A conversion table can be found in Kearns and Inouye, 1993.

Nectar sugar mass is calculated using the following equation:

**References**

Kearns, C. A. and Inouye, D. W. 1993. Techniques for Pollination Biologists. University Press of Colorado, Niwot, CO

Primary source: LaBare K. M., S. B. Broyles, R. L. Klotz. 2000. Exploring nectar biology to learn about pollinators. The American Biology Teacher, 62(4):292-296