#### **Petiole Nitrate Protocol**

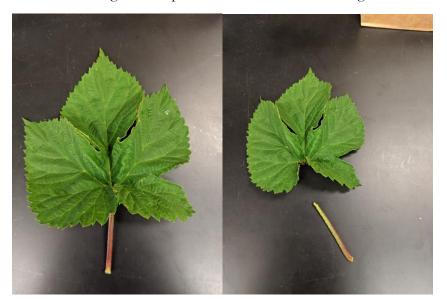
We have made some quick how-to videos that demonstrate these steps. Check out **go.osu.edu/hops-trials** to see the entire process.

# Step 1. ASSEMBLE THE NITRATE METER (video: go.osu.edu/hop-meter)

- 1. Follow the provided instructions to assemble the meter and turn on.
- 2. Briefly, insert batteries "+" side up and attach the sensor to the probe.
- 3. Turn probe on with on/off switch
- 4. Open cover, exposing the sensor and place a few drops of 2000 ppm standard solution on the sensor to condition. You should add enough to cover bottom of sensor area and let sit for an hour or so. You only need to do this when you first assemble your meter.

### Step 2. SAMPLE COLLECTION (video: go.osu.edu/hop-sample)

- 1. Identify sampling area by locating four corners you staked when you established your sampling area.
- 2. Record approximate average height of bine (top leaves) at the time of sampling on datasheet.
- 3. Collect 20 leaves with petioles attached from across your sampling area. Leaves should be the most recently matured leaves and should be collected 5-6' from the ground. You will want to select 'representative' plants and leaves avoiding stunted plants or leaves that have a large amount of insect damage or disease.



Hop leaf with petiole (left) and with petiole detached (right). The petiole is piece that attaches the leaf to the bine. Be sure to collect the entire petiole when you complete the next steps.

4. Pinch and pull the petiole and leaf off the bine, pull the leaf off and discard and put petiole into the corresponding paper bag. Repeat until you have no less than 20 petioles.

5. After collection is complete, immediately go to a clean area that you can process the petioles and take readings with your meter.

# Step 3. CALIBRATE THE METER (video: go.osu.edu/hop-calibrate)

- 1. The meter comes with two standard solutions to calibrate the probe. This step is essential so your probe can give you accurate values. **Please perform this step before each sampling**.
- 2. If you still have standard solution on the sensor from step 1, use tap water to rinse the probe for 3 seconds. Dry sensor by dabbing gently with a soft tissue.
- 3. Turn on meter. Press and hold the CAL switch until CAL appears on the top of the LCD and "150 ppmNO<sub>3</sub>" starts flashing.
- 4. Open the light shield cover and add several drops of 150 ppm standard solution to the flat sensor, ensuring sensor is covered completely. Close the shield cover and press and hold the CAL switch. A smiley face will appear on the display and both the CAL and smiley face will flash for a few seconds. Once the calibration value is achieved, the flashing will stop and the solid smiley face and number indicate your first calibration is complete.
- 5. Open the light shield cover, rinse the low standard off the sensor and gently dry the sensor with tissue.
- 6. Press and hold the CAL switch until CAL appears on the top of the LCD and "2000 ppmNO<sub>3</sub>" starts flashing.
- 7. Open the light shield cover and add several drops of 2000 ppm standard solution to the flat sensor, ensuring sensor is covered completely. Close the shield cover and press and hold the CAL switch. A smiley face will appear on the display and both the CAL and smiley face will flash for a few seconds. Once the calibration value is achieved, the flashing will stop and the solid smiley face and number indicate your first calibration is complete.
- 8. Open the light shield cover, rinse the high standard off the sensor and gently dry the sensor with tissue.
- 9. Your calibration is complete. Press the MEAS switch to enter measurement mode.

# Step 4. EXTRACT THE PETIOLE SAP AND MEASURE (video: go.osu.edu/hop-petiole)

- 1. Before beginning, calibrate your nitrate meter following provided manufacturer instructions. Ensure meter is recording in ppm units (ppmNO-3).
- 2. Cut petioles into small pieces to make pressing easier. Be sure to clean scissors (or other cutting tool) between samples.
- 3. Place enough pieces into garlic press so that it is full without overflowing. Holding press over the supplied Al foil container, or a saucer, squeeze press until sap stops flowing. Continue this process until all petioles for that sample are processed. Remove any solid pieces/ particles.

- 4. Using the kit syringe, load the syringe and gently shake to mix. Ensure meter is in measurement mode (MEAS displayed). Holding syringe over the sensor, gently push syringe to release a few drops of sap onto the meter ensuring the bottom of the sensor is covered by sap.
- 5. Close the light shield cover.
- 6. A flashing smiley face will appear and continue to blink until a consistent reading is measured. Once the blinking stops, record the final concentration on your datasheet. If meter is not displaying a value, press the MEAS button until it begins to read.

**Note:** If the meter reads "Or" this means you have reached the upper range and the nitrate concentration is too high for the meter to read (>10,000 ppm). If this happens you can still get a reading by diluting equal parts of the sap and water (1ml water + 1ml sap in syringe) and reading. It is imperative to note in the datasheet if you needed to dilute your sap as your value will be ½ the real concentration.

- 7. Repeat steps 4 -6, until you have 3 readings recorded.
- 8. Thoroughly clean meter, dropper, press, and scissors before beginning next sample then repeat steps in Step 4 until all samples have been analyzed.