HIGH SCHOOL TEACHER GUIDE

THERE'S SUGAR IN GRASS?
BRIX REFRACTOMETER LAB

MATERIALS:

Engage Activity

- 5 100 mL beakers or clear plastic cups (4 oz. or larger)
- 5 50 mL beakers or clear plastic cups (2 oz. or larger)
- Warm tap water
- Granulated sugar
- 5 straws or colored rods
- Wax crayon or tape

Lab Investigation (per group)

- Plants for collection
- 1 refractometer*
- 1 garlic press
- 1 soft cloth
- 1 plastic transfer pipet
- 1 small collection container

  (3 oz. plastic cups work well, small bowl, Tupperware container)
- 1 bowl, paper bags, grocery sacks (to mix grass)
- 1 small screwdriver
- Paper towels

* *Refractometers can be purchased from numerous vendors. The refractometers that were used in developing this lab were purchased from Amazon for $17 each (0-32% Brix Meter Refractometer). The kit includes a transfer pipet, screwdriver, wipe cloth and case.*

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LESSON OVERVIEW:

In this lesson students will use a Brix refractometer to determine sugar concentrations in forages.

LESSON OBJECTIVES:

Students will be able to:

1. Demonstrate proficiency in using a refractometer.
2. Determine the approximate amount of sugar in forages.
3. Evaluate the relationship between amount of sugar, photosynthesis and harvest time.

ESSENTIAL QUESTION:

How does photosynthesis in forage plants affect nutrient quality?

TOPICAL ESSENTIAL QUESTION:

Can we determine the optimal time to harvest forages to improve cattle performance?

PRECAUTIONS:

- If students will be outside, make sure that they are in a safe area and aware of traffic or other hazards.
- Refractometers cannot be submerged in water. Water can be placed on the prism to clean them after use, however.

TOTAL DURATION: 15 min. prep time; 45-90 min. class time

STANDARDS:

HS-LS1-5 From Molecules to Organisms: Structure and Processes
Students who demonstrate an understanding can use a model to illustrate how photosynthesis transforms light energy into stored chemical energy.

HS-LS1-6 From Molecules to Organisms: Structure and Processes
Students who demonstrate an understanding can construct and revise an explanation based on evidence for how carbon, hydrogen and oxygen from sugar molecules may combine with other elements to form amino acids and/or other large carbon-based molecules.

HS-LS2-3 From Molecules to Organisms: Structure and Processes
Students who demonstrate an understanding can construct and revise an explanation based on evidence for the cycling of matter and flow of energy in aerobic and anaerobic conditions.

HS-LS 2-5 From Molecules to Organisms: Structure and Processes
Students who demonstrate understanding can develop a model to illustrate the role of photosynthesis and cellular respiration in the cycling of carbon among the biosphere, atmosphere, hydrosphere and geosphere.

KEY VOCABULARY:

Refract
Refractometer
Quantitative
Qualitative
Photosynthesis
Cellular respiration
Brix scale
Forages
Ruminant
Cattle Performance

NOTE TO THE TEACHER:

1. This investigation can be done in the field or in the classroom in several ways.
   a. Students can collect plant material from the schoolyard or a nearby field.
   b. Teacher can collect samples of plants from schoolyard or nearby field and have them ready for class.
      i. If plants are collected in the field, any type of grass, legume or broadleaf forbs will work for this experiment. Just make sure to collect all of the same type of plant.
         1. If sampling from a small, specific area, several handfuls of plant material is sufficient.
         2. If sampling an entire field or pasture, walk in a Z or W pattern while taking samples.
   c. If the time of year is not conducive to collecting plants outside, common vegetables can be easily substituted (spinach, lettuce, kale, collard greens, different colored peppers).
2. The Engage activity highlights the concept of refraction. You will prepare solutions prior to class. Further instructions are in the Engage section. The sugar solutions are used again in the lab activity so if you choose not to do the Engage activity, be sure to have the solutions ready for lab activity 1.

LAB BACKGROUND INFORMATION:

Plants play a critical role in providing food and fiber for humans and animals. In agriculture, we harness nature's food production and use it to our benefit to meet the food, fiber and beverage needs of a demanding consumer market. Photosynthesis and cellular respiration are two of the most important chemical reactions on our planet. They are also ones that students learn about throughout their education in science. Many students have a basic understanding that the plant uses water and carbon dioxide and makes sugar and gives off oxygen and that during respiration the equation is reversed. However, few can give any concrete examples of why these processes are important beyond producing oxygen for the planet.

One of the tools used by agriculturalists, winemakers, fruit and vegetable buyers, food processers, beekeepers and many more is a Brix Refractometer. This tool uses the science of refractometry to measure the soluble solids concentration of a plant to gauge the amount of sugar in it. The soluble solute can include different compounds like lipids, acids, pectins and minerals, so the sugar content is only an estimate. The Brix score is one of the quantitative values used to determine if a plant product (fruit, vegetable, grass) meets the standard for harvesting.

The **Brix scale**, developed by Adolf Brix in the mid-1800s, determines the soluble solid concentration of a solution using a refractive index. In other words, it creates a scale for dissolved solids based on changes in the direction of a light beam as it passes through a liquid containing suspended solids. In simple terms, the greater the amount of suspended solids in a liquid, the more light is bent as it passes through. A Brix unit is equal to a percentage of dissolved solids, so a 100g solution that measures 30° Brix contains 30 percent (30g) soluble solids (sugar) and 70 percent (70g) water. Temperature can also affect the amount of refraction, so the industry uses a standard temperature of 20°C (68°F) as a base.

The refractometer itself is comprised of a prism, a sun shield and a tube students can look through. A few drops of a liquid removed from a plant is placed on the prism and covered with the sun shield before looking down the tube toward light. Inside is a scale with the Brix value. The point at which the blue and white areas meet is the percent Brix of the sample.

It should be noted that while the Brix score is widely used, it only provides an estimate; and the estimate can range widely depending on environmental factors like barometric pressure, temperature, moisture, time of day, fertilization, crop species and maturity of the plant. Most professionals will use the Brix value in conjunction with other, more qualitative, methods to determine product readiness.

In the agricultural industry, the soluble solids level influences commercial uses and consumer reactions to fruits, vegetables and the products made from them. Brix scores are used by many areas within the industry, from farmers and ranchers to food processors. On farms and ranches, producers can use Brix scores to determine when **forages**, plants that are fed to livestock, contain maximum nutrition and are ready for animals to eat or are ready to be cut, dried, and turned into hay. Other on-farm uses include routine testing of fruits and vegetables to determine quality and readiness for harvest, such as checking to see if the sugar content in grapes is high enough to harvest for wine production. In the food production process, Brix scores are used to create tomato sauce. This is an energy-intensive process, most of which is used to remove water from the tomatoes. So manufacturers look for tomato crops and varieties with a higher Brix value. The higher the value, the less water will need to be extracted from the tomatoes.

ENGAGE:

This activity is designed to review the concept of refraction of light. The basis of the Brix refractometer is the use of a prism that measures how much light is refracted by the plant sap.

Teacher Preparations Prior to Class:

1. Label the five 100 mL beakers or cups 1-5.
2. Label the five 50 mL beakers or cups 1-5.
3. Create each solution below in the appropriately labeled 100 mL beaker or cup.
   - Solution 1: 100 mL plain water
   - Solution 2 (4% solution): 100 mL water, 4 grams of sugar (1 teaspoon)
   - Solution 3 (8% solution): 100 mL water, 8 grams of sugar (2 teaspoons)
   - Solution 4 (16% solution): 100 mL water, 16 grams of sugar (4 teaspoons)
   - Solution 5 (20% solution): : 100 mL water, 20 grams of sugar (5 teaspoons)
4. Pour 40 mL of each solution into the appropriately labeled 50 mL beaker or plastic cup.
5. Place the 100 mL beakers somewhere they will not be disturbed. These will be used in the Explore portion of the lesson.

PROCEDURE:

1. Place a colored straw or rod in each beaker, turning the beakers and straws in the same direction.
2. Ask students to draw the setup and describe what they see. Students should look at the beakers by getting their eye level on the level of the meniscus, not by lifting the beakers up or looking down at them.
3. Guide students to notice the angle of the rod in each solution, and ask them what causes the optical displacement effect in the straws that they are observing. The straws appear to be bent where they enter the solutions. Ask students where they have seen this effect before, and what causes it.
   a. The density of each solution causes light to **refract** or bend as it passes through the solution. The plain water has the least refraction, while the water with 20% solution of sugar (5 teaspoons) has the most. The density of the sugar-water increases the optical displacement in this demo and causes the light to bend as it passes through the solution.

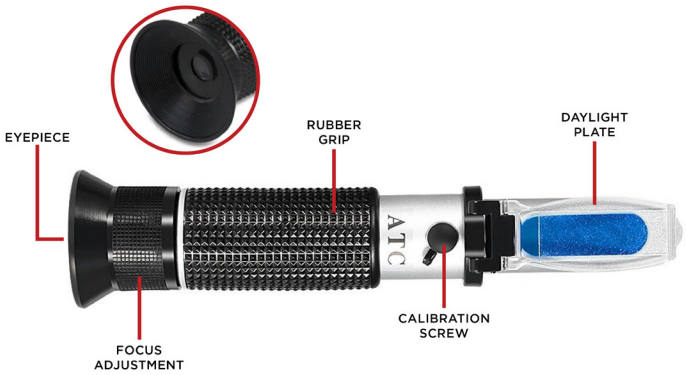
INTRODUCTION:

In this lab you will be investigating the sugar content of plant materials. Scientists, like you, can use light to estimate the amount of sugars in a plant with tool called a refractometer. A refractometer measures how much light bends, or refracts, as it passes through a liquid. This angle is then used to estimate the sugar concentration in the plant, fruit or vegetable. Refractometers are used by agriculturalists, winemakers, fruit and vegetable buyers, food processers, beekeepers and many more. The refractometer uses a unit called a Brix value or score. This is one of the **quantitative** (numeric data) values used to determine if a plant product (fruit, vegetable, grass) meets the standard for harvesting.

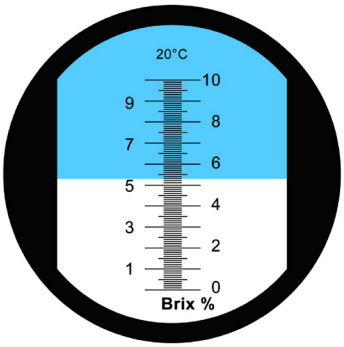
PROCEDURE:

Activity 1: Calibrate the Refractometer

1. Place two to three drops of distilled water on the prism surface.
2. Close the daylight plate so the water spreads across the whole surface without air bubbles or dry spots. Wait 30 seconds.
3. Look through the eyepiece toward a light source. You should see the graduations clearly. If you need to focus, turn the eyepiece to the left or right until it focuses. The upper portion of the field should be blue and the lower white.
4. Use the small screwdriver to turn the screw on the top of the refractometer until the blue and white fields meet at the zero graduation.
5. Clean the plate with a soft, damp cloth before placing another sample on the prism.



Parts of a Refractometer



Reading a Brix Scale

https://www.kibeland.com/products/pd_31

Activity 2: Practicing Reading the Brix Scale

1. Make sure that the refractometer has been cleaned with a soft, damp cloth if used before.
2. Place two to three drops of the sugar solution on the prism surface.
3. Close the daylight plate so the water spreads across the whole surface without air bubbles or dry spots. Wait 30 seconds.
4. Look through the eyepiece toward a light source and read the scale to determine the Brix reading (where the blue field and white fields meet). Record your information in Table 1.
5. Clean the refractometer with a soft, damp cloth.
6. Repeat steps 2-5 for each of the sugar solutions.

Table 1: Brix Reading of Sugar Concentration in a Solution

Sugar Solution	Brix Reading	Actual
1		
2		
3		
4		
5		

Activity 3: Taking the Brix Measurement of Forage

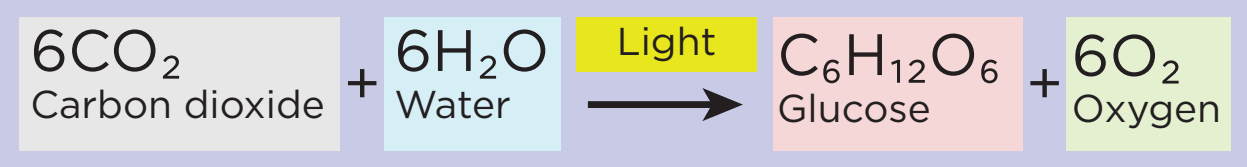
1. Record the relevant sampling information (location, time of day, etc.) in Table 2.
2. Select samples from plants that are healthy and are free of dirt and moisture. If necessary, dry with paper towel. Try to use only one variety or plant at a time (collect all alfalfa or all Bermuda grass).
3. Grasp a handful of the plant you have selected to sample from the approximate height that the animal would graze and tear them off the stem.
   a. If the plants are taller than 6 inches, take the top 4-6 inches of leafy materials.
   b. If the plants are shorter than 6 inches, remove the top 4-5 inches leaving 1-2 inches on the plant.
   c. Gather all the plant material together in a large container, then mix well.
4. Place a sample of mixed plant material just larger than the bowl of the garlic press. You will need to press the plant material down to fit inside the bowl. Hold the garlic press over the collection bowl and firmly press the handle down to squeeze the sap from the plant materials. Collect the sap.
5. Use a plastic pipet to transfer two to four drops of sap onto the daylight plate.
6. Close the sunlight cover and point refractometer toward light source. Focus the eyepiece by turning the ring to the right or left. Locate the point on the graduated scale where the blue and white fields meet. Take a reading and record in Table 2.

Table 2: Brix Readings in the Field

	Sample 1	Sample 2	Sample 3	Sample 4
Location				
Time of Day				
Temperature				
Weather Conditions (cloudy, humid, windy, etc.)				
Plant Sampled				
Brix Reading				

EXPLAIN:

Photosynthesis is the process of transforming light energy into chemical energy that a plant uses for life processes. Photosynthesis is one of the most important processes on our planet. Without it, life as we know it wouldn't be possible. While the equation below is correct, the process is much more complex .



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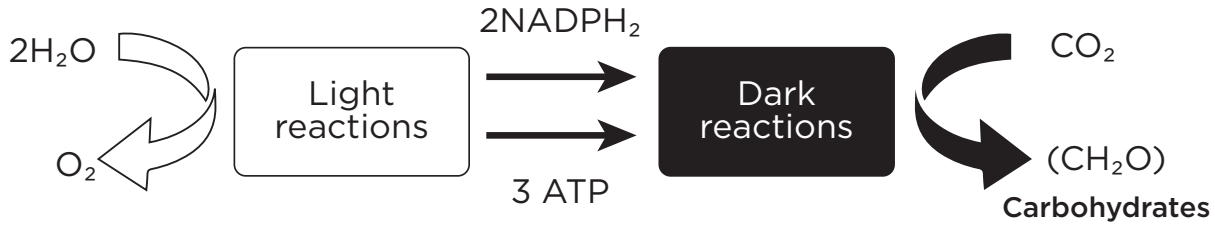
Photosynthesis occurs in two phases: light dependent and dark. In the light-dependent phase, the energy from light is captured by plant pigments and used to break the bonds of water to get electrons to create ATP and NADPH. These products are then used in the dark phase to break down carbon dioxide, build glucose and other macromolecules in the Calvin-Benson cycle. Perhaps a better way to depict the process is:



The entire point of photosynthesis is to produce the glucose needed for the plant to maintain cellular respiration, which occurs all day and all night. **Cellular respiration** is the process that uses glucose produced during photosynthesis to create ATP that provides the energy cells need to function. The plant allocates part of the glucose produced during the day into storage as starch. During the night, this storage reserve is used for cellular respiration. Research has shown that plants maintain a constant rate of respiration during nighttime and have run out of most of their starch stores by morning.

It is important to understand the photosynthetic process because it is critical for the growth and reproduction of plants. This is at the heart of the global agricultural system — the one that provides food, clothing, shelter and fuel to everyone on the planet.

ELABORATE:

From an agricultural standpoint, it is important to realize that rates of photosynthesis will vary throughout the day. Levels of photosynthetic activity are lowest in the early morning and highest in the late afternoon hours, around 6 p.m. This is important information for ranchers. Livestock like cattle eat forages, grasses, grains and legumes, to gain weight. Cattle are ruminants, meaning they have a divided stomach that contains microbes that break down food to release nutrients needed for growth. Research has shown that cattle fed a diet higher in plant sugars gain weight and produce milk in greater quantities (**cattle performance**). This means that if ranchers are growing grass to make hay, mowing in the late afternoon will produce a better forage. It should also be noted that sugar concentrations within the plant will vary from the bottom to the top of the plant. The top of the plant will have more sugar than the bottom.

While the Brix score is widely used in agriculture, it only provides an estimate of the amount of sugars in a plant. The estimates can range widely depending on environmental factors like barometric pressure, temperature, moisture, time of day, fertilization, crop species and maturity of the plant. Most professionals will use the Brix value in conjunction with other, more **qualitative**, methods to determine a plant product's readiness.

EXTEND:

You can use a refractometer for a variety of investigations with students. Here are some ideas to extend the lesson:

- Have students sample store-bought vegetables such a spinach and compare to Brix values from the sap of a growing plant.
- Have students sample different varieties of tomatoes to determine which has the highest Brix value and would be best for canning or processing.
- Compare the Brix values in different leafy greens to determine which would make a good salad mix.
- Compare various plant parts (leaf, stem, root, growing tips) from the same plant. Compare samples from the sunlit to the shaded parts of the plant.
- Compare time of day and season to find patterns of variance in the glucose produced. After 5 p.m., many plants move their glucose from the leaves to the roots. Help students discover the symbiotic relationship plants have with soil bacteria to get the nutrients they need.
- Make readings in adverse weather conditions. When the barometric pressure drops due to an oncoming storm, many plants shift their glucose storage into their roots, an adaptive mechanism that allows them to survive above-ground damage and maintain the strength to restart growth.
- If any disease-ridden or pest-damaged plants are available in your garden or sampling field, have students compare the Brix values for those plants compared to healthy plants and explain what they find. Insects cannot digest most plant proteins and prefer plants with high amino acids in sap. The sugar produced in photosynthesis is used by plants to build proteins, giving the plant insect resistance.
- Compare crops to weeds in Brix values. Higher Brix values in weeds indicate the growing conditions are favoring weeds over crops and need to be adjusted.
- Compare their results to published Brix charts and ask students to explain differences.

  Many Brix charts are readily available through online sources. Here is one from Ohio State https://ohioline.osu.edu/factsheet/HYG-1651

  If you prefer to purchase charts this source has laminated charts for $4.99: https://www.nisupply.com/products/laminated-refractometer-brix-chart-4-fruits-vegetables-1
- Compare plants as the first frost approaches. Sugar produced in photosynthesis gives plants some frost resistance. Have students make predictions and follow-up observations.

EVALUATE:

1. What might cause differences in Brix values between two plants of the same species in the same pasture?
   - **Different nutrient availability**
   - **Different water availability**
   - **Different relationship with microbes**
   - **Disease**
   - **Drought**
   - **Fertilizer**

2. What factors can affect the Brix value of a plant?
   - **Weather conditions**
   - **Barometric pressure**
   - **Temperature**
   - **Moisture**
   - **Time of day**
   - **Moisture**
   - **Stage of growth**
   - **Species**

3. Explain the role of the light-dependent and dark phases of photosynthesis in the production of glucose.
   - **The light-dependent phase is responsible for the breakdown of water to get H+ ions (electrons) and create ADP and NADPH.**
   - **The dark phase is responsible for breaking down carbon dioxide to build glucose and other macromolecules in the Calvin-Benson cycle**

4. Why does sugar content of forages vary throughout the day?

   In the morning, the plant's sugar content is low because the plant doesn't photosynthesize at night and must use its starch stores to power cellular respiration. The plant is only beginning to produce glucose again as the sun rises. As the day goes on, photosynthetic activity increases and more sugar is being produced so that, by the evening, the plant has replenished its stores for the coming night.

5. Why do sugar values vary between parts of the plant (leaf, upper stem, lower stem, roots)?

   Sugars are transported to the parts of the plant that need them. The leaves of the plant have higher concentrations of sugar, because it is the site of production. The upper portion of the stem has high concentrations because it is the growth point, and in order to produce more cells, the cells need fuel for respiration.

6. Why is it advantageous for agriculturalists to know the sugar content of their crops?
   - **Determines best time to harvest fruits, vegetables, forages**
   - **Can be used to determine honey sweetness**