INQUIRY & INVESTIGATION

A Question-Based Approach to Teaching Photosynthesis, Carbohydrate Partitioning, and Energy Flow

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Abstract

To help high school students develop a deeper understanding of energy and matter flow in biological systems, a key goal of the Next Generation Science Standards (NGSS), we have designed a series of inquiry-driven modules that experimentally explore photosynthesis in the context of carbohydrate partitioning. Carbohydrate partitioning refers to the distribution of photosynthesis products from source organs, such as leaves, to growing or storage tissues. These cost- and time-effective modules help students develop a more integrated understanding of how energy flows from light into leaves before moving outward to other parts of the plant and ultimately into other organisms. Our approach of teaching carbohydrate partitioning along with photosynthesis greatly expands the number of NGSS core ideas and cross-cutting concepts in an integrated manner, empowers students to see how photosynthesis and carbohydrate partitioning are central to their existence, and provides a rich platform for experimentally addressing questions related to photosynthesis, crop production, global climate change, plant physiology, and the carbon cycle.

Key Words: *photosynthesis; carbohydrate partitioning; NGSS.*

○ Introduction

The Case For Including Carbohydrate Partitioning When Teaching Photosynthesis

Photosynthesis is a suggested topic in the Next Generation Science Standard (NGSS, 2013), is prominent in most high school curricula, and is arguably the most important metabolic pathway in our global ecosystem. Essentially all energy that flows into biological systems ultimately originates from this process. We are all familiar with photosynthesis, and it touches our lives every day. When we eat, turn on our car, or walk into our homes, we are utilizing food, fuel, and building materials produced through photosynthesis. Superficially, most of our students know this, but few have a deep understanding of how energy flows out of photosynthetic tissues to the rest of the plant before entering into other biological systems. The disconnect between photosynthesis and energy flow within and out of plants may result simply because photosynthesis is often taught in the absence of carbohydrate partitioning—the process of distributing photosynthates in plants. As a result, what happens in photosynthetic tissues and organs such as a leaf often seems isolated and disparate—a separate topic on energy capture in plants instead of the central energy source for our own lives that it is.

To help high school students and teachers gain a deeper understanding of energy flow from leaves to plant parts, we created a series of inquiry-driven modules that experimentally explore photosynthesis in the context of carbohydrate partitioning. These modules were developed over the course of five years in an annual four-day workshop for high school teachers and students. Collectively, over the duration of this Leaves of Green project (2011–2015), creative input was integrated from 30 different teachers and 91 high school students, representing 22 schools in New York, New Hampshire, and Vermont.

Simply defined, photosynthesis is the biological process of converting light energy into chemical energy (i.e., reduced hydrocarbons).

Simply defined, *photosynthesis* is the biological process of converting light energy into chemical energy (i.e., reduced hydrocarbons), and *carbohydrate partitioning* describes how sugars produced during photosynthesis are distributed from source organs (primarily leaves) to sink organs (e.g., roots, stems, flowers, and fruits). In sink structures, these sugars are catabolized, anabolized, or stored (Bihmidine et al., 2013). When introducing this idea to students, we describe the leaf as a sugar factory, the collective phloem as an interstate highway system that transports the sugars to consumers (i.e., distal organs), and the phloem companion cells as on- and off-ramps that load and unload the phloem sieve tubes (avenues of translocation). This effective analogy helps students see that sink organs, like roots and fruits, are dependent upon imported sugars just like consumers are dependent

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upon the delivery of goods. Understanding that sugars are transported out of leaves, can be redirected, or even fail to be delivered helps students realize that carbohydrate partitioning is essential to plant functions. For example, if a flower or developing fruit fails to receive the energy it needs in the form of sugars, it will not mature properly as demonstrated in developing apples that are girdled (Beruter & Feusi, 1997). Likewise, growth rates are a function of how well source-to-sink transport works, analogous to slow or blocked traffic in our sugar interstate analogy. Sink-to-source transport can be slowed by biotic and abiotic factors such as heat, insects, and water availability, and slowing this process has profound impacts on humans by lowering crop yields or reducing the speed at which an area can be reforested, for example (Osorio et al., 2014). Furthermore, changes in atmospheric carbon dioxide (CO_2) levels and climate are expected to affect photosynthesis and carbohydrate partitioning (Long et al., 2004).

Experimental Modules to Conceptually Link Photosynthesis, Carbohydrate Partitioning, and Flow of Energy and Matter into Other Biological Systems

Teaching carbohydrate partitioning alongside photosynthesis helps students understand how energy and matter flow from leaves to other parts of the plant. In our workshop teachers and students learned and helped develop informative ways to follow carbon as it moves from CO_2 to sugars, then to other plant molecules, and eventually back to CO_2 if respired or fermented by the plant or after consumption by other organisms. The goal of these modules is to help students develop a more mechanistic understanding of how energy and matter move out of a leaf and into the rest of the plant. It is then a small step to connect how the energy captured during photosynthesis flows as reduced hydrocarbons to other biological systems, where it is consumed through cellular respiration, fermentation, or biosynthesis of new material.

For each experimental module we follow the gradual release of responsibility framework (Pearson & Gallagher, 1983) by first demonstrating a technique, then posing a seed question, and concluding with independent learning through experimentation (Figure 1). By design, our seed questions are easily addressed experimentally in an hour or less, are not intuitive, and lead to more inquiry. For these reasons, these modules can be adopted as stand-alone labs to be completed in 60 to 90 minutes. They also can be used as springboards into further inquiry. In our workshop, teams of teachers and students were given an additional 60–90 minutes (the equivalent of a lab period for most high schools) to pose and answer a second question of their choosing using the same technique. The strength of this approach is that teachers are able to customize modules to better fit their individual curricular needs and professional strengths, while students are able to explore their own interests.

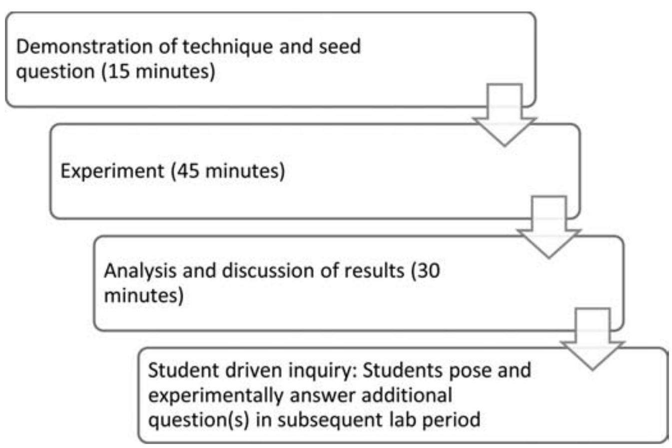

Figure 1. Flowchart for introducing each experimental module.

Following Carbon to Understand Energy Flow

Below we outline how we conceptually "follow the carbon" as it moves from CO_2 to a source leaf, then to a sink organ, and finally into another organism (yeast), where it is fermented and released as CO_2, thereby completing the carbon cycle (Figure 2). Each module can be completed in 60–90 minutes using the referenced techniques and protocols in the Leaves of Green manual, which can be downloaded at http://www.smcvt.edu/pages/get-to-know-us/faculty/lubkowitz-mark.aspx.

○ Lab 1: Stomata

What Occurs

Carbon enters into plants as CO_2 through pores called stomata, where it is then fixed through the Calvin cycle. Stomatal pore size is regulated by a pair of cells called guard cells. Guard cells regulate the pore opening and closing, allowing CO_2 to diffuse into leaves, while O_2 and H_2O diffuse out of leaves.

Seed Questions & Answers

- *Does stomatal density differ between the photosynthetic (green) and non-photosynthetic (white) tissues on a variegated leaf?* There should be no difference, because the stomata developed prior to when the sections became white or green.
- *How might stomatal density differ between the adaxial (top) and abaxial (bottom) sides of the leaf?* Stomata are typically only on the abaxial side—the shady side—in dicots to minimize water loss. Monocots often have stomata on both sides since their leaves generally grow in a more upward direction, and one side does not necessarily receive more light than the other.

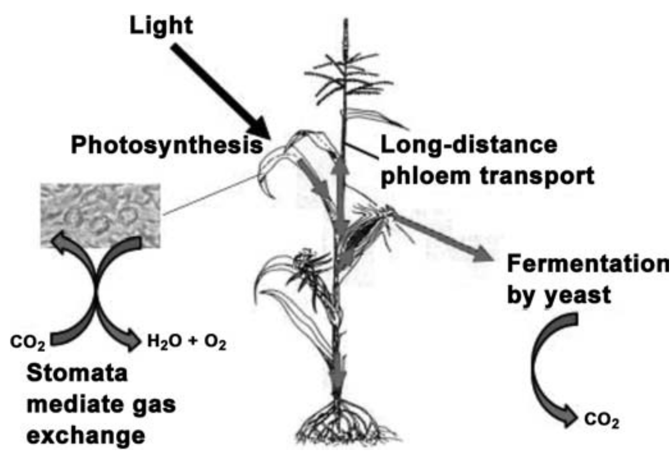

Figure 2. Experimental modules "follow the carbon" as it moves from source to sink in plants before being used as an energy source by yeast.

Plant Material to Collect Prior to Lab

- Variegated leaves from ornamental grasses, *Coleus* plants, or other species with green and non-green leaf sectors
- Mature dicot leaf from a plant (including broad-leafed trees) growing in full sun

What to Measure & Why

Stomatal density, to see the influence of development or environment.

How to Measure

Dermal impressions are made using Superglue, which is reliable, fast, and shows remarkable detail (Freeman, 2010). Impressions are then viewed under a microscope at 100–400X.

○ Lab 2: Transpiration

What Occurs

CO_2 enters a leaf through stomata, where it is dissolved in water provided during transpiration. Water evaporates and exits via stomata and, along with adhesive and cohesive forces, creates the negative pressure that drives transpiration.

Seed Question & Answers

- *How might transpiration rates differ with respect to canopy position in a tree?* The outer canopy is typically a harsher environment because of increased sun, wind, and heat exposure—all of which can affect transpiration. Depending upon the time of day and weather, the stomata on leaves of exterior branches that receive more light and experience higher temperatures may be closed, which will greatly reduce transpiration. The state of the stomata (open or closed) can be assessed with dermal impressions.

Plant Material to Collect Prior to Lab

- Woody branches with leaves from the interior and exterior of a tree canopy

What to Measure & Why

Transpiration rates and stomatal density, to explore how environmental conditions affect both of these, and to observe the relationship between stomatal density and transpiration.

How to Measure

Transpiration is measured using a pressure sensor (Collins et al., 2012) or a Potometer (http://www.phschool.com/science/biology_place/labbench/lab9/design.html), and the state of stomata (open or closed) is observed through dermal impressions.

○ Lab 3: Photosynthesis

What Occurs

Light energy is captured in photosystems I and II to produce ATP and NADPH, and O_2 is formed as a byproduct. The ATP and NADPH are subsequently used to fix CO_2 and produce sugars for export to sink organs, where they are used as building blocks for other organic molecules, stored, or respired in the mitochondria.

Seed Question & Answers

- *How might light intensity during development affect photosynthesis?* Leaves on the outside of a tree canopy are typically exposed to more sunlight than those of the interior and, as result, have higher chlorophyll concentrations and a greater photosynthetic output per unit area when in bright light.

Plant Material to Collect Prior to Lab

- Leaves from the interior and exterior (preferably sunny side) of a tree canopy

What to Measure & Why

Photosynthesis of both interior- and exterior-canopy leaves under the same bright-light condition, to explore how light intensity during development affects photosynthetic output. Repeating the experiment with the leaves in the dark can help illustrate that plants also metabolize carbohydrates, as shown by the production of CO_2 and the consumption of O_2 as a result mitochondrial respiration.

How to Measure

Photosynthesis is measured through the production of O_2 and/or the consumption of CO_2 in a closed chamber using sensors produced by Vernier.

○ Lab 4: Sucrose in sink and source organs

What Occurs

Sucrose (the primary sugar that is transported by most plants) is exported via the phloem to sink organs where it can be (a) stored (often as starch), (b) used as a building block for synthesis of other plant molecules, or (c) metabolized during respiration in the mitochondria.

Seed Questions & Answers

- *How might sucrose concentration differ in sun versus shade leaves?* Sun leaves receive more light and will typically produce more sucrose per unit area than a shaded leaf on the same plant.
- *How might developing and mature apples or bananas differ in sucrose concentration?* An unripe apple or banana will have a lower sugar concentration than a ripe one because carbohydrates are stored as starch while these fruits develop. During ripening this starch is rapidly converted to sugars.
- *Which sink, garlic or any sweet fruit, will have a higher sucrose concentration and why?* Garlic has a surprisingly high concentration of sucrose but does not taste sweet because it is protected from herbivores by an arsenal of sulfur-containing compounds.

Plant Material to Collect Prior to Lab

- Shade and sun leaves from the same plant
- Mature and developing fruits (sink organs) from the same plant
- Garlic cloves and any sweet fruit (sink organs)

What to Measure & Why

Sucrose levels in source and sink organs, to see how light influences sucrose production in leaves, and to quantify the extent that fruits and other sink organs accumulate sugars.

How to Measure

Low-cost refractometers (Atago Master 20PT) are used to directly measure sucrose concentration in liquid squeezed from plant material.

○ Lab 5: Starch localization in sink and source organs

What Occurs

Sucrose is produced in source leaves and then exported. In many plants carbohydrates are stored as starch in sink organs. Sucrose that is not exported from source leaves is also stored as starch in the leaf.

Seed Questions & Answers

- *How might the different sectors in variegated leaves show differences in starch accumulation?* White sectors do not undergo photosynthesis or accumulate starch.
- *Do leaf starch levels differ in the morning and at the end of the day?* Starch levels peak at the end of day/beginning of night since sucrose not exported from source tissues during the day is converted to starch and temporarily stored in these same tissues. Starch levels will decrease throughout the night as it is broken down and exported as sucrose, achieving a minimum starch level at dawn.
- *How might sweet and field corn differ with respect to starch staining and sucrose concentration?* Field corn stores its carbohydrates as starch, whereas sweet corn contains a mutation in the starch biosynthetic pathway that causes it to accumulate simple sugars. If given enough time, these simple sugars are eventually converted to starch in sweet corn, which is why freshly harvested sweet corn is generally considered tastier. If fresh sweet corn is available, a nice follow-up experiment is to analyze kernels from the same ear over the course of a week to see how the sugar-to-starch ratio changes over time.

Plant Material to Collect Prior to Lab

- Variegated leaves
- Leaves collected at near dawn and kept in the dark and leaves collected during the day from the same plant
- Ripe sweet corn and field corn (not frozen or dried)

What to Measure & Why

Starch in source and sink organs, to see where plants store carbohydrates spatially and temporally.

How to Measure

Starch can be reliably visualized in plant tissues and cells using Lugol's stain (Sigma Catalog #62650) on hand sections viewed under a microscope at 100–400X (Florine et al., 2010; www.practicalbiology.org/areas/introductory/energy/photosynthesis/testing-leaves-for-starch-the-technique,73,EXP.html).

○ Lab 6: Fermentation

What Occurs

Carbohydrates produced through photosynthesis flow into another biological system—yeast—where they are utilized as an energy source through fermentation.

Seed Question & Answers

- *How might the type of carbohydrate—e.g., starch or glucose—influence fermentation?* Yeast is able to ferment glucose more easily than starch.

Plant Material to Collect Prior to Lab

- Sweet corn and field corn (fresh, dried, or frozen)

What to Measure & Why

Fermentation of sugars, to illustrate how energy and matter flows from plants into other biological systems. Parallels can be drawn to respiration in animals and how humans use carbohydrates as biofuels.

How to Measure

Carbon dioxide is captured over time and used to calculate the rate using a fermentation tube (Caroline Biological # 732345).

Independent Projects

Our experimental modules help students develop a schema for seeing energy and matter flow from leaves outward to other biological systems. To reinforce, build upon, and allow students to explore their interests related to energy flow, we use this same suite of techniques for independent projects in our workshop. Students and teachers are given an additional five hours to pose and answer a question that is an extension of the previous experiments (Table 1). Each student group then presents their findings in a short research presentation. Based on our exit survey, 51 percent of students ($N = 85$) stated that this was the most helpful part of the workshop. To quote one student, "The fact that we could run wild with our ideas (was the best part). . . . we were taught the basic concepts and techniques so we had absolutely everything we needed and all information to be able to answer any question we wanted."

As shown in Table 1, the experimental modules described here are quite flexible and can be used to investigate a broad range of topics. Furthermore, our approach successfully integrates the three domains of the NGSS: the practice of science, the cross-cutting concepts, and the disciplinary core ideas. One of the goals of the NGSS is to help students see beyond disciplinary boundaries and understand the integrated nature of science. Photosynthesis is a common topic in high school biology, but it also lends itself to exploring ecological and physiological topics as well as core ideas in chemistry and physics; after all, understanding photosynthesis requires a working knowledge of many concepts, including electrons, covalent bonds, light, and free energy, to name a few. Teaching photosynthesis along with carbohydrate partitioning and energy flow into other biological systems greatly expands the number of core ideas and cross-cutting concepts in an integrated manner (Table 2). Finally, because our modules expand upon the number of core topics and cross-cutting concepts, participating teachers have been able to partially or completely adopt these

Table 1. A sample of independent project titles from the Leaves of Green workshop. Each of these questions was posed by a teacher + student team, then experimentally answered in three to five hours. This time frame is comparable to from one-half to a full week of lab time in most high school schedules.

- The future of stomatal behavior and transpiration in a CO_2-rich environment
- Biofuel from an invasive species?
- Effect of aphid infestation on goldenrod as measured by sucrose concentration in plant tissue and transpiration rates
- How do changes in temperature affect transpiration rates?
- How does proximity to a developing fruit affect the photosynthetic rate?
- Is photosynthesis affected by a plant's proximity to a road?
- Is photosynthesis affected in leaves that are proximal or distal to a flower?
- Comparison of photosynthesis in C4, C3, and CAM plants.
- How does carbohydrate partitioning change along the axis of a plant?
- Does maturity of fruit affect photosynthesis in neighboring leaves?
- Are green flower petals sources or sinks?

Table 2. Teaching photosynthesis in the context of carbohydrate partitioning greatly increases the number of disciplinary core ideas addressed.

A. Disciplinary Core Ideas covered through the teaching of photosynthesis	**Example of how this disciplinary core idea might be addressed while teaching photosynthesis**
LS1.A: Structure and Function	The structure of the chloroplast inner membrane is critical to establishing the proton motive force.
LS1.C: Organization for Matter and Energy Flow in Organisms	CO_2 is fixed by leaves and reduced using light energy. These reduced molecules (primarily sugars) are the fuel that flows through biological systems.
PS1.B: Chemical Reactions	The reactions of the Calvin cycle
B. Additional Disciplinary Core Ideas covered through the teaching of photosynthesis alongside carbohydrate partitioning	**Example of how this disciplinary core idea might be addressed while teaching photosynthesis and carbohydrate partitioning**
LS1.B: Growth and Development of Organisms	Sugars move from source organs (leaves) to sink organs (developing leaves, shoots, roots, flowers, and fruits), providing the energy and matter for plant growth and development.
LS2.B: Cycles of Matter and Energy Transfer in Ecosystems	Energy captured through photosynthesis is used to produce carbohydrates, which are consumed through cellular respiration or fermentation as they flow trophically through ecosystems.
LS3.B: Variation of Traits	Genetic variation can influence how plants store carbohydrates (e.g., sweet corn versus field corn). Genetic variation also influences where and how plants use their photosynthates, which can influence their morphology. This can be seen in familiar cultivars of *Brassica oleracae*, such as kohlrabi, cabbage, Brussels sprouts, and broccoli. These diverse structures are produced by small genetic variations within the same species.
ESS2.D: Weather and Climate	Weather and climate (e.g., drought) can affect plant growth by altering photosynthesis and carbohydrate partitioning, which in turn can be seen in crop yields.
ESS3.D: Global Climate Change	Changes in CO_2 as well as temperature affect photosynthesis and carbohydrate partitioning.
ETS2.B: Influence of Engineering, Technology, and Science on Society and the Natural World	The role of breeding and GMOs as it relates to crop yields is a natural extension of carbohydrate partitioning.

modules in many of their courses, including: Biology, Global Sciences, Environmental Systems, Earth Science, Science of Food, Botany, AP Biology, and AP Chemistry.

○ Summary

Teaching carbohydrate partitioning alongside photosynthesis has allowed our experimental modules to become successful platforms for helping students develop a deeper, integrated, and more holistic understanding of energy flow in plants. Taking the additional step of examining plants as an energy source completes the conceptual jump of energy flow to other biological systems. These modules are cost-effective, technically accessible, and malleable enough for student-driven inquiry. Finally, our approach helps students develop a framework for scientific thinking by learning content through experimentation.

○ Acknowledgements

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