Green Means Go: The Role of Pigments in Photosynthesis as Measured by Starch Storage

I. Abstract

The level of photosynthetic activity as determined by the presence of starch stores was measured for pigmented leaves of seven plant species. The plants were exposed to morning sunlight, then the leaves were picked and pigments extracted by boiling the leaves in an 80% ethyl alcohol solution. A potassium iodide solution was used to stain the starch stores dark blue, and the levels of starch accumulation were compared with leaf color. The highest levels of starch accumulation were found in the green chlorophyll regions, followed by the orange-to-red carotenoid regions. The violet-red and pink anthocyanin regions, while expected to be free of starch accumulation, were found to have low levels of starch storage. These regions may include carotenoids or chlorophyll whose pigment was masked by the presence of the anthocyanins, and levels of pigments may be varied due to the different species of plants used.

II. Introduction

The process of plant photosynthesis creates all the food the world consumes, either directly as plant sources of food, or indirectly as feed for animals. Nearly 300 million acres of farmland in the US are planted with food crops intended for human or animal consumption (US EPA 2009). Perhaps even more important than providing the base of the food web, photosynthesis also removes carbon dioxide and releases oxygen into the atmosphere, providing clean air for life (Hall and Rao 1999).

However, photosynthesis is a relatively inefficient process. For most plants only one to two percent of sunlight falling on the leaves is actually converted into the products of photosynthesis (Gust 1996). Determining the photosynthetic contribution of each type of pigment has applications for increasing efficiency of food production, cleaning carbon dioxide from the atmosphere, and even possible wider application, such as medical technology and environmental clean-up (Vermaas 2007).

Chlorophyll is the main pigment involved in photosynthesis, but at least two other pigments, carotenoids and anthocyanins, play supporting roles in photosynthesis (Vermaas 2007). Chlorophyll produces green colors in leaves, while carotenoids produce yellow to orange and bright red colors, and anthocyanins produce violet and dark red to pink colors (Morgan et al. 2010).

This experiment explored the question of which pigments support photosynthesis as measured by levels of starch stored in a multi-colored leaves of various plant. Because chlorophyll is the main photosynthetic pigment, the highest starch levels were expected in the leaves with the most chlorophyll, as judged by the most green color on the leaves.

According to this hypothesis, if chlorophyll is the main contributor to photosynthesis, then the green areas of the leaves will have the highest starch content. Anthocyanins are not active in photosynthesis, so the areas of the leaf with violet, blue, pink, or dark red colors are not expected to have starch stores (Nielsen et al. 2011). Cartenoids play a role in regulating temperature as well as photosynthesis, so the yellow-to-red areas of the leaf will likely have starch content lower than the green areas but higher than the areas pigmented by anthocyanins (Deming-Adams and Adams 1996).

III. Materials and Methods

The procedure for this experiment was based on the Investigating Biology lab manual with one major exception: the experiment outlined in the manual specified using one *Coleus* leaf, but no *Coleus* plants were available (Morgan and Carter 2010). Instead of the specified *Coleus*, seven multi-colored leaves from different plant species were used to include a range of pigments in the experiment.

The leaves were collected between 9am and 10am, allowing for exposure to two to four hours of normal morning sun. The coloring of each leaf was documented, and the pigments were extracted by boiling the leaves in an 80% ethyl alcohol solution until they became colorless. After boiling, the leaves were rinsed with deionized water and stained with a solution of potassium iodide. Starch stored in the leaves reacts with potassium iodide, staining the starch dark blue while leaving the rest of the leaf unstained, thus making the stored starch visible.

The stained areas of starch storage on each leaf were examined and related to the original pigments of the leaf to determine which areas of pigment had the most photosynthetic activity.

The important measured variables in this experiment included the colors and related pigments and accumulated starch stores in each leaf. The independent variable studied was the pigments in the various leaves, and the dependent variable was the accumulated starch stores, an indicator of photosynthetic activity.

Using one leaf from the same plant would allow for many variables to be controlled, including plant species, light and temperature exposure, soil and pot environment, amount of fertilizer and water received, and age of plant. Unfortunately this experiment was not able to use only one plant species, so these variables were all uncontrolled. In addition to these uncontrolled variables, sources of error include inexpert pigment extraction and starch measurement. Repetition was accounted for by recording data for the leaves studied by other groups in the lab.

IV. Results

Starch accumulation was greatest in the green areas of the leaves studied. For leaves with several green areas, such as leaf 6 seen in Figure 1, the starch accumulation was greater in the darker green regions than the lighter green areas.

Figure 1.
Sample of leaves prior to pigment extraction (1a) and after pigment extraction (1b)

Starch accumulation was also seen in orange and bright red areas of the leaves, such as in leaves 2 and 7 (Figure 1), though to a lesser extent than in the green regions. Very little starch storage was present the pink and red-violet leaves, and no starch storage was evident in white regions (Table 1).

Table 1
Leaf Color, Pigment, and Levels of Starch

Leaf Color	Associated Pigment	Starch Accumulation
Dark Green	Chlorophyll	Very Heavy
Light Green	Chlorophyll	Heavy
Bright Red	Carotenoids	Moderate
Orange	Carotenoids	Moderate
Pink	Anthocyanins	Light
Red-Violent	Anthocyanins	Light
White	None	None

V. Discussion

The hypothesis of the green pigment chlorophyll being the greatest contributor and the carotenoids being a lesser contributor to photosynthesis in leaves was borne out by this experiment. The findings that the white regions of the leaves had no starch accumulation were also in line with expectations.

However, the presence of starch was detected in small amounts in the pink and red-violent regions of some of the leaves. This was an unexpected result, as the pigments responsible for this coloring, anthocyanins, aren't active in photosynthetic starch production. It's possible that other pigments, perhaps carotenoids, were also present in these areas and are responsible for the low levels of starch storage observed.

The varied levels of starch storage seen in the regions where color indicated the presence of carotenoid or anthocyanin pigments may be due to the different species of plants used in this experiment. If leaves from the same plant were used, the results might show a more linear relationship, because while they are certainly related, leaf color and pigment are not always accurate predictors of photosynthetic activity (Rouhani 1977).

Because it used leaves from various plant species, this experiment was able to include a wide range of leaf colors and pigments, but this variety also introduced a number of sources of error. Such potential error sources include a number of uncontrolled variables for the plants, including plant species, light and temperature exposure, soil and pot environment, amount of fertilizer and water received, and age of plant. Even the light received the morning of the experiment was varied, since some plants had been inside while others were outside. Using leaves from one plant, or from several plants of the same age and species, would correct these sources of error.

In addition, the replication of results depended on different laboratory groups who may have boiled the leaves for different periods of time, potentially extracting different levels of pigments. Another possible source of error involves the subjectivity in reading the stained starch stores in the leaves. A standard way to measure the starch accumulation would help to increase the precision of this the experiment.

While the experiment introduced a number of sources of error into the results, it also raised some interesting questions and ideas for future experiments.

Some plants respond to changes in atmospheric carbon dioxide concentrations with similar changes in photosynthetic activity—in other words, more carbon dioxide actually causes these plants to grow and produce more oxygen (Gust 1996). Exploring the connection between the rate of photosynthesis in leaves of different pigments and exposure to various levels of carbon dioxide would be an interesting direction for future research. Discovering plants that thrive in high levels of carbon dioxide would have immediate applications in areas where carbon dioxide pollution is an issue, such as cities, along roadways, or near factories.

The link between photoprotection of leaf pigments, temperature, and efficiency of photosynthesis could also be explored in future experiments. Plants enter a cycle of photorespiration when temperatures rise, but certain levels of sunlight are needed for oxygen and sugar synthesis (Vermaas 2007). Anthocyanins have been shown to play a role in photoprotection as well as temperature regulation, with the implication that levels of these pigments might expand the range of conditions that permit photosynthesis (Zeliou 2009; Gould 2002). An experiment exploring the balance of sunlight and temperature could help find the best environment for photosynthetic efficiency, leading to an increased yield of photosynthesis products—the food and clean air the human world depends on.

VI. Literature Cited

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