Laboratory 4: Plant Pigments and Photosynthesis

*understand the principles of chromatography
*calculate R_f values
*design an experiment in which chromatography is used as a separation technique
*describe a technique for determining photosynthetic rate
*understand the relationship between dependent and independent variables
*describe how light intensity, light wavelength, and temperature can affect photosynthesis
*design an experiment to measure how light intensity, light wavelength, and temperature can affect photosynthesis

Paper chromatography allows you to separate molecules (plant pigments in this case) based on solubility in the particular solvent, differing attractions to the cellulose of the paper (due to H bonds), molecular size and weight. Chlorophyll a is the primary photosynthetic pigment at the reaction center of all photosystems: other pigments (chlorophyll b, carotene, xanthophyll) are parts of the antennae system to funnel extra energy to "a" plus carotenoids function like "sunscreen" to protect pigments from damage by bright light. In chromatography, pigments can be identified by color, but also by R_fs - ratio of fronts - the distance the molecule traveled divided by the distance the solvent traveled. R_fs remain constant for the same molecule given the same conditions (the largest R_f possible is 1.00 -- why???)

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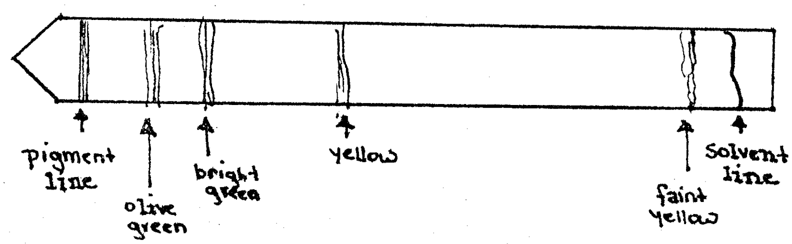
Here are the visual results of the chromatograph:



Beta carotene is carried quickly, close to the solvent front because it is very soluble in this solvent and no atoms sticking out to form H bonds.....xanthophyll is slowed by the H bonds it forms. Chlorophylls contain more exposed O and N, and are therefore bound more tightly to the paper and travel more slowly. Measurements yielded the following R_fs:

$\frac{8.2}{8.7} = .94$ carotene

$\frac{3.5}{8.7} = .40$ xanthophyll

$\frac{1.7}{8.7} = .20$ chlorophyll a

$\frac{1.0}{8.7} = .12$ chlorophyll b

	1 Blank	2 Dark	3 Unboiled	4 Boiled
Phosphate Buffer	1 mL	1 mL	1 mL	1 mL
Distilled H_2O	4 mL	3 mL	3 mL	3 mL
DPIP	—	1 mL	1 mL	1 mL
Unboiled Chloroplasts	3 drops	3 drops	3 drops	—
Boiled Chloroplasts	—	—	—	3 drops

Wavelengths of light in the visible spectrum are used for photosynthesis. There are two photosystems in the light reactions: during the non-cyclic phase, electrons are passed from photosystem II to photosystem I and finally to NADP (which also accepts an H+ from the splitting of water). The rate of these reactions might be measured several ways, but one involves dye reduction. The dye DPIP (2,6-dichlorophenol indophenol) more readily accepts e^- and H+ than NADP, and in the process will change from a dark blue to a clear solution. This change can be quantified visually by relative color intensity changes, or more precisely with a spectrophotometer (a machine that measures % light transmittance through a sample (dark blue will transmit little light, clear almost all the light).

This experiment will involve a control (no chlorophyll present so no photosystems, no change of reducing the dye), a chloroplast setup kept in the dark (how essential is light to the process?), a chloroplast setup boiled before starting (does high heat affect the process?), and a normal chloroplast setup.

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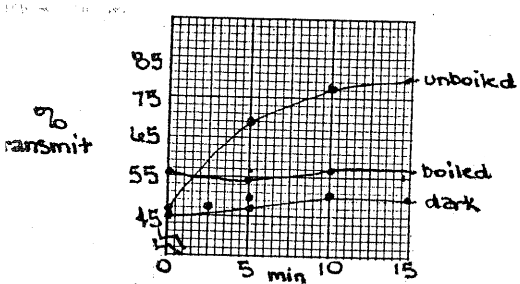
Cuvette	Time (minutes) 0	5	10	15
2 Dark	45.3	48.5	50.3	49.8
3 Unboiled	47.9	70.3	78.5	79.8
4 Boiled	56.2	54.0	57.1	56.4



Conditions vs. Rate of Photosysthesis

This is a Spec20 machine; solutions to be tested are placed in special tubes called "cuvettes" and entered into the chamber; wavelength of light to be shown through it is chosen (this lab involved 680 nm) and a meter reads what % actually gets through (% transmittance)!

Obviously, the control shows constant transmittance; the dark may receive some light as the tube is put into the Spec20 for testing, so may show some change but not much; the boiled should show no significant change; and the normal setup should show significant dye reduction and lightening over the time period!

Lab #4
Photosynthesis

1999

The rate of photosynthesis may vary with changes that occur in environmental temperature, wavelength of light,
and light intensity. Using a photosynthetic organism of your choice, choose only ONE of the three variables
(temperature, wavelength of light, or light intensity) and for this variable

- design a scientific experiment to determine the effect of the variable on the rate of photosynthesis for the organism;
- explain how you would measure the rate of photosynthesis in your experiment;
- describe the results you would expect. Explain why you would expect these results.

Question 1 Scoring Guidelines

Question 1 is the laboratory question for 1999; it focuses on **designing** an experiment to test the effects of one of three possible environmental variables on the rate of photosynthesis. After designing the experiment, students are asked to **explain** how they would measure photosynthetic rate in their experiment. The third part of the question asks students to **describe and explain** their expected results. Designing an experiment is not a new task on a free-response question, and the three-part design of the question is typical of such questions in recent AP Biology Exams. The standards were set in such a way that students could garner 7 points for experimental design, 2 points for describing expected results, and 3 points for biological explanation of results. Our typical scoring requires students to get points from all parts of a multipart question before achieving a maximum score of 10.

A. **Experimental Design:** (7 points maximum)

The following experimental characteristics may earn 1 point each:

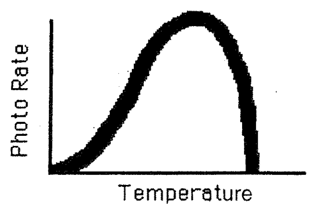
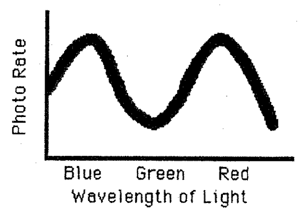
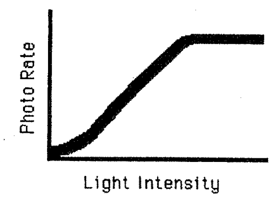
Score only the ***first*** *independent variable (temperature, λ, intensity) manipulated, and the* ***first*** *factor used by the student to measure photosynthetic rate (O_2, CO_2, etc.).*

A 3-point maximum in Section A if the experiment will not work biologically. Examples: using an organism that is not photosynthetic, or using an apparatus that biologically will not measure photosynthesis as designed (i.e. potometer or respirometer). 0 points lost for a minor flaw in technical design.

- State **hypothesis** (clear statement of a hypothesis, identifies it as a hypothesis, uses "If/then" statement
- Specify a **control group** for comparison
- Identify and **hold constant at least one experimental factor** that can affect photosynthetic rate
- Manipulate **the independent variable** (change the temperature, λ of light, intensity of light)
- Describe **what is being measured** to determine rate (CO_2 or H_2O consumption, O_2 or carbohydrate production, growth, e^- flow measured with dye reduction, production of an intermediate product, etc.)
- **Quantify** the measurement of the variable (method **and** time frame of measurement)
- Rate calculation or definition
- Verify results through sample size (>1) or repetition
- Utilize **statistical application** of data (mean, t-test, ANOVA, etc.)
- Design an **exemplary** experiment

B. **Describe expected experimental results** (2 points maximum)

- Verbal or graphic description of expected experimental results (1 point)
- Verbal or graphic description of expected results across the entire range of biological activity (1 point)
- The graphs below represent 2-point graphs, but to earn **any** points, graphs must be accurately labeled

Temperature	Wavelength	Intensity
• Rate rises with temperature to an optimum and then falls	• An "action spectrum" with highest rates in the blue and red regions of the spectrum	• Rate increases steadily to a maximum and levels off
		

How students could earn points in this section:

- One point for a reasonable **description of expected results** from experimental set-up, and a second point if description included what to expect if the independent variable extended across the range of biological activity.
- To earn points for **temperature results,** a photosynthetic rate that rose with temperature to an optimum and then fell. A description or a graph similar to the one in the table above could earn the student 2 points.
- To earn points for **wavelength results,** a photosynthetic rate showing an "action spectrum" with highest rates in the blue and red regions of the spectrum and a pronounced dip in the green. A description or a graph similar to the one in the table above could earn the student 2 points.
- To earn points for **light intensity results,** a photosynthetic rate that rose with temperature to an optimum and then leveled off. A description or a graph similar to the one in the table above could earn the student 2 points.

C. **Biological explanation of results** (3 points maximum)

- **Temperature**
  - Enzyme kinetics or metabolic changes
  - Enzyme denatures
  - Photorespiration
  - Stomatal closing with high temperature, limits CO_2, and lowers rate
  - Excessive water loss, less reactant available for reaction
  - Elaboration

- **Wavelength**
  - Absorption/reflection of light by chlorophyll
  - Accessory pigments absorbing green light
  - Relation between wavelength and energy
  - Elaboration
- **Intensity**
  - More photons hit photosystems
  - More e^- flow in the electron transport system/time
  - Plateau caused by limiting factors
  - Elaboration

How students could earn points in this section:

- In this section, students could earn points by giving solid, biological explanations for the changes in photosynthetic rates they would expect.
- The simplest way to earn 2 points was by explaining both the rise and fall of the temperature curve, both "peaks" and the "valley" of the action spectrum, and both the rise and the plateau of the light intensity curve.
- There was also an elaboration point to be given at the readers' discretion for very strong, in-depth answers.
- Answers were expected to contain some sophistication, and answers such as, "In the high temperature, the plant dies so photosynthesis is lower" or "Since light is important to photosynthesis, with higher intensities, photosynthesis goes faster," did not receive points.

1

Write in the box the number of the question you are answering on this page as it is designated in the examination.

1R1

The experiment would test ~~the effect~~ how varying wavelengths of light affect the rate of photosynthesis in a young maple tree. ~~I hypothesize~~ Four young plants would be used, each placed in a glass tank in a black closet. Each closet would have a ventilation system that maintains an air content of 79% nitrogen, 20% oxygen, and ~~10%~~ 1% carbon dioxide. The first maple tree (young, so that it would be small and able to fit in the tank that contains ~~air~~ soil and is open to the air of the closet) would be [a control] exposed to visible light from ~~a~~ a lamp positioned one foot above the plant. The lamp would simulate the sun, giving off a full spectrum of wavelengths of light. The second tree would be exposed to red light, set up like the control. The third would be exposed to blue light, and the forth to green light. These light wavelengths represent the two ends of the visible light spectrum, while green has a wavelength in between the wavelengths of red (longest) and blue (shortest) ~~(along with~~ (except violent). My first hypothesis would be that the ~~trees exposed to blue light would~~ tree exposed to blue light would have a higher rate of photosynthesis than the tree exposed to red light. My second hypothesis would be that the tree exposed to green light would have the lowest rate of photosynthesis. My third hypothesis would be that the control exposed to "sunlight" would have the highest rate. Thus, the variable of my experiment would be the wavelength of light.

① Eliminate other variable

① control

① man.

① Hypothesis

Each tree would be exposed to its respective light source for [three weeks] After this time period, each plant would

time

Write in the box the number of the question you are answering on this page as it is designated in the examination. | 1 | 1R2

be ground up and [tested for starch content] since ① what tested photosynthesis produces carbohydrates stored as starch, mostly in the roots but also in the leaves themselves. A simple test would be to use iodine, which detects the presence of starch, although it is likely that all four plants would contain some starch. ~~[illegible]~~ To really ~~[illegible]~~ measure the rate of photosynthesis, the specific number of grams of starch should be found by analyzing the plant content. ~~Before beginning the~~ ① Quantify: how & when.

I would expect the tree exposed to the full spectrum of light to have the highest rate of photosynthesis because the light-absorbing pigments in a plant chloroplast differ in which wavelengths of light they absorb. ① Results Chlorophyll a absorbs light of wavelength 700 and 680 nm, while [other pigments like chlorophyll b absorb light of different wavelengths] to use as energy for photosynthesis. ① Ex. accessory pigments Either the red or blue-light exposed tree would have the next highest starch content because chlorophyll tends to have the [highest absorption of light of these wavelengths] Results ① rate over whole spectrum Finally, I would expect the tree exposed to green light to wither and die because [chlorophyll a green pigment, reflects green] light rather than absorbs it. The energy is not taken in, so this tree cannot ~~be~~ carry out photosynthesis. ① Explanation It would have the lowest starch content of the four plants. The overall order, from highest to lowest, of photosynthesis rates would be: control, red or blue, green.

[This experiment should be repeated numerous] ① verify

1

Write in the box the number of the question you are answering on this page as it is designated in the examination.

1R3

times -at least 50- to prove its validity. Though maple trees are apt specimens because of their broad leaves and tap roots, the experiment should be tried with maple seeds from the beginning as well as starting with young plants.

10