Procedure and general instructions (for students).

Title: Energy Storage in Plants: Should Biofuels Be Made from Seeds or Leaves and Stems?

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Narrative:

What is energy? Physicists define **energy** as the capacity to do work. **Work** is defined as any force acting through a distance. A **force** is a push or a pull. Thus, energy can be defined as the capacity to have a push or a pull act through a distance. **Thermodynamics** is the study of how energy can change form. The workings of an internal combustion automobile can be used to illustrate many of the principles of thermodynamics. When fuel is ignited, the combustion changes the fuel from a liquid to a gas. The gas pushes the piston, which drives a crankshaft, which in turn causes wheels to rotate allowing the car to move. This illustration shows that energy comes in two basic forms, potential energy, and kinetic energy. **Potential energy** is any static form of energy that can be converted to kinetic energy. **Kinetic energy** is the energy of movement. The fuel, in our illustration, is chemical potential energy. The fuel's ignition converts the potential energy into the energy that created the force that pushed the piston. The moving piston is kinetic energy. Thus, engines are devices that convert potential energy into kinetic energy that can be used to do work.

Thermodynamics

There are two major principles that have been developed in the study of thermodynamics, known as the first law and the second law. The **first law of thermodynamics** states that in a closed system, energy is neither created nor destroyed. Though the energy may be changing form, the total amount of energy remains the same. In our car illustration, the fuel is chemical potential energy and the moving piston is kinetic energy. The kinetic energy is only possible because stored chemical potential energy was released when the fuel was ignited. The kinetic energy changes form too. For example, the engine makes sound waves (a form of energy) and heat. **Heat** is the random molecular vibration of substances. You can think of heat as a form of kinetic energy, even though the object itself is not moving.

The **second law of thermodynamics** states that during energy transfers, **entropy**, which is randomness, is always increasing. Since heat is random molecular motion, you can think of heat as a form of entropy. Internal combustion engines create entropy, primarily in the form of heat. Heat is released when the fuel is ignited. Additional heat is created due to the friction created between the moving parts of the engine. Because entropy is disorganized energy, it is not useful for creating additional work. Thus, the second law of thermodynamics explains why a perpetual motion machine, that is a machine that keeps going without adding energy, is not possible. The consequence of the second law of thermodynamics is automobile engines require potential energy (i.e., fuel) to keep the vehicle moving.

Photosynthesis

Photosynthesis does follow the laws of thermodynamics. The summary equation for photosynthesis is written below:

Equation 1: Summary Equation for Photosynthesis

$$\underset{\text{(Carbon dioxide)}}{6\,CO_2} + \underset{\text{(Water)}}{12\,H_2O} + \underset{\text{(Radiant energy)}}{\text{Light}} \xrightarrow{\text{Chlorophyll}} \underset{\text{(Glucose) (Potential energy)}}{C_6H_{12}O_6} + \underset{\text{(Water)}}{6H_2O} + \underset{\text{(Oxygen)}}{6\,O_2}$$

From the equation one can see that light, a form of energy is converted to chemical potential energy in the form of the sugar **glucose** ($C_6H_{12}O_6$). Plants accomplish this energy transfer by using a green pigment, known as chlorophyll, which captures light rays. The absorbed energy is used to split water to release electrons and produce an unwanted byproduct, molecular oxygen (O_2). The captured light energy and the resulting high energy electrons are used to convert six carbon dioxide molecules (CO_2) into one glucose molecule ($C_6H_{12}O_6$). Additionally, six new water molecules are made. Thus, the first law of thermodynamic is followed because the chemical energy came from light energy. The second law of thermodynamics is also followed because the net entropy of the system is increasing. Thermonuclear reactions destroy matter in the Sun, creating both light and creating entropy.

The matter captured by photosynthesis is used to make molecules in the plant. For example, glucose is made into larger molecules, like **cellulose** and **starch** (Figure 1), by joining glucose molecules together. Plants use cellulose to make structures, like the cell walls in leaves and stems, while plants use starch to store energy. The plant can release the stored energy when needed by breaking down starch.

Biofuels

In the past few years, liquid fuels coming from plants have been developed that can be used in internal combustion engines. These **biofuels** come mainly in two forms, **E85 ethanol** (85% ethanol, 15% gasoline) and **biodiesel** (diesel fuel made from vegetable oil). The goal of using biofuels is to make America more energy independent.

The laws of thermodynamics are followed when biofuels are used to power vehicles. In the process of photosynthesis, plant leaves use light energy to capture CO_2 from the atmosphere. The carbon atoms captured by plants are used to make carbohydrates (sugars like starch and cellulose), oils known as triglycerides, and other molecules. The resulting carbon-carbon bonds in the molecules store the captured light energy. In other words, plants take light energy and convert it into chemical potential energy. Plants use these molecules to build structures, like stems, leaves, and seeds. Additionally, plants can use starches and oils as stores of energy. Thus, you can think of plants as storehouses of energy coming from the Sun.

The seed is one of the great evolutionary advances of plants. They allow the offspring to remain dormant for long periods of time until environmental conditions are conducive for seedling survival. Additionally, seeds provide energy for the germinating seedling, to allow it to become sufficiently established to support photosynthesis. Plants store energy in seeds primarily in the form of starches like amylose and oils.

Seeds from crops like corn and soybean are used to make ethanol and biodiesel, the two main forms of liquid biofuel (Figure 1). Biodiesel is made by extracting vegetable oil from oilseeds like soybean. The oil is then chemically modified into biodiesel which then can be used in any diesel engine. The ethanol in E85 fuel is made by fermenting starches extracted from corn seeds. Since yeast only ferments simple sugars, the starch is treated with enzymes to break down the starch into glucose (a.k.a., dextrose and blood sugar). Glucose is then used by yeast to produce ethanol in a process known as **fermentation**[1]. After fermentation, the ethanol is purified and concentrated by the process of distillation.

People supporting the use of ethanol fuel argue that biofuels will reduce the amount of CO_2 that automobiles place into the atmosphere. The carbon in the fuel is from the CO_2 captured by photosynthesis (Figure 1). In theory, biofuels will create no net CO_2 because the CO_2 is cycled between cars and plants. Currently, most ethanol is made from fermenting starches found in corn. Corn production typically involves the addition of nitrogen fertilizer (made by burning natural gas) and application of insecticides and herbicides. Machinery is used to till the fields, plant seeds, apply agricultural chemicals, harvest the crop, process the crop, and transport the crop. All of these inputs involve the use of fossil fuels. **Fossil fuels** (coal, oil, and natural gas) are stored energy captured by photosynthetic organisms millions of years ago. Burning fossil fuels put CO_2 into the atmosphere. By using fossil fuels to make biofuels, most of the environmental benefits of using biofuels are lost. A study by the economist, David Pimentel concluded that using E85 fuel puts more CO_2 into the atmosphere than burning petroleum gasoline[2].

Scientists are trying to develop another method of creating ethanol fuel, called **cellulosic ethanol**. Plant cell walls are made of a carbohydrate known as cellulose (Figure 1).

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[1] Microorganisms make many substances other than ethanol by fermentation. For example, most antibiotics, soy sauce, yogurt, and raised bread are made by fermentation.

[2] *For more details, read the article at* http://www.card.iastate.edu/iowa_ag_review/fall_07/article1.aspx, or read Pimentel, David. 2003. Natural Resources Research, 12(2):127-134.

Like starch, cellulose is made from the products of photosynthesis. It can be converted into glucose by enzymes. Yeast cells are then used to ferment the glucose into ethanol. Since plants store a lot of carbohydrate in the form of cellulose, using more of the plant in the fuel synthesis will make ethanol production more efficient. If liquid biofuels are to become an important energy source for the automobile, it is important to know where the majority of the stored energy is found in a plant. Biodiesel is made from oils extracted from seeds. Ethanol is made from starches found in corn seeds. Cellulosic ethanol is made from cellulose isolated from stems and leaves. You will be conducting experiments to determine what the best starting material for making biofuel is. You will be evaluating seeds, leaves, and stems.

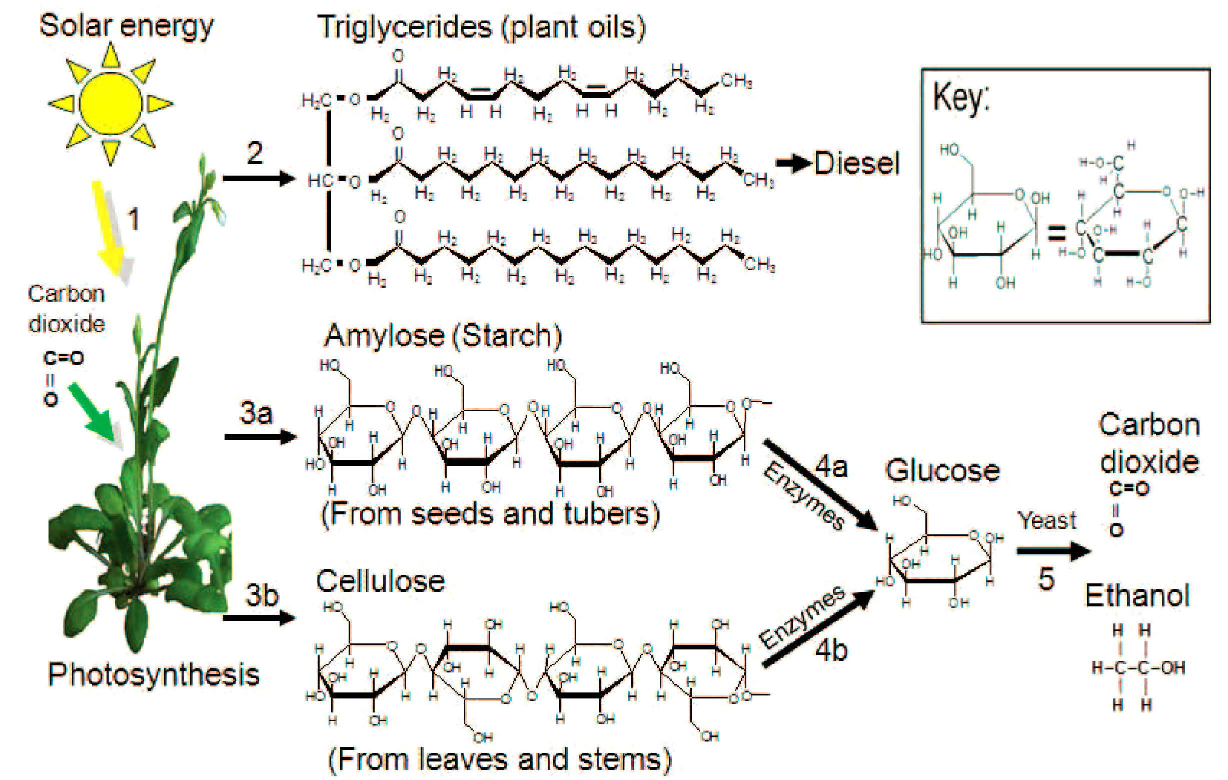

Figure 1: The biology of making liquid biofuels. Part 1: Plants use the energy from sunlight to capture CO_2 and convert it into glucose and other small organic molecules.
Part 2: Some of the molecules created by photosynthesis are used to make larger molecules, such as triglycerides (oils). Plants make these oils to store energy in seeds. Vegetable oil is extracted by pressing oily seeds. Biodiesel is then made by chemically modifying vegetable oil. Part 3: Plants use some of the glucose made by photosynthesis to create glucose polymers, in the form of starches (3a) or cellulose (3b). Plants make starches, like amylose, to store energy. They accumulate in storage organs like seeds and tubers. Cellulose (3b) is used to make cell walls. Part 4: To make ethanol from glucose polymers, it needs to be converted into a monosaccharide, usually by treating with a collection of enzymes to release glucose. Part 5: Yeast is then used to ferment glucose to produce ethanol and carbon dioxide (a by-product).

Calories

Potential energy is measured in a unit called the **calorie**. One calorie is defined as the amount of heat needed to increase the temperature of one gram of water (equivalent to 1 mL of room temperature water) one degree Centigrade. A device, known as a **calorimeter**, is used to measure the calories released by the burning of materials. To measure calories, the temperature of a known mass of water is first measured. Then the material being tested is ignited. The resulting fire raises the temperature of the water. After the material is completely burned, the temperature of the water is measured again. The calories are then calculated using the following formula:

$$\text{Calories} = [2^{nd}\text{ temperature } (^{o}C) - 1^{st}\text{ temperature } (^{o}C)] \text{ X water mass (g)}$$

Pre-Activity Questions:

1) Where does the potential energy found in plants come from?
2) Where does the carbon found in plants come from?
3) Why do plants make carbohydrates and oils?
4) If all methods of making biofuels cost the same, then what plant materials should be used to make liquid biofuel?

Activity description:

In this activity you will:

- Measure how much net CO_2 is captured by plants.
- Measure how much potential energy is stored in a plant.
- Determine which part of the plant stores the most energy, the seeds or the stems and leaves.

Materials List:

- Rapid-cycling *Brassica rapa* seeds.
- Small pots. One of the advantages of rapid cycling *Brassica rapa* is it can grow in a confined space. Thus, 2-inch diameter plastic cups with a pencil size hole punched through the bottom is sufficient to grow the plants to maturity. To aid in watering, a short segment of string can be passed through the hole. The string acts as a wick and draws water from the drip pan back into the pot. Thus, the plants do not need to be watered as often. Plants are irrigated by pouring water into the reservoir instead of watering on top of the pots.
- Pan to hold pots and to collect dripping water from the pots. A plastic dishwashing pan works well.
- Potting soil.
- General purpose fertilizer. Apply at the rate recommended by the manufacturer. Pellet fertilizer works well and is not messy to use.
- Desk lamp with fluorescent bulbs.
  Warning: Fluorescent light bulbs contain mercury, a toxic chemical. If a fluorescent bulb breaks, inform your teacher immediately.
- Aluminum foil.
- Laboratory scale or balance that can measure weights within 0.01 g.
- Weighing paper or weighing boats.
- Forceps.
- Metric ruler.
- Graph paper.
- Popped popcorn. It is best to use popcorn where oil has not been added. An easy way to make the popcorn is to place a few dozen popcorn kernels in a brown paper lunch bag and heat in a microwave until popped. Do not overcook the popcorn or it will burn. An alternative method of making popcorn is to use a hot air corn popper.
- Sample holder made by alligator clip attached to bent paper clip (Figure 3A).
- 2 wire gauze alcohol burner stands per calorimeter.

- Aluminum mailing canister, foil cupcake wrappers, or aluminum catch bucket.
- Small, light, non-mercury, metric thermometers.
- Small tin can with both ends removed. A tomato paste can works well.
- Tin snips to cut vents into the can. Wear leather gloves while cutting metal.
- Distilled water or tap water.
- 50 mL graduated cylinder.
- Wooden matches or butane lighters designed to light barbecue grills.
- Non-flammable surface to set up your apparatus.
- Fire extinguisher.
- Chemical goggles.

Procedure A: Measuring the amount of matter captured by photosynthesis.

In this experiment, you will measure the amount of matter captured by plants from the process of photosynthesis. To demonstrate the effect of light on photosynthesis, one set of plants will not be exposed to light.

1. Determine the average mass of a rapid cycling *Brassica rapa* seed, by counting out 50 to 100 seeds. Measure the mass of the weighing paper or weighing boat. This weight is called the **tare mass** (a.k.a., tare weight). Place the seed on the weighing paper or weighing boat and measure the total mass. Record your data below and in Table I. The average seed mass[3] can be calculated using the following formula.

   Average seed mass (g/seed) = (total weight – tare weight) / (number of seeds)

   Average seed mass _______ = (_________ – _________) / _______________

[3] This value will be considered the dry weight of the seed. Dormant seeds typically have a water content of ~5%. If this water was removed, then the seed would not be viable.

2. Label two small pots containing fertilized potting soil[4] with your name. Label one pot "+ light". Label the other pot "no light". Sow[5] two to three rapid cycling *Brassica rapa* seeds 1/2 inches deep into potting soil containing fertilizer. Detailed growing instructions can be found at the Fast Plants™ website: http://www.fastplants.org. Once sown, wrap the entire "no light" pot with aluminum foil. Make sure there is a space above the soil to allow the plants to grow.

3. Place the pots under fluorescent lights[6] that are on 24 hours per day. Keep the soil moist by watering periodically. The plants grow best at temperatures between 18°C and 26°C (65°F and 78°F). Germination should occur in two to three days.

4. Periodically (at least once a week) measure the heights of your plants, including the plants covered with aluminum foil. Record the results in Table II. Make notes of the appearances of your plants. Your teacher may ask you to pool your data with the entire class and calculate averages. After you measure the heights of the no-light plants, recover the plants with aluminum foil. Make sure to leave growing room and that light leaks have not been introduced. Record your data in Table II. Rapid-cycling *Brassica rapa* will start to flower in about 14 to 20 days. It is an insect-pollinated species. Since bees are not likely to be found in your classroom, you will need to hand pollinate the plants. *Brassica rapa* is a self-incompatible species. This means pollen from a plant cannot be used to fertilize itself. Thus, you will need to use pollen from another person's plants to fertilize your plants. Use forceps (a.k.a., tweezers) to pick stamens (Figure 2). Choose the stamen with yellow grainy looking anthers. The yellow grains are

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[4] A wide variety of potting mixes can be used. If the potting soil is not already fertilized, use a general purpose fertilizer. Apply at the rate recommended by the manufacturer. Pellet fertilizers work well.

[5] Sow means to plant seeds or to scatter seeds.

[6] To grow well, the plants require fairly bright lighting. Fluorescent lights can be used to grow the plants indoors. The bulb should be placed 6 to 12 inches away from the plants. Compact fluorescent light bulbs work well. Do not use incandescent lamps because they are too hot and can kill the plants.

the shedding pollen. Place the anther on the stigma[7]. Pollen from the anther will stick to the stigma. Repeat the process on all the flowers showing stigmas. The plants should be pollinated for two to four consecutive days[8].

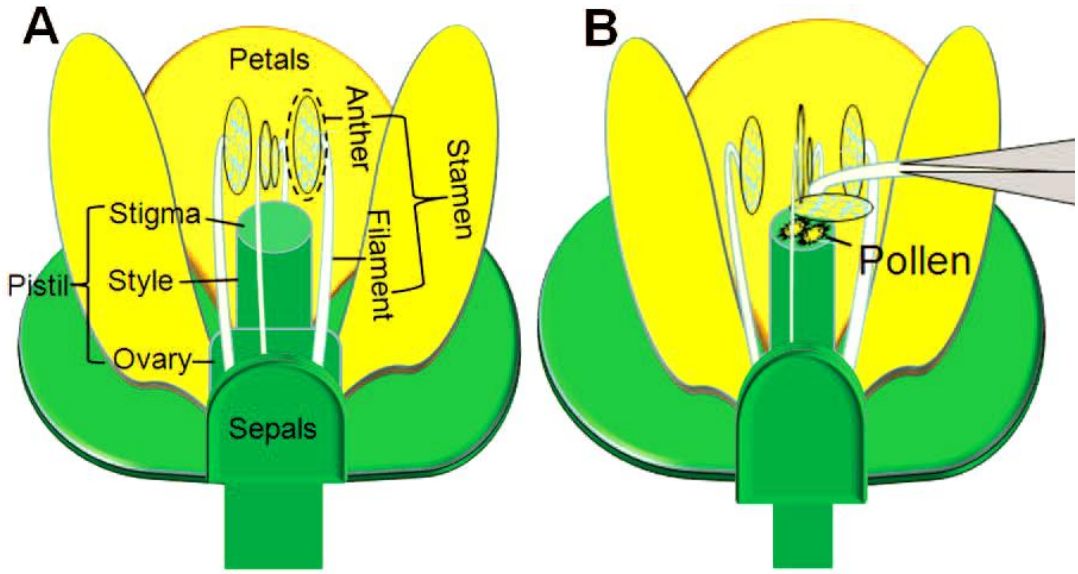

Figure 2: Anatomy and pollination of *Brassica rapa* flowers. Panel A shows the external anatomy of a flower. Flowers are arranged as whorls of modified leaves. The outer most whorl consists of sepals (three of the four green sepals are shown), followed by the petals. (Note: One petal has been removed to show the inner whorls). The next whorl contains the male organs called stamens. A stamen consists of a filament and an anther. The anther produces the pollen. The central whorl is the female organ called the pistil, consisting of a stigma, style, and ovary. When pollen lands on the stigma, the pollen will grow a pollen tube that passes through the style to the egg found in the ovary. The ovary becomes the flower's seed pod as the seeds mature. Panel B show how to hand pollinate a flower. Forceps (a.k.a., tweezers) are used to remove a mature pollen shedding stamen. This stamen is used to place pollen onto the stigma of another plant. Alternately, you can use a small soft paint brush to collect pollen from shedding anthers and then lightly stroke the stigma with the same paint brush.

[7] An alternative method is to use pollination wands. Pollination wands can be made from pipe cleaners, small soft paint brushes, or cotton swabs. Premade pollination wands can be purchased from biological supply houses. Brush the wand on several anthers that are shedding pollen. Yellow grains of pollen should be visible on the pollination wand. To transfer the pollen to different plants, gently place the pollination wand onto the stigmas of receptive flowers.

[8] Consecutive days of pollination increases the number of seeds produced.

5. Optional: Repeat Step 5 for three to five days to ensure a large number of seeds are produced.

6. Seed pods become mature about 35 days after planting. When the seed pods start to turn yellow or brown, stop watering the plants. The seeds mature quicker when the soil is dry. Maturation takes about 5 days. (See http://www.fastplants.org for details)

7. Harvest the seed pods. Remove, count, and weigh the seeds, as described in Step 1. Record your data in Table I.

8. Cut off the rest of the plant at the root crown (where the stem meets the soil).

9. In most plant cells ~ 90% of the mass is water. To measure the amount of matter captured by photosynthesis, water needs to be removed. Fashion small plates out of aluminum foil. Put the leaves and stems on small plates. Place them under a 60 to 100 watt incandescent light bulb. Let the plant material dry for several days[9].

10. Measure the dry mass (a.k.a., dry weight) of each sample and record in Table I. Calculate the biomass gained, as described in Table I.

11. Make a graph of plant growth using the data in Table II.

Table I: Biomass Gained by Photosynthesis

	Light			No light		
	Tare mass (g)	Total mass (g)	Dry mass (g)	Tare mass (g)	Total mass (g)	Dry mass (g)
Seeds:						
Stems + Leaves:						
Total biomass:						
Sown seed average mass (from Step 1):						
Biomass gained = total biomass - sown seed mass:						

[9] If a 68°C to 100°C laboratory oven is available, the plant materials can be placed on pieces of heavy gauge aluminum foil (tare weight measured) and dried in the oven. One to three days is sufficient, depending on the temperature of the oven.

Table II: Growth of Brassica rapa					
	Days since	Light grown		No light	
Date	sowing	Height (cm)	Appearance	Height (cm)	Appearance
			No seedlings		*No seedlings*

Post-activity questions:

1. Question: Look at your graph of plant growth. At the beginning of the experiment, which seedlings grew the fastest, the seedlings in the light, or the seedlings kept in the dark? Propose a hypothesis (i.e., an explanation) to explain your results.

2. Question: Why were the seedlings able to grow in the dark? Where did the energy come from? Where did the matter come from? [Hint: Look at the data in Table I.]

3. Question: Where is the majority of the matter in a plant, in the seeds or in the leaves and stems? Based on this data, where do you think the majority of the potential energy of a plant is located?

Procedure B: Measuring potential energy in seeds and stem/leaves.

In this experiment, you will measure the amount of potential energy in seeds and leaves/stems. Dried leaves from Procedure A will be used. Since the seeds of *Brassica rapa* are difficult to handle due to their small size, popped popcorn will be used to estimate the potential energy found in seeds. From this information, you will able to calculate the total amount of potential energy available to make biofuels in both seeds and stem/leaves.

1. Measure the mass of the dried leaves/stem and air-popped popcorn that will be tested. Record the results in Table III.

2. Construct the calorimeter shown in Figure 3.
    - Work on a non-flammable surface.
    - Attach the material to be tested onto the alligator clip holder.
    - Place the alligator clip holder on top of the wire gauze stand.
    - Place the chimney over the alligator clip holder.
    - Place the second wire gauze holder on top of the chimney. Place the metal water container on top of the second wire gauze stand.
    - Place thermometer into the water holder.

3. Add 25 mL to 50 mL of room temperature distilled water or tap water into the metal container. Record the amount of water you used in your experiment in Table III. Note that 1 mL of water has a mass of 1 g.

4. Measure the temperature of the water. Record your readings in Table III.

5. Obtain permission from your teacher before starting fires. Ignite the plant material with a match (Figure 3D).

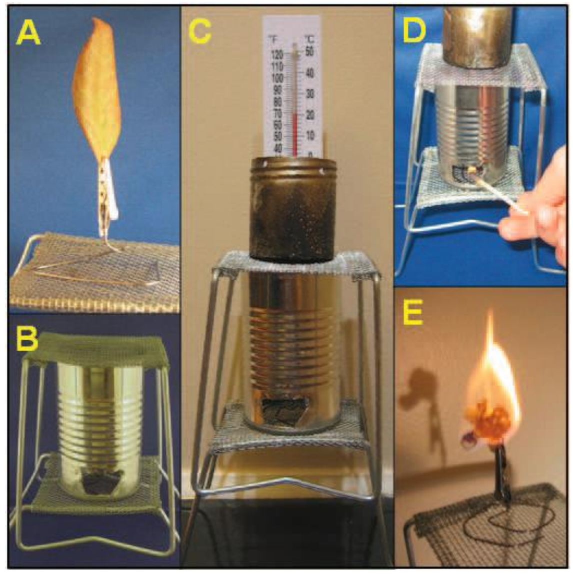

Figure 3: Calorimeter used to measure chemical potential energy in plant materials. Panel A: Sample holder is made by attaching an alligator clip to a bent paper clip. After leaf or popcorn sample is attached, it is placed on the wire gauze attached to an alcohol burner stand. Panel B: A chimney is made by cutting out both ends of the can. Tin snips are used to make access hole on one end. The chimney is placed over the sample to be tested. A second alcohol burner stand with wire gauze is placed over the chimney. Panel C: An aluminum can is placed over the wire gauze. After adding 25 mL of room temperature distilled water, a thermometer is inserted. Panel D: A match is used to ignite the plant sample. Panel E: Popcorn sample is being burned. The chimney was removed for the photograph.

Warnings:

- Use caution. The flame can burn you.
- Do not have flammable materials like loose paper near the apparatus.
- Do not wear loose fitting clothes. Do not wear plastic gloves. Fabric and plastics can catch on file.
- If you have long hair, tie it back. Hair is very flammable.
- Wear chemical splash goggles.

6. Measure the maximum temperature of the water. Record your results in Table III.

7. Carefully discard the hot water in the metal container.

8. To measure additional samples, repeat Steps 2 through 7.

9. Complete the calculations outlined in Table III. Make sure to include units of measure in all of your calculations. Note how the units of measure cancel out.

Table III: Calculating Total Caloric Content of Seeds and Stems + Leaves

	Math operation	Seeds	Stems + Leaves
Water temperature after ignition (°C):			
Water temperature before ignition (°C):	–		
Change in temperatrure (δ °C)[10]:	=		
Mass of water (Hint: 1 mL of H_2O = 1 g):	X		
Calories (calories = δ °C X g H_2O):	=		
Dry mass of plant sample ignited (g)	÷		
Energy density (calories/g):	=		
Total dry mass of plant organs (g): (Seeds or stem + leaves, from Table I)	X		
Total energy in organs (calories):	=		

[10] δ is the Greek letter delta. In science, it symbolizes change. Thus, δ °C mean change in temperature in degrees Celsius.

Post-activity Questions:

1. Where is the majority of the potential energy stored in a plant, the seeds or in the leaves/stems?

2. What form is most of the potential energy stored as, oils, starches or cellulose? What is the basis for this conclusion?

3. If the goal of making biofuels is to make the United States of America energy independent, what is the best liquid biofuel, biodiesel or ethanol? What is the basis of your answer?

4. If the goal of making biofuels is to reduce the impact automobiles have on global warming, what would be the best liquid fuel? What is the basis of your answer?

5. If you want to reduce the impact that liquid biofuels will have on food supplies, what would be the best method of making liquid biofuels?

6. Puffed popcorn was used in the calorimetry experiment because it is easier to handle and ignite than *Brassica rapa* seeds. Is this a fair comparison? The following nutritional information will help. 100 g of popcorn contains 350 kilocalories, 8.6 g of protein, 5.0 g of fat, and 70.6 g of carbohydrate. 100 g of rapeseed (a type of *Brassica rapa*) contains 35 kilocalories, 4.1 g of protein, 0.4 g of fat, and 6 g of carbohydrate. How should you change the calculation in Table III to take into account for the difference between popcorn and *Brassica rapa*?