Growing Bioenergy

Bioenergy Education Initiative

Levels:
Grades 3-10

Content Areas:
Biology; Botany; Genetics

Lesson Time:
50 minutes to introduce unit and plant cuttings. Add time for regular watering and maintenance over several weeks, up to three months.

Lecture 30 minutes plus 30+ minutes weekly follow-up periods for up to six weeks to take measurements and record observations; variable time to maintain plants.

Next Generation Science Standards:
MD.A.2; MD.B.3; MD.B.4

LS2.A

MS-LS2-1; HS-LS2-2

Objectives & Outcomes:
~ Students will understand the process to grow hybrid poplar trees.

~ Students will learn what phenotypes and genotypes are; how to identify phenotypes in plants and basic plant structures.

~ Students will be able to observe; take meaningful notes; make measurements; graph findings; draw conclusions from data.

Contact:
Bioenergy Education Initiative
agsci.oregonstate.edu/bioenergy-k-12

Description:
In this multi-week unit, students grow a selection of hybrid poplar trees from cuttings and identify different phenotype characteristics of the trees. They then use this information to match the trees to their genotypes. During the course of the unit, additional activities are done to demonstrate fermentation of biomass into ethanol and how DNA is extracted from plants.

Using This Lesson:
Classroom testing of this unit found it provides significant cross curricular opportunities in math, biology, language arts and visual arts. The whole unit, with growing time, takes approximately 10-12 weeks. Therefore, the poplar cuttings should be planted by early February. Links to related activities to enhance the unit are provided under Resources.

GreenWood Resources has offered to provide a limited number of hybrid poplar cuttings to Oregon and Washington teachers. Orders need to be received by October. Background information is provided and can be used as reading material for older students. Also, advanced student activities are included.

Energy from Hardwoods:
This unit allows students to learn about plant biology, genetics and breeding crops. It also introduces the concept of producing locally grown renewable energy.

Hybrid poplars grow quickly, six to ten times faster than similar trees. This is a primary reason researchers have been using hybrid poplars as a source of renewable biofuels and biochemicals. Growing hybrid poplars, however, is not without challenges. For example, researchers are working to breed trees that are draught tolerant *and* grow quickly. Breeding trees which use less resources, like water, as well as thrive and produce crops is part of a successful bioenergy future.

Growing Bioenergy

Getting ready to plant the trees

A kit of cuttings containing different varieties of hybrid poplar can be ordered from GreenWood Resources (see Resources).GreenWood Resources is an Oregon-based tree breeding company with tree farms worldwide. They have generously offered to provide Oregon educators with cutting samples to use in this lesson. Supplies are limited and teachers need to make their orders by November 1.

Directions:

Part I: Planting poplar cuttings

1. While growing the cuttings is easy, it will take approximately eight weeks before the cuttings have grown large enough (showing leaves, etc.) for students to begin the phenotype activity. It is best to plant the cuttings in late January/early February to be ready for the lesson in spring.
2. Plant cuttings in one gallon pots. Local nurseries often donate pots to schools. If you are reusing pots, make sure they have been washed before you plant the trees. This will prevent spreading of any disease or fungus to the trees.
3. Check the bottoms of containers to ensure there are ample drainage holes.
4. Randomize the cuttings. Create a key that gives each cutting a random number that matches the taxon code that came with the cutting. See chart example.

Taxon Code	Hybrid Combinations	Cuttings Numbers
D x D	*P. deltoides x P. deltoides (pure hybrid)*	1, 7, 13, 19
T	*P trichocarpa (pure species)*	2,8,14,20
T x D	*P. trichocarpa x P. deltoides (mixed hybrid)*	3,9,15,21
D x M	*P. deltoides x P. maximowiczii (mixed hybrid)*	4,10,16,22
D x N	*P. deltoides x P. nigra (mixed hybrid)*	5,11,17,23
D x N	*P. deltoides x P. nigra (mixed hybrid)*	6,12,18,24

5. Place a piece of masking tape around each cutting and mark them with their assigned number. This number should correlate to the key you made. Let students plant the cuttings. Then make another tape label for the pots with the cutting number on it. Remove the tape on the cutting to prevent any damage.
6. To plant, fill the pots full of potting mix. Push the cutting down into

Materials for Planting

- Hybrid poplar tree cuttings
- 24 one gallon pots and water catching trays
- Potting soil; best to use soil with a time release fertilizer
- Masking tape and sharpie maker
- Watering cans with measuring units
- Rulers with inches and cm
- Notebooks; craft paper and drawing supplies

Planting Time Line

- **September/October:** Place order for cuttings kit in fall for late winter/spring lesson.
- **Late January/Early February:** Plant cuttings
- **Week 1:** Bud movement "flushing" begins
- **Week 2-3:** Minimal maintenance, watering
- **Week 4-5:** Plants start developing root systems and shoots, begin leaf development.
- **Week 6-7:** More leaves appear; trees can be fertilized once a week at this point if leaves start to yellow.
- **Week 8-:** Enough stem and leaves should have appeared at this point that students can start the phenotype activity.

the soil, leaving one bud above the soil surface.

7. Water thoroughly and allow the soil to drain. Then, finish filling the pot with soil. Lightly pack the soil around the base of the cutting to remove any air pockets. Water the cutting thoroughly until the water drains out the bottom of the pot.
8. Place cuttings in a sunny, well lit area inside or, even better, in a greenhouse. Make sure to keep the soil moist. You should begin to see growth within four to five weeks. See Planting Time Line and Expected Outcomes for more growing tips.

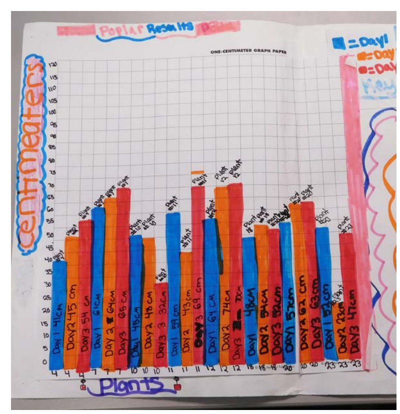
Part II: Preparing to be a scientist and background lecture

When the trees' leaves are showing students can begin the next part of the unit, observing phenotype traits. This part of the unit provides students an opportunity to learn about botany, long-term observation, record keeping, measurement, and graphing findings.

Prepare students for this by providing a lecture on phenotypes, genotypes and basic plant anatomy. Background reading material has been provided for support. Older students can use it as reading material. Students should record their observations in a notebook daily. Samples of materials created by pilot teachers to enrich and organize students are provided.

Phenotype to Genotype Matching:

1. Tell students that over the coming weeks, they will be observing the phenotypes or characteristics in the trees. Based on their observations, they will determine which trees have similar traits and then match them to their genotype.
2. Explaining how the numbers on the pots are part of a code system that only the teacher knows. These numbers refer to the trees' genotype. Using phenotyping, students will match the trees to the genotype and crack the code.
3. Have students brainstorm about the types of phenotype traits they can see in the trees (height, leaf shape, color, etc.). List the traits on the board. A list of phenotypes is provided on the next page.
4. Work with students to determine which phenotypes from the list they will observe and how they will make their measurements. For example, height can be measured in centimeters and inches and be taken twice a week. Students can then graph these measurements.
5. Break students into teams and assign them to specific trees. Establish set days and times for plant care and taking measurements. Have students keep regular notes. This observation period can be up to six weeks.
6. After four weeks, provide students with a list of the phenotype characteristics of each genotype (see attached sheets). Have students try and match the trees, based on their phenotype observations, to the correct genotypes used to make the hybrid and then submit that list to you.
7. Record the class' responses in a chart and compare conclusions.
8. Finally, have an unveiling and reveal how the trees are grouped by genotype. Discuss students' reasoning for the matches they made.

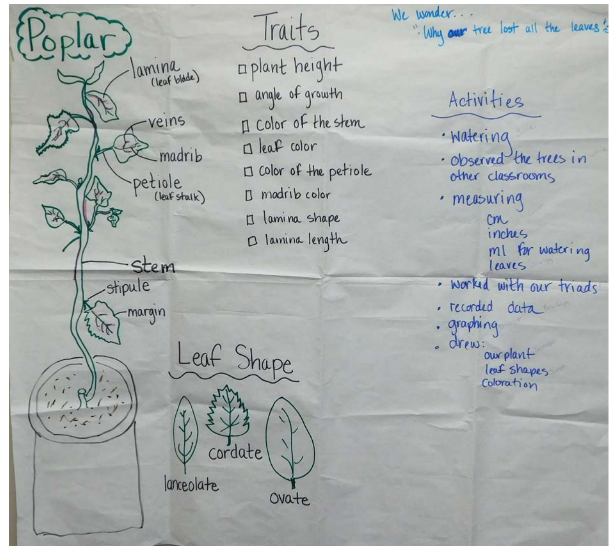


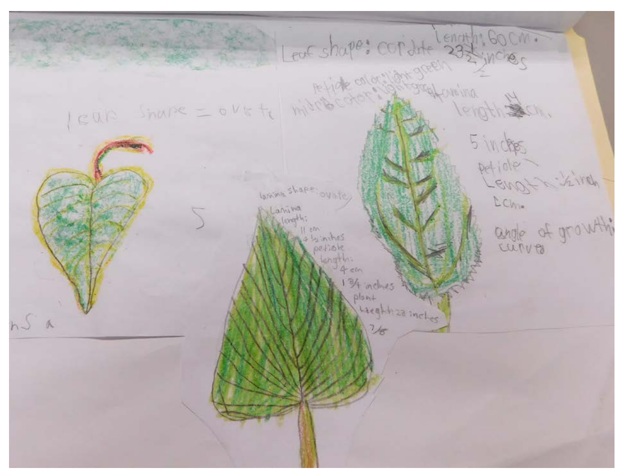
In the classroom trial, teachers found they were able to incorporate multiple, cross curricular activities such as graphing, measurements, language arts and art. Above is an example of a third grade student's drawing of a tree with measurements of the leaves. The bar graph shows the growth of many trees over time.

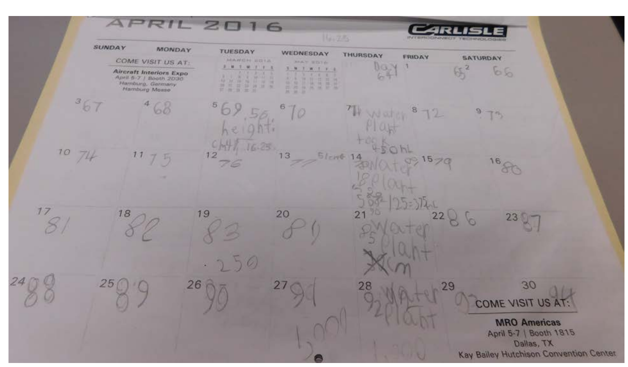
Notes from the Test Classrooms

The team of third grade teachers tested this lesson with approximately 90 students, divided into three separate classrooms. The trees were divided among the classes for students to care for and monitor. Students took weekly "field trips" to each other's classes to observe the trees.

For consistency among students, the teachers used common materials including diagrams of the trees, lists of traits students were to observe, and "I wonder" posters with sticky notes from students. The teachers also created folders for each team of students (averaged three students per group) and assigned their tree. The folders were numbered to match the number on the tree. Calendars for the period of the lesson were included and the students labeled the days of growth. They also used the calendars to mark watering days, amount of water used, and mark when measurements would be taken. The folders also held the students' drawings of their plant, leaves, observation notes, conclusions and "wonderings" they had about the tree, the experiment, etc. Below are some images from the class materials.







Enhancement Activities

The two lessons below to round out the unit and connect it to how biomass from sources like hybrid poplars, is turned into fuels. Also, it links some of the techniques tree breeders use to study the genotypes of trees for growing trees that produce high yields. These two hands-on lessons are interactive and each take about 30-40 minutes to complete.

Brewing for Bioenergy - In this fermentation lesson students observe chemical changes caused by fermentation and test a variety of feedstocks. The experiment gives insight into the fermentation process and the challenge of producing ethanol and other biofuels from cellulosic materials, like hybrid poplars.

DNA from Strawberries. In this activity students isolate the DNA in strawberries using common household materials. It introduces students to the concept of DNA, the code in all living things that contains instructions which directs the activities of cells and ultimately the organism to which they belong.

Expected Outcomes:

Tips for Growing Cuttings:

- Bud movement or ***flushing*** will start within one week of planting. In two to three weeks the buds will be using the energy reserves in the cuttings. If the cutting accepts its surroundings, the roots and leaves will continue to grow. If the flushing buds wilt and die, it means the entire cutting is dead.

- It should take approximately eight weeks to grow up the cuttings into plants with characteristics visible enough for students to observe phenotypes.
- The ideal temperatures for growing the cuttings in a classroom is 65˚-70˚ F. While direct light is best, indirect light will work as well.
- Establish a classroom calendar for watering and determine the amount of water the students should use. A Monday, Wednesday, Friday routine, with 250-500 ml of water for each plant, works well and insures the plants are watered before the weekend.

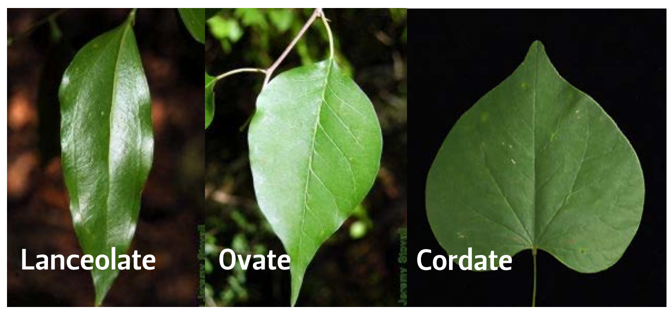

Cuttings after 64 days.

- Have students use a small plastic fork to score the soil around the base of the trees. This will keep fungus and moss from forming on top of the soil. Students should do this gently. Make sure they do not dig too deep, or they will disrupt the tree roots.

Phenotype Types and Terms:
The following is a list of possible phenotype traits students could observe for this lesson.

Leaf Shapes - Being one of the more visible features on a plant, leaf shape is commonly used for plant identification. Leaves may be ***simple*** (a single leaf blade or lamina) or ***compound*** (with several leaflets), have a regular or irregular border, be smooth, hairy, have bristles or even spines.

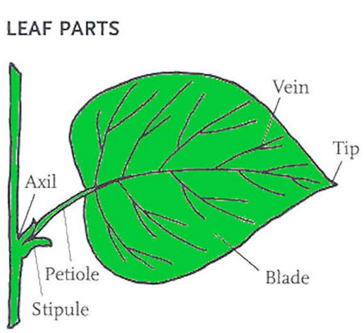
- ***Lanceolate Leaf*** - a leaf shaped like a lance head; tapers to a point at each end
- ***Ovate Leaf*** - an egg-shaped lead with the broader end at the base.
- ***Cordate Leaf*** - Heart shaped leaf that is attached to the petiole at the notched end.



Plant Height - Plant height is the shortest distance between the upper boundary of the main photosynthetic tissues (excluding inflorescences) on a plant and the ground level. Plant height is strongly correlated with life span, seed mass and time to maturity, and is a major determinant of a species' ability to compete for light.

Branching structure and angle of lateral branches- The branching structure of trees varies among species and it gives them distinctive shapes, such as a spruce's pointed spire silhouette or the large, rounded canopy of an oak. A lateral branch is any secondary branch that grows off of the main trunk or scaffolds. The growth angle of the lateral branches is an important characteristic and recognizable property of plants.

Stem Color- The color of the stem of different species can vary. This is an often used way to tell species differences.



Leaf color - Leaves color comes from the three natural pigments - chlorophyll (green color), carotenoid (orange, yellow and brown colors) and anthocyanin (red, purple and crimson color).

Petiole Color and length – The petiole is the stalk that joins a leaf to a stem. It is also the transition between the stem and the leaf blade. The color and length of the petiole are traits for phenotyping plants.

Midrib Color - The central or middle rib of a leaf.

Lamina Shape, Size and Color- The broad expanded part of the leaf that is generally green in color.

Veins – Are the vascular structure in a leaf that provides supports for the leaf and transports both water and food. The veins on monocots are almost parallel to the margins of the leaf. The veins of dicots radiate from a central midrib.

Stipule – The outgrowths on each side of the petiole. Not all plants have these so the presences of a stipules are traits for phenotyping plants.

Margin – The margin refers to the shape and structure of a leaf's edge. This trait is a common phenotype used to identify plants. A chart of the types of leaf edges can be found on Wikipedia at https://en.wikipedia.org/wiki/Leaf.

Experiment Questions:

The questions below are included to promote critical thinking as students work through the lesson. Some questions may need to be adapted for grade level.

1. How well can you characterize each phenotype? *This depends on students' power of observation. Traits such as height can be easily measured. Traits which vary in terms of quality (e.g. color) are more difficult to characterize. Encourage student to find ways to make observations less subjective and more objective.*
2. Which traits are the ones most helpful for determining genotypes? *Leaf shape, ratio of lamina size to petiole length and stem color.*
3. Are there differences in phenotypes among the different varieties? *Yes, the collection of the six clonal varieties was assembled to maximize the differences in phenotypes. The expression of the gene(s) that the clonal varieties carry differentiate the taxa and the genotypes within the taxa.*
4. Are there differences in phenotypes among the plants of the same variety? What causes this? *Yes, there can be differences, but the magnitude of differences within a variety should be less than the variation among different genotypes. These differences can often be caused by environmental factors, such as the amount of water, light exposure and temperature a plant experiences.*
5. Which traits are inherited vs. which traits are acquired? *Have students look at environmental factors (acquired traits) that could affect the trees' growth patterns. Inherited traits are transmitted genetically and acquired traits are the result of external influences in the environment. Examples of acquired traits in plants bent because of the wind or growths caused by insect bites (galls).*
6. Why do researchers need both phenotype and genotype tools? *Breeders use both to develop new and improved varieties. Breeders can manipulate the genes/genotype by making crosses, but they then have to observe the phenotypes to see the effects of changing the genotype.* ***Note:*** *In this lesson, because many of the trees are a cross between two different taxon, you will not know exactly what phenotypes will be expressed until the plants are grown up and can be observed. This is why phenotyping is such a valuable tool in combination with genotyping. When an exceptional tree has been breed and identified by phenotyping, then tree breeders will make copies of that tree using* ***serial propagation****.*

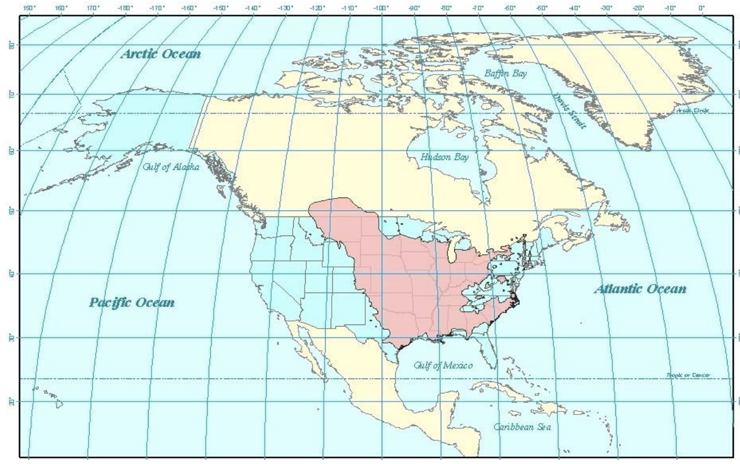
ADVANCED OPTION

GreenWood Resources will be providing four cuttings of the six different species combinations. This replication will allow your students to grow the plants in different locations (sunny/shaded) or under different conditions (wet/dry) and compare the heartiness of the trees under those conditions. To learn how the different hybrid poplars grow in different conditions, set up an experiment growing the plants under different variables (water, light, fertilizer, etc.) Make sure to have a control group. Chart growth and make a recommendation, based on findings, of the best type of poplar to grow in different environments to produce the most biomass.

ADDITIONAL ADVANCED ECONOMIC/SOCIAL QUESTIONS:

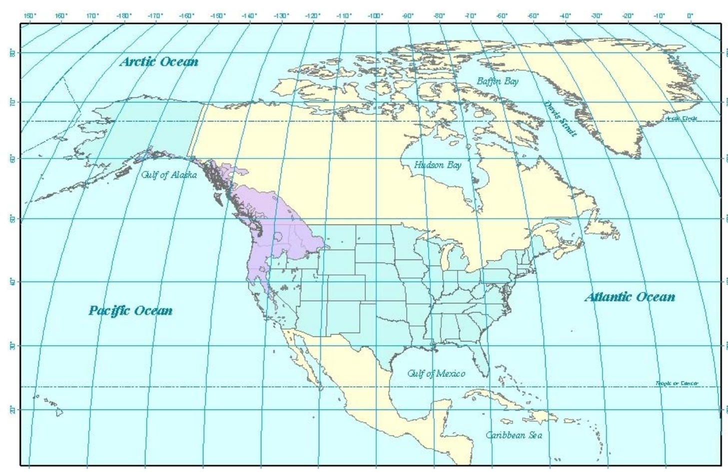
- What do farmers need to consider when planting a large crop of poplar trees?
- What are the economic considerations?
- Are there social issues that farmers need to contend with?
- Based on the experimental design, what factors affected the growth of the trees?
- What would you have done differently?

Genus: Populus
Section: Aigeiros
Species: P. deltoides (Taxa D)
Common Name: Eastern Cottonwood
Native: North America, eastern, central and southwestern
Leaf Shape: Alternate, simple (single leaf on petiole), triangular



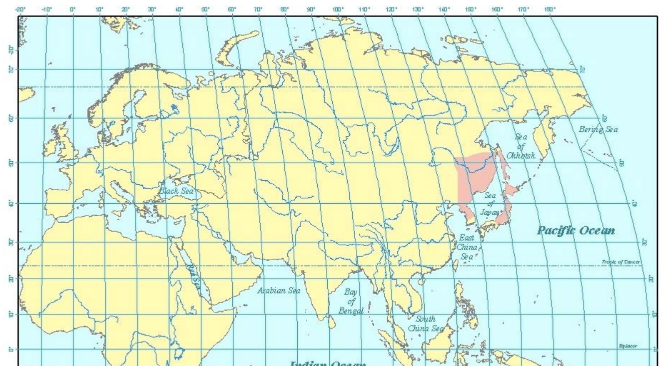
Leaf Length: 3-5"
Leaf Edge: Serrated margins
Leaf Color: Dark green
Leaf Petiole: Flat
Buds: Stout, scaly and resinous

Genus: Populus
Section: Tacamahaca
Species: P. trichocarpa (Taxa T)
Common Name: Black Cottonwood
Native: Western North America
Leaf Shape: Alternate, simple and ovate (can have variability of size and shape on the same tree)
Leaf Length: 3 to 6 inches
Leaf Edge: No serrations, wavy
Leaf Color: Dark green, silvery white bottom with brownish smears of resin

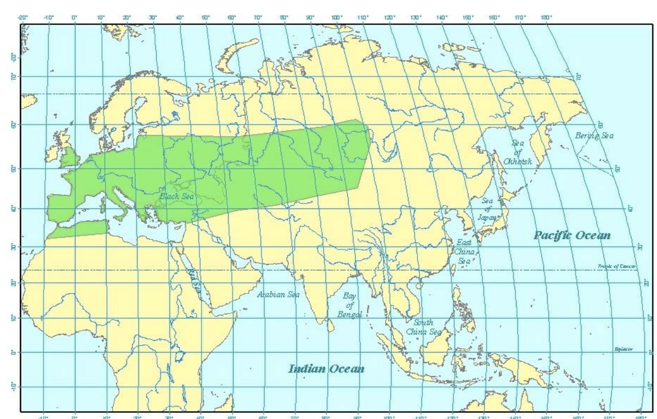


Leaf Petiole: Long and round, but can also be flat
Buds: Long and sharp pointed with high resin and sweet aroma fragrance

Genotypes with Phenotype Traits



Genus: Populus
Section: Tacamahaca
Species: P.maximowiczii (Taxa M)
Common Name: Japanese or Asian poplar
Native: Asia
Leaf Shape: Alternate, simple (single leaf on petiole), ovate-orbicular broad
Leaf Length: 3-5"
Leaf Edge: Small serrated margins
Leaf Color: Deep dark green
Leaf Petiole: Round, slight twist (sometimes) cardoid
Buds: Glossy, reddish/brown, stout



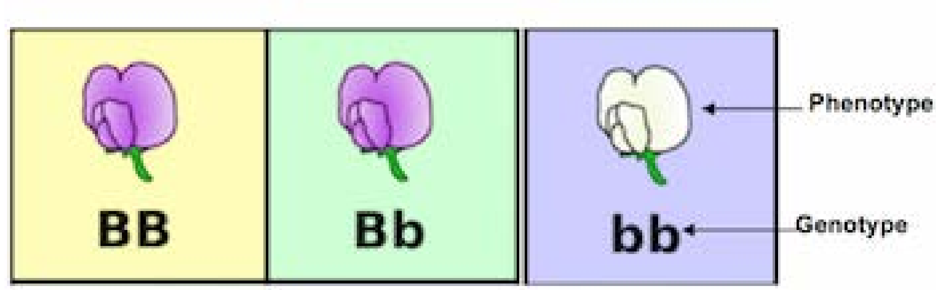
Genus: Populus
Section: Aigeiros
Species: P.nigra (Taxa N)
Common Name: Black poplar
Native: Europe
Leaf Shape: Alternate, simple (single leaf on petiole), triangular
Leaf Length: 2-4"
Leaf Edge: Notched - slightly serrated
Leaf Color: Glossy green
Leaf Petiole: Round
Buds: Pointed, thick, brown color

What is Genotype and Phenotype?

Genotype is the genetic makeup of an organism or a group of organisms that share specific traits, constitution or physical makeup due to their genes. Genotype can also refer to particular alleles present in an organism. ***Alleles*** are any of the possible forms in which a gene for a specific trait can occur. The genotype of an organism influences many of its traits, but it is not the only factor responsible.

For this lesson we are using the term genotype as a synonym for clonal variety. Often the genotype can be used to refer to a specific set of alleles, but we are using it to refer to the makeup of the entire genome. Poplars are grown from cuttings. All of the cuttings that originate from the same plant (or whose ancestors came from the same plant) are considered to be ***clones***.

A ***phenotype*** is a set of visible or expressed traits, such as hair color or leaf shape. The phenotype depends upon the genotype, but it can also be affected by random variables and environmental factors like water, temperature or available nutrients.



B= Purple Allele ; b= White Allele

Genotypes Used to Identify Different Plants Species

A ***taxon*** (plural is ***taxa***) is a representation of the species used to produce a hybrid cross. In this lesson there are six genotypes, one for each taxon with the exception of the DxN taxon which has two.

The species' symbols are represented by D (*Populus deltoides*), N (*P. nigra*), T (*P. trichocarpa)* and M (*P. maximowiczii*). The DxN taxon is produced by cross pollinating *P. deltoides* with *P. nigra*. The DxD taxon is produced by crossing two *P. deltoides* selections. The T taxon is representative of a *P. trichocarpa* mother that was open pollinated and the parent is unknown, but assumed to be another T.

What is a Hybrid Poplar?

A ***hybrid*** plant is produced when pollen of one species is used to fertilize flowers of another species. A ***hybrid poplar*** is a tree resulting from combining, either naturally or artificially, poplar species into a hybrid.

Hybrid poplars (*Populus spp.*) are among the fastest-growing trees in North America and well suited for certain conditions. Poplar hybrids are not desirable in many landscapes, but they can be of major importance under certain forestry conditions.

Why Grow Hybrid Poplars?

Hybrids grow six to ten times faster than similar species. As a result, tree farmers can see economic returns in 10 to 12 years.

Hybrid poplar research has reduced many disease problems, and there are now commercially available disease resistant trees.

Hybrid poplars are also easy to plant. You can grow the trees by planting a rootless dormant cutting and it will root and grow in a few weeks. The trees can also be cut off at the base and they will regrow from the remaining stump. These new sprouts insure future trees can be grown with little planting costs to farmers.

What are the Primary Commercial Uses of Hybrid Poplar?

Pulpwood: There is an increasing need for the production of wood products, and hybrid poplars can be substituted for slower growing hardwoods.

Engineered Lumber Products: Hybrid poplars can be used in the process of making oriented strand board (OSB) and structural lumber.

Bioenergy: Bioenergy is a renewable form of energy produced from biomass, such as hybrid poplars. A hybrid poplar absorbs as much carbon monoxide (CO) over its lifetime as it gives off when its burned. Therefore, it does not increase atmospheric CO. Bioenergy can contribute to long-term environmental and economic sustainability, and help mitigate the impact of using fossil fuels on the climate.

Growing Hybrid Poplars for Fuels and Chemicals

As energy demands increase, the search for alternative fuels becomes a top priority.

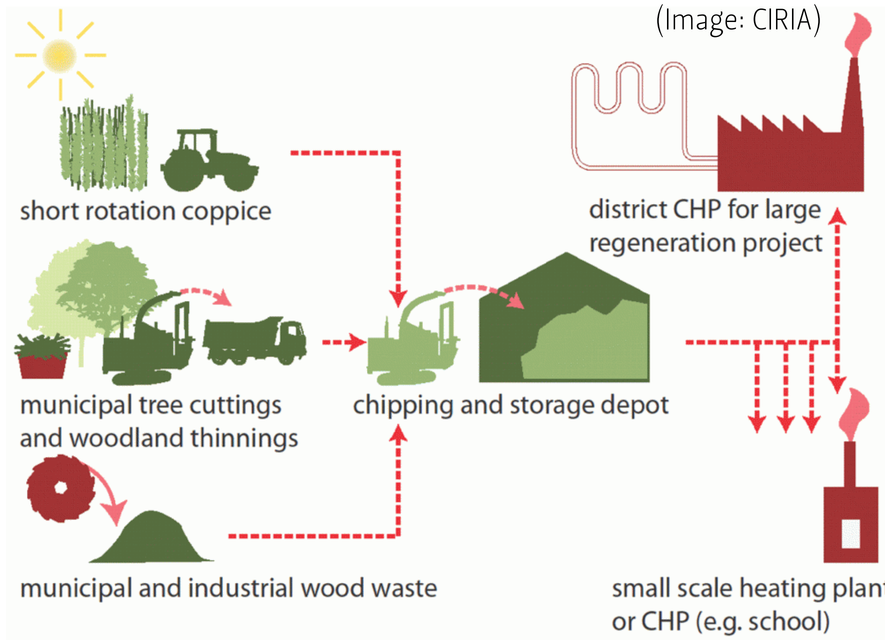
Researchers are studying trees, as well as other sources of biomass to find sustainable resources able to produce ethanol and biochemicals.

Biomass is biological material from living, or recently living organisms. In the context of producing bioenergy, it typically refers to agricultural byproducts and residues, wood and wood waste, and crops and microbes grown specifically for fuel.

Fast-growing poplar trees show potential as a fuel stock, however they do require pretreatment before being processed into ethanol and this increases the cost of production.

Cellulose is the plant material used to make ethanol. When compared with gasoline, ethanol from cellulosic biomass could dramatically reduce emissions of the greenhouse gas carbon dioxide.

While burning gasoline and other fossil fuels increases atmospheric carbon dioxide concentrations, the photosynthetic production of new biomass takes up most of the carbon dioxide released when ethanol is burned.



The Big Picture for Biomass Energy in the U.S.

Another feature of growing poplars and other biomass feedstocks is that these crops can be raised on poor quality agricultural land or land that has been previously developed. Renewable energy crops offer a unique opportunity to produce carbon-neutral biomass fuels without impacting food production.

According to a 2011 report sponsored by Department of Energy (DOE), the U.S. could sustainably produce about 1.1–1.6 billion dry tons of biomass annually by the year 2030, and still meet its demands for food, feed and fiber.

This amount of biomass could be used to produce enough biofuels to displace more than 30% of the country's current petroleum consumption.

To help make this biomass future a reality, researchers are working with selected plants, like hybrid poplars, to increase their yields and minimize the plants water and fertilizer needs.

The DOE and the U.S. Department of Agriculture are working to accelerate plant breeding programs by characterizing the genes, proteins, and molecular interactions that influence biomass yields and breed plants that are easier to turn into biofuels and biochemicals.

The DOE is also researching approaches to increase the amount of biofuel that can be made from biomass feedstocks. This work will help each region of the country to select the best feedstocks for sustainable biofuels production, and potentially making use of marginal lands that are not well suited for agriculture.

Next Generation Science Standards

DISCIPLINARY CORE IDEAS:
LS2.A: Interdependent Relationships in Ecosystems

PERFORMANCE EXPECTATIONS:
MD.A.2; MD.B.3; MD.B.4

MS-LS2-1: Analyze and interpret data to provide evidence for the effects of resource availability on organisms and populations of organisms in an ecosystem.

HS-LS2-2: Use mathematical representations to support and revise explanations based on evidence about factors affecting biodiversity and populations in ecosystems of different scales.

PRACTICES:
- Asking questions/defining problems
- Planning/carrying out investigations

CROSSCUTTING CONCEPTS
- Analyzing/interpreting data
- Structure and function

VIDEO RESOURCES
Phenotype - Science Rap Academy
What is a Phenotype - Study.com

GRANT SUPPORT
This work is part of the Advanced Hardwood Biofuel Northwest project (hardwoodbiofuels.org) and is supported by Agriculture and Food Research Initiative Competitive Grant no. 2011-68005-30407 from the USDA National Institute of Food and Agriculture.

Resources:

GreenWood Resources: A limited number of cuttings is available for teachers in Oregon and Washington. Orders will be filled on a first-come-first-serve basis. To order supplies, contact GreenWood Resources by November 1. They will send you a packet of cuttings to use for a winter planting. Contact Kat Haiby at Kathy.Haiby@gwrglobal.com.

Leaf, Wikipedia (June 22, 2016). Retrieved from https://en.wikipedia.org/wiki/Leaf

Bioenergy Frequently Asked Questions, U.S. Office of Energy Efficiency and Renewable Energy (2016) Retrieved from http://www.energy.gov/eere/bioenergy/bioenergy-frequently-asked-questions#biomass.

Biomass Fuel, CIRIA: Opportunities for Previously Developed Land (2016). Image retrieved from http://www.opengreenspace.com/opportunities-and-challenges/environmental/biomass-fuel/.

Special thanks to the third grade teaching team at Jackson Elementary School for piloting this lesson and offering valuable insights. Also, heartfelt appreciation to the crew at GreenWood Resources. Thank you for generously providing trees for the classes, as well as your team's time and expertise.