Plant Growth: Allelopathy

SC Academic Standards: 4.L.5B; 6.L.4A; 6.L.5B; 7.L.3A; 7.L.3B; H.B.2B; H.B.2C

NGSS DCI: 4-LS1.C; 5-LS2.A-B; MS-LS1-A-C; MS-LS2.C; MS-PS3.D; HS-LS1.A-C

Science and Engineering Practices: S.1A.1; S.1A.2; S.1A.3; S.1A.4; S.1A.5; S.1A.6; S.1A.7; S.1A.8

Crosscutting Concepts: Cause and Effect: Mechanism and Explanation; Structure and Function; Scale, Proportion, and Quantity; and Systems Models.

Focus Question(s): How do plants defend themselves against other plants encroaching upon their space? How do plants minimize competition for space?

Background: Flowering plants (and gymnosperms, but not ferns or mosses) come from **seeds**. Each seed contains a tiny plant embryo waiting for the right conditions to **germinate**, or start to grow. Seeds wait to germinate until three needs are met: water, correct temperature (warmth), and a good location (such as in soil). During its early stages of growth, the seedling relies upon the food supplies stored with it in the seed until it is large enough for its own leaves to begin making food through photosynthesis – so it doesn't necessarily need the sun right away, though it will eventually. The seedling's roots start growing first, and push down into the soil to anchor the new plant and to absorb water and minerals from the soil. And its stem, with new leaves, pushes up toward the light. The germination stage ends when a shoot emerges from the soil. But the plant is not done growing. It's just started. Plants need water, warmth, nutrients from the soil, and light to continue to grow. Water is obtained from the soil, through the roots, and contains many dissolved nutrients. Light comes from the sun and is absorbed by chlorophyll in the leaves (and green stems). Oxygen is generally absorbed via the roots from the soil as well, but CO_2 is absorbed (and excess oxygen released) from the leaves, through small pores called stomata.

However, even if all the "right stuff" is present for seeds to begin to grow, sometimes they just don't grow. Nurturing the garden doesn't stop with addition of seeds, water, and fertilizer. In order to get lots of fruits and vegetables, the gardener must eliminate factors that can hurt the plants. Sometimes, chemicals in the soil from other plants and trees can hurt a garden**.** In order to reduce competition for water, light, and nutrients, some plants produce chemicals that prevent other seeds from germinating. This is called allelopathy. Secondary metabolites cause these allelopathic effects. The most commonly known plant to exhibit this is the black walnut. Where a black walnut tree is present, most other plants cannot grow.

Walnut trees produce a compound called Juglone, which stops other plants growing (it inhibits H+ ion transfer and disrupts the electron transport chain, both essential elements of cellular respiration!) In the tree itself, most of the juglone exists in a non toxic form, but if the tree is damaged by insects or disease or mechanical injury the non-toxic hydrojuglone is converted to juglone rapidly. Juglone leaves the tree when leaves and husks fall and decay, when roots leak it out, or when rainfall washes it from leaves. Even when the black walnut tree is removed, the soil may remain toxic for years. The upshot is that the juglone may be a great natural herbicide!

Competition is used by both plants and animals to assure a place in nature. Plants will compete for sunlight, water and nutrients and, like animals, for territory. Competition, like parasitism, disease, and predation, influences distribution and amount of organisms in an ecosystem. The interactions of ecosystems define an environment.

When organisms compete with one another, they create the potential for resource limitations and possible extinctions. Allelopathic plants prevent other plants from using the available resources and thus influence the evolution and distribution of other species. One might say that allelopathic plants control the environments in which they live.

In this lab we will study the toxic effects that some plants can have on other plants. **In particular, we will investigate walnut allelopathy.**

Materials: black walnut hulls, black walnut leaves, water, cheesecloth, flasks or plastic storage containers (2 qt.), disposable gloves, 1oz. jigger (shot glass), filter paper or absorbent white paper towel, presoaked corn, tomato, lettuce, and winter rye seeds.

Previous Knowledge: Ecology is the study of interactions between organisms and their environment. One type of interaction frequently studied by ecologists is the competition between two or more species for limited resources. When a resource such as water is in limited supply, those species that need it will compete for it.

Competition may be defined as one organism having a negative effect on another by restricting its access to a resource that is in limited supply. Competition may be intraspecific, occurring between individuals of the same species, or interspecific, occurring among different species. One possible result of competition is the extinction of a species. The Russian biologist G.F. Gause demonstrated this possibility with two species of *Paramecium*. When grown together in a laboratory culture, one species always became extinct, even though each species grew well

alone. Another possible outcome of competition is specialization of each species in their use of resources. As this happens, their resource requirements no longer overlap entirely and they are able to coexist in the same environment. Competition between species can be an important factor influencing the distribution and evolution of species.

There are two general ways an organism can limit the resources available to another organism—exploitation or interference competition. In exploitation competition, one species is better adapted for using the limited resource. This indirectly deprives the other species of the resource. An example is a desert plant that has an extensive root system which allows it to use water that would otherwise be available to another plant species. The second type of competition, interference, occurs when one species directly prevents the other from using the resource by either physical or chemical means. Allelopathy is a form of chemical interference competition utilized by plants.

Procedure: (* note: Touching black walnut tree parts and removing nut husks will not normally injure people. Some people are very sensitive to the toxin. To these people, just small amounts of black walnut sawdust on the skin can cause small blisters. Wash!! First aid: Rinse with cold water. Also, the husks will stain clothing and hands. Second, pre-soak all seeds overnight in a cup of water. Last, the teacher may wish to make the allelopathic solutions ahead of time).

1. Make a concentrated slurry of black walnut hulls (you can also do this with leaves instead). Add 25 g of hulls to the food processor or blender with 50 ml of water. Blend to pulp. Strain through a cheesecloth into a flask.
2. Place 5 ml of allelotoxin (walnut "tea") into one petri dish lined with filter paper. For a control, take 5 ml of water (no allelotixin) and add it to a 2nd petri dish lined with filter paper.
3. In dish 1, place 20 lettuce seeds. Cover and seal to prevent evaporation.
4. In dish 2, place 20 lettuce seeds. Cover and seal.
5. Wait one week, then count the number of seeds that have germinated, and measure the radicle (embryonic root) of any seed that has germinated.

Petri Dish	# Seeds Germinated	Radicle length (mm)					Average Radicle Length (mm)
Petri Dish 1 – treatment with allelopathic tea (20 lettuce seeds)							
Petri Dish 2 - Control (20 lettuce seeds)							

Table 1. Germination and growth of radicle in seeds treated with allelopathic walnut solution.

6. Now lets look to see if all seeds react the same. Take 4 petri dishes and as before cover with filter paper or white paper towel and to the first dish add 5 ml water and to dishes 2-4 add 5 ml of allelotoxin "tea".
7. Add 2 tomato, 2 rye, and 2 corn seeds to dish 1, 6 tomato seeds to dish 2, 6 winter rye seeds to dish 3, and 6 corn seeds to dish 4.
8. Cover all dishes and seal to prevent evaporation.
9. Wait one week and then count number of germinated seeds and measure the radicle of any germinated seed.

Data Analysis: You can graph germination by placing treatment on the X (Dish #) and Germination on the Y - in part 1, you will have two bars, one for control and one for treatment. In part 2, you will have 4 bars, one for the control and one for each of 3 seed types. You can do the same with avg. radicle length on the Y and treatment on the X.

Petri Dish	# Seeds Germinated	Radicle length (mm)	Average Radicle Length (mm)
Petri Dish 1 – control 2 tomato , 2 rye, 2 corn seeds			
Petri Dish 2 - 6 tomato seeds			
Petri Dish 3 – 6 winter rye seeds			
Petri Dish 4 - 6 corn seeds			

Table 2. Germination and radicle growth of 3 different seeds grown in allelopathic walnut "tea"

<u>Extensions:</u> Here are some ideas to start a discussion with your students – let them come up with their own question and design their own experiment! You, the teacher, are a guide and facilitator here, let the students be the scientists.

Try using different seeds to see which plants are sensitive to juglone and which are not: Sensitive plants include many domestic grapes, tomatoes, root-crop vegetables, blackberries, hackberry, birch, willow, cottonwood, pine, basswood, cypress, and many types of broad-leaved forbs. Some plants which get along well with walnut include most grasses, lima beans, beets, corn, oak, red cedar, hawthorn, black cherry, locust, maple, and some types of clover.

Or, try using different plants to discover if they also have allelopathic effects: grinding up lemon or orange peel often inhibits seed germination, alfalfa leaf tea is used as an herbicide, and tobacco roots have been known to have root allelotoxins. Alfalfa leaf tea is made by placing alfalfa sprouts (shoots, not roots) in a beaker of water, submerge, and steep for 2-3 days in a cool place).

You can also try using different parts of the walnut tree: the edible nut, the hull

(note, these stain skin and clothes!), leaves, and roots (try using these products from trees over 7 years of age – and note that removal of roots is invasive and should be undertaken with care and permission). Hulls are easy to find in the late summer and fall. Crush the plant part (food processor), add water and steep overnight, then strain through cheesecloth to get a nice liquid.

You can also try this with established plants – grow some tomato seedlings until they are about 8 inches tall. Keep some seedlings for a control, apply the juglone (walnut "tea") solution to some shoots with a spray bottle, and try adding juglone solution to the soil of a third set of plants (is it more effective to spray on or soak the soil?) Generally leaves treated with juglone will turn yellow, and the plant will then wilt and die..

Last, you may want to try a dose – response curve – with serial dilutions of one plant part (hulls). The stock solution can be diluted (with serial dilutions) if you want to test concentration (pour 25 ml into a second flask and add 25 ml water – mix. Take 25 ml from that second flask and add to a third flask with 25 ml water. Mix. Add 25 ml from third into 4th flask, add 25 ml water, mix …. and so on, finally removing 25 ml from the last flask, after mixing, and throwing it away. This makes 1:2 (flask 2) and 1:4 (flask 3) and 1:8 (flask 4) and 1:16 (flask 5) and 1:32 (flask 6) and so on.

Reflection Questions:

- **Which parts of the walnut plant produce allelotoxins How would we test this? (**Most parts! You could make a solution using just leaves, just hulls, just roots etc and test in the same was as above).

- **What do you think would happen if we made serial dilutions of the allelopathic tea? (**As the "tea" became more dilute, the number of germinated seeds should increase, and avg. growth (length) of the radicle should increase**)**.

- **Which seeds seem to be able to resist the allelopathic chemicals more readily? Which seeds are not tolerant of the allelopathic chemicals?** (the corn seed should do ok with walnut "tea" but the tomato is very intolerant of the allelopathic chemicals found in walnuts).

- **What is the advantage for a plant which produces allelotoxins?** (reduced competition- no other plants could grow nearby and thus compete for space, sunlight, water, or soil nutrients. In fact, plants usually have some sort of mechanism for seed dispersal for this same reason – you don't want your babies to grow right next to you, because they would be competing with you for slight, nutrients, water etc. So most seeds need to be dispersed – and there are a lot of adaptations for this (sticky "burrs", maple "helicopters",

tasty fruit with seeds that pass through the digestive system and end up in a pile of "fertilizer", floating coconuts, etc).

- **Is there a tradeoff when producing allelotoxins?** (Sure. Energy spent making these secondary metabolic compunds means energy not available for growth or reproduction).

Models and Explanations: In this lab we explored plant allelopathy. **A student who demonstrates understanding of this concepts can explain why** it is beneficial to some plants to produce allelopathic chemicals – in that they reduce competition from other plants. **This student can discuss the experimental protocol** used to discover if walnut hulls produce allelopathic chemicals, including identification of dependent, independent and controlled variables, and the control experiment, and can defend his conclusion. **Further, this student will be able to describe** an experimental design and generate a hypothesis for testing if other plants show allelopathic properties, if some seeds might be less sensitive to allelopathy, and if concentration of the allelopathic solution is important.

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Student Worksheet:

In order to reduce competition for water, light, and nutrients, some plants produce chemicals that prevent other seeds from germinating. This is called **allelopathy**. Secondary metabolites cause these allelopathic effects. The most commonly known plant to exhibit this is the black walnut. Walnut trees produce a compound called Juglone, which stops other plants growing (it inhibits H+ ion transfer and disrupts the electron transport chain, both essential elements of cellular respiration). In the tree itself, most of the juglone exists in a non toxic form, but if the tree is damaged by insects or disease or mechanical injury the non-toxic hydrojuglone is converted to juglone rapidly. Juglone exits the tree when leaves and husks fall and decay, when roots leak it out, or when rainfall washes it from leaves.

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Question:

Hypothesis:

Prediction:

Control = _____ Treatment = _____

Independent variable:

Dependent variable:

Controlled variables:

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Conclusion: