Induced Plant Defenses
Teacher Instruction Sheet

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Goal: To allow students to see that plants can change the presence of chemicals as a means of defense.

Supplies: (based on 25-30 student class)

25 Runner bean seeds
25 10 cm pots
Multipurpose Compost
25 Snails
25 Containers with lids or plastic film to cover
5 scales
Gauze or Cheese Cloth
Distilled Water
Mortars and Pestles
Scissors
Push Pins
Rulers
Plastic Transfer Pipettes (VWR)
Filter Paper (Whatman # 1)
Squirt Bottles Filled with Distilled Water

Growing Time:

Allow ~ 2 weeks for the runner beans to get to the 4 leaf stage (2 cotyledons + 2 standard leaves).

Directions:

Day 1-14:

1) Grow the beans to the four leaf stage as stated above:
    a) Try to keep pest free and try to avoid using pesticides
    b) Rotate the plants during growth to keep growth even
    c) You will need a total of 20 plants, but a few extra plants should be grown in case of any difficulties.
2) Divide the plants into two evenly numbered groups:
    Group 1: Control
    Group 2: Treated
3) Have the students subject the treatment group to physical damage such as making a 2 cm tear from the side of the leaf to the midrib on each side of the two standard leaves (not the cotyledons), burning the standard leaves, or placing the two standard leaves too close to a growth light. Choose one type of physical damage for the entire class.

Day 14-21:

1) Allow the plants to grow to the eight leaf stage (when the fourth pair of leaves are expanded).

Day 21:

1) Divide the students into groups of 2-4 and have each group cut two pieces of filter paper (Whatman # 1) per snail into two different shapes of equal area (size ~ 16 cm2). For example, cut one piece of filter paper into a triangle with a base of 8 cm and a height of 4 cm, cut the other piece of filter paper into a square with height and base 4 cm.
2) To test the changes in chemical defenses in the leaves, have each group make an extract of the leaves by:
    a) Removing and weighing all of the leaves above the damaged pair in the treated group.
    b) Removing and weighing the same leaves on the control plants. **While producing the extracts, make sure to keep the leaves from control and treatment groups separate!**
    c) Adding 3 milliliters (cm3) in distilled water per gram of leaf.
    d) Using the mortar and pestle to blend the water and leaf material until the water becomes bright green.
    e) Filtering the mixture using the gauze or cheese cloth.
3) Have each group soak one shape with the extract from the treated leaves and the other shape with the extract from the control. (Have each group observe 1-2 snails) **Be sure to record which shape corresponds to control and which shape corresponds to the treatment group.**
4) Have the students then place the soaked filter paper at alternate corners of a plastic container and place the snail in the middle .
    a) The students should mist the tops and sides of the container with water and cover with lid (students should introduce holes in the top with the push pins or may use plastic film – allow for some air). The students should avoid misting the extract soaked filter paper.
    b) The containers should then be stored in the container in a dark, warm area for 4 – 24hr. (Be sure not to allow so much time that all of the material gets eaten).
    c) Before observing the snails feeding pattern, the students should answer question #1 at the end of this packet before leaving.

Day 22:

1) After the feeding period, have the students remove the extract soaked filter papers, measure the eaten areas of the remaining pieces of paper, and record the areas on the data sheet provided (Table 1). The students should then use Table 2 to calculate the total area consumed.
2) Pool the class results and conduct a statistical analysis of the data.

a) Snails that have either eaten nothing or everything should not be included in the sample.
b) The appropriate test is a paired sample, one – tailed student's t-test

Notes on the snails: Keep snails in a cool, moist (wet paper towels help), dark location. Feed the snails bran and/ or lettuce until ready to conduct the activity.

Extension: you may want to have the students keep detailed notes of the conditions of their control and treatment plants. This is a good observational exercise and may help with data interpretation (especially if some of the controls become damaged in some way).

3) Have the students finish answering the questions at the end of this packet.

Induced Plant Defenses
Teacher Data Sheet with Rubric and Answers

Data: 1 pt.

Group Members: 1 pt.

Control Shape: 1 pt.
Treated Shape: 1 pt.
Starting areas: 16 cm^2

Table 1: Total Area of the Paper After Consumption

Area of Eaten Section of Filter Paper	**Control**	**Treatment**
1	1 pt.	1 pt.
2		
3		
4		
5		
Total Area	1 pt.	1 pt.

*Students will have a variable number of sections of the filter paper eaten by the snails. Table 1 provides space for the students to measure and record multiple individual sections eaten by the snails. The total area should be calculated by summation of the areas of eaten sections from control or treatment groups.

Table 2: Area of the Paper Consumed

	Control	**Treatment**
Total Start Area	1 pt. - from Table 1	1 pt. - from Table 1
Minus Total End Area	1 pt. - 16 cm^2	1 pt. - 16 cm^2
Total Area Consumed	1 pt.	1 pt.

This Page Worth a Total of = 14 pts.

Induced Plant Defenses
Teacher Class Data Sheet with Rubric

Table 3: Pooled Results for the Class

Total Area Consumed (calculated in Table #2)		
Group #	**Control**	**Treatment**
1	1 pt.	1 pt.
2		
3		
4		
5		
6		
7		
8		
9		
10		
Total	1 pt.	1 pt.

*Each group should record their total area consumed from Table 2 above in the proper column. The teacher may want to make an overhead of this table in order to pool the class results.

Table 4: Statistics

	Control	**Treatment**
Average	1 pt.	1 pt.
Standard Deviation	1 pt.	1 pt.
P Value (from t-test)	1 pt.	1 pt.

Induced Plant Defenses
Discussion Questions: Rubric and Answers

1) Write down a hypothesis (a guess) predicting the most likely outcome of this experiment. Do you think the snail will eat more of one paper than the other? Why or Why not? (2 pt.)

(Example hypothesis only, these answers will vary)

I predict that the snail will eat more of the undamaged plant that the damaged one because the damaged plant will produce a chemical as a defense which will repel the snail.

2) What is a significant difference? In other words what does this term mean? (1 pt.)

A significant difference refers to a difference between results that are too unlikely to accept as resulting from chance.

3) On day 22 of the experiment, was there a significant difference between the areas consumed in the control and treatment groups? If so, which filter paper had the greatest amount eaten by the snail? (2 pt.)

Yes, control treatment
No, neither control nor treatment

4) Why would a plant need the inducible defenses? (1 pt.)

(These answers will vary)

A plant might need induced defenses in order to ward off predators when they attack.

5) How could induced plant defenses bring about the formation of a new species? (1 pt.)

Consistent attack of a particular plant species by a predator might stimulate a plant to produce chemical defenses. The plants able to produce this chemical most readily might be most likely to survive. Eventually plants might arise which produce that chemical all the time, forming a new species.

6) What should we do with snails after the experiment? Why or why not? (2 pt.)

(The answer to this question will also vary)

Your class could do a number of things with the snails such as, releasing them, killing them, keep them as pets etc. This question allows a good opportunity to talk to the students about environmental issues. For example, if released, the snails may not survive or the snails may survive but may eat a lot of plants and ruin the landscape around the school.

7) What other types of treatments could be tested for induction of defenses in these plants? (Name two) (2 pt.)

Grow the plants too close to the grow lamps
Introduce a pest (such as caterpillars)

Induced Plant Defenses
Student Instruction Sheet

Supplies:

1 Control Runner Bean Plant
1 Treated Runner Bean Plant
1 Snail
1 Container with lid
Scales
Distilled Water
1 Mortar and Pestle
Scissors or Push Pins
1 Ruler
2 Pipettes
1 Filter Paper (Whatman #1)
Squirt Bottle Filled with Distilled Water

Growing Time:

Allow ≈ 2 weeks for the runner beans to get to the 4 leaf stage (2 cotyledons + 2 standard leaves).

Directions:

Days 1-14:

1) Grow the beans to the four leaf stage as stated above:
    - Try to keep pest free and try to avoid using pesticides
    - Rotate the plants during growth to keep growth even
2) Divide the plants into two evenly numbered groups:
    Group 1: Control
    Group 2: Treated
1) Subject the treatment group to physical damage by making a 2 cm tear from the side of the leaf to the midrib on each side of the two standard leaves (not the cotyledons).

Day 14-21:

1) Allow the plants to grow to the eight leaf stage (when the fourth pair of leaves are expanded).

Day 21:

1) Cut two pieces of filter paper (Whatman # 1) per snail into two different shapes of equal area (size ~ 16 cm2). For example, cut one piece of filter paper into a triangle with a base of 8cm and a height of 4 cm, cut the other piece of filter paper into a square with height and base 4 cm.
2) To test the changes in chemical defenses in the leaves, make an extract of the leaves:
    a) Remove and weigh all of the leaves above the damaged pair in the treated group.
    b) Remove and weigh the same leaves on the control plants. **While producing the extracts, make sure to keep the leaves from control and treatment groups separate!**
    c) Add 3 milliliters (cm3) in distilled water per gram of leaf.

d) Use the mortar and pestle to blend the water and leaf material until the water becomes bright green.
e) Filter the mixture using the gauze or cheese cloth.

3) Soak one shape with the extract from the treated leaves and the other shape with the extract from the control. (Have each group observe 2 snails) **Be sure to record which shape corresponds to control and which shape corresponds to the treatment group.**
4) Place the soaked filter paper at alternate corners of a plastic container and place the snail in the middle .
5) Mist the tops and sides of the container with water and cover with lid (students should introduce holes in the top with the push pins or may use plastic film – allow for some air). Avoid misting the extract soaked filter paper.
6) Store the container in a dark, warm area for 4 – 24hr. (Be sure not to allow so much time that all of the material gets eaten).
7) Answer question # 1 from the questions at the end of this packet.

Day 22:

1) After the feeding period, remove the extract soaked filter papers, measure the eaten areas of the remaining pieces of paper, and record the areas on the data sheet provided (Table 1).

***Snails that have either eaten nothing or everything should not be included in the sample.**

2) Use table 2 to calculate the total area of the paper consumed.
3) Record your group's and the rest of the class's results on the class data sheet and conduct a statistical analysis of the data by calculating the average, standard deviation of the class data and performing a student's t-test on the data.

Induced Plant Defenses
Student Data Sheet

Data:_____

Group Members:_____

Control Shape:_____
Treated Shape:_____
Starting areas: 16 cm^2

Table 1: Total Area of the Paper After Consumption

Area of Eaten Section of Filter Paper	**Control**	**Treatment**
1		
2		
3		
4		
5		
Total Area		

Table 2: Area of the Paper Consumed

	Control	**Treatment**
Total Start Area		
Minus Total End Area		
Total Area Consumed		

Induced Plant Defenses
Class Data Sheet

Table 3: Pooled Results for the Class

Total Area Consumed (calculated in Table #2)		
Group #	**Control**	**Treatment**
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
Total		

Table 4: Statistics

	Control	**Treatment**
Average		
Standard Deviation		
P Value (from t-test)		

Induced Plant Defenses
Discussion Questions

1) Write down a hypothesis (a guess) predicting the most likely outcome of this experiment. Do you think the snail will eat more of one paper than the other? Why or Why not?

2) What is a significant difference? In other words what does this term mean?

3) On day 22 of the experiment, was there a significant difference between the areas consumed in the control and treatment groups? If so, which filter paper had the greatest amount eaten by the snail?

4) Why would a plant need the inducible defenses?

5) How could induced plant defenses bring about the formation of a new species?

6) What should we do with snails after the experiment? Why or why not?

7) What other types of treatments could be tested for induction of defenses in these plants? (Name two) (2 pt.)