

## **Plants can get sick from a virus too: How you can transmit a virus to young wheat plants**

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**Idea:** Living things can become sick when they are infected with certain very tiny creatures scientists call microbes. Microbes are so small that you need a microscope to see them. Bacteria and viruses are microbes.

### **Material**

- wheat seeds
- small pots (at least 4)

(You can make good pots from 2 L cardboard milk cartons. Mark a line on each side about 15 cm from the bottom. Cut along the lines so the bottom part will be your pot. Punch a few holes in the bottom and cover the inside of the bottom with some folded over kitchen paper towel. The holes and absorbent paper towel are important for drainage).

- potting soil
- small mortar and pestle
- carborundum grit powder
- cotton-tipped applicators (Q-tips)
- pot of wheat plants with "Wheat Streak Mosaic" (WSM) disease

### **Methods**

#### **PART I - Growing the test plants**

1. Place soil in pots but don't fill them right up. Leave about 5 cm of free space in each pot.
2. If you are using the milk carton pots, place 4 wheat seeds on top of the soil in each pot so that they are evenly spaced from each other and not too close to the walls. An easy way to do this is to place one seed about 3 cm away from each corner.
3. If you are using another kind of pot, simply place seeds so they are evenly spaced and not closer than 2 cm away from each other.
4. Cover the seeds with about 2 cm of soil. Pat the soil down gently. You can use the bottom of a paper or styrofoam cup to do this.
5. Water gently so that the soil becomes thoroughly moist - but not flooded! Until green shoots poke through the soil, keep the pots in a cool spot away from direct sunlight. Water gently every day to keep soil moist.
6. After about a week, green shoots will poke through the soil. When this happens, move the pots to an area where the plants will receive daylight but make sure that it doesn't get too hot. The temperature should never be higher than 25 C. Water gently in the early morning and in the late afternoon to keep the soil moist.
7. The wheat plants will continue to grow. When they have 4 to 6 leaves, you are ready to try and transmit the virus that causes wheat streak mosaic disease.

#### **Part II - Transmitting the virus to the test plants**

Note: It is important to do the steps in the order given. Otherwise you might transmit virus to plants that you do not want to become infected!

Make labels for the 4 groups of pots in the experiment

- Group 1 ... Control Group
- Group 2 ... Healthy plant source for "Mock Inoculum"
- Group 3 ... Mock-inoculated Group

- Group 4 ... Virus-inoculated Group
1. Take the pots labelled "Control Group" to one side. The plants in these pots will receive no treatment except for normal watering.
  2. Assemble the pots labelled "Mock-inoculated". The plants in these pots will have the sap of healthy plants rubbed onto their leaves.
  3. Take a pot labelled "Healthy Plant Source" and remove a total of 2 leaves from the wheat plants in that pot. Place the leaves in the mortar and add 2 mL of water. Grind and scrape the leaves with the pestle. The water will turn green. Add carborundum and more water. Grind some more until the leaves are completely torn up. This green liquid with the carborundum grit in it is called "mock inoculum".
  4. Gently shake the mortar to make sure some carborundum is suspended in the inoculum. Dip a Q-tip into the inoculum and gently rub onto the two largest leaves of a plant in a pot labelled "Mock-inoculated". Dip the same Q-tip into this inoculum again and rub (inoculate) the two largest leaves of another plant in a pot labelled "Mock-inoculated". Repeat until all plants in the pot(s) labelled "Mock-inoculated" have been rubbed with mock inoculum.
  5. Rinse the excess mock-inoculum off the rubbed leaves. The easiest way to do this is to hold the pot almost horizontally over a sink and rinse the leaves with a gentle stream of cool tap water.
  6. Assemble the pots labelled "Virus-inoculated". The plants in these pots will have the sap of virus-infected plants rubbed onto their leaves.
  7. Take the pot labelled "Wheat Streak Mosaic" and remove 2 large leaves that show the yellow streaks. The leaves show the yellow streaks because they are infected with the virus that causes Wheat Streak Mosaic Disease.
  8. This virus has NO EFFECT on humans and is perfectly safe to handle.
  9. Place the leaves in the mortar and add 2 mL of water. Grind and scrape the leaves with the pestle. The water will turn green. Add carborundum and more water. Grind some more until the leaves are completely torn up. This green liquid with the carborundum grit in it is called "virus inoculum".
  10. Gently shake the mortar to make sure some carborundum is suspended in the inoculum. Dip a Q-tip into the inoculum and gently rub onto the two largest leaves of a plant in a pot labelled "Virus-inoculated".
  11. Dip the same Q-tip into this inoculum again and rub (inoculate) the two largest leaves of another plant in a pot labelled "Virus-inoculated". Repeat until all plants in the pot(s) labelled "Virus-inoculated" have been rubbed with virus inoculum.
  12. Rinse the excess virus-inoculum off the rubbed leaves. The easiest way to do this is to hold the pot almost horizontally over a sink and rinse the leaves with a gentle stream of cool tap water.

### **PART III - Completing the experiment**

You will be comparing 3 groups of plants:

- Control ... these plants were not rubbed
  - Mock-inoculated ... these plants were rubbed with the sap of healthy plants
  - Virus-inoculated... these plants were rubbed with the sap of plants that were sick because of virus infection.
1. Make sure the plants from one group are not touching those from another group. You don't want to transmit the virus by accidental rubbing from plants in one group to plants in another.
  2. Continue to grow the plants. Water carefully. Keep the plants by day in a warm (20 to 25 C), well lighted or sunny place.

### **Observations:**

Describe the appearance of the plants in the pot with the "Wheat Streak Mosaic" label. Write down the ways in which these plants are different from the wheat plants you have grown from seed. A good way to do this is to make a table something like the one below. (It is only an example. Make your own table using your own observations.)

Plant Character Colour of Leaves	Healthy Wheat Plant Dark green	Wheat Streak Mosaic Plant (Put in your own description)
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Shape of Plant

Grows upright

(Put in your own description)

Record the date on which you rubbed (inoculated) the plants. This is called the "Date of Inoculation".

Measure the heights of plants in the 3 groups.

You will be recording how the plants in the 3 groups develop over a period of time. Use a table like the one below to make such a record (The numbers in the table are there only as an example. Your numbers will probably be different).

#### Plant Height (cm)

Date	Days After Inoculation	Control Group	Mock-Inoculated Group	Virus-Inoculated Group
Oct. 1	0	10,11,10,10	10,11,10,10	10,11,12,10
Oct. 4	3	13,14,14,12	13,14,14,13	13,13,14,13
Oct. 8	7	16,17,16,17	16,17,16,17	16,15,16,16
Etc.				

Record the appearance of the plants and the leaves, especially the leaves that come out after your inoculation date.

Use a table like the one below to make such a record. (The notes in the table are there only as an example. Your observations could be different. Leave enough space in the table to make it possible for you to record longer and more detailed observations).

#### Plant Appearance

Date	Days After Inoculation	Control Group	Mock-Inoculated Group	Virus-Inoculated Group
Oct. 1	0	Green	Green	Green
Oct. 4	3	Green	Green, some brown spots	Green, some brown spots
Oct. 8	7	Green	Green	Light green and yellow streaks
Oct. 11	10	Etc.	Etc.	Etc.

You should record observations for at least 2 weeks after inoculating the plants. If you take proper care of the plants you may record observations for the complete life of the plants, that is until they produce heads of ripe seed. This will take about three months.

#### Conclusions and Questions:

1. Did rubbing alone (mock inoculation) affect the plants compared to the plants that were not rubbed at all (the 'Control' plants)?
2. What happened to the plants that were rubbed with sap from the virus-infected plant? Describe the changes.
3. How could you prove that the virus is still able to infect wheat plants? If you have time, do an experiment to test your idea.

4. Someone tells you that heating the virus will stop it from being able to infect plants. What experiments could you do to test this idea?
5. Another person tells you that freezing the virus will stop it from being able to infect plants. What experiments could you do to test this idea?