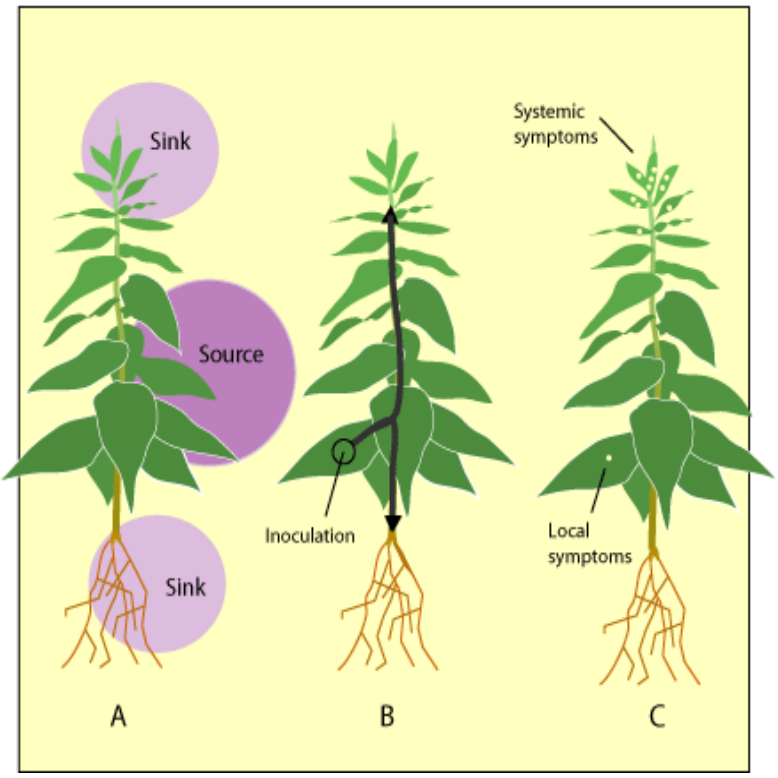
Lab#8 Symptoms and Mechanical Inoculation of Plant Viruses

The effects of viruses on plants are various and expressed as “symptoms” which are the observable signs of virus infection on plants and provide first clue in the field. Viral symptoms can be produced in a range of test plants which help in infectivity assays and selection of indicator and propagative hosts for different viruses.

Primary symptoms: develop at the site of virus entry in mechanically inoculated leaves; called as Local lesions.

Secondary or systemic symptoms: occurs when the virus is not confined to the site of inoculation but it spreads to other cells within leaf mesophyll, usually through plasmodesmata connections, and reaches vascular system, phloem in most of the cases. Therefore, severe symptoms may develop when a virus has infected a plant **systemically.**



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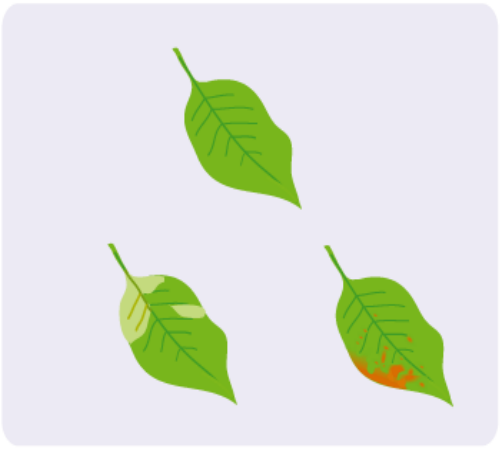
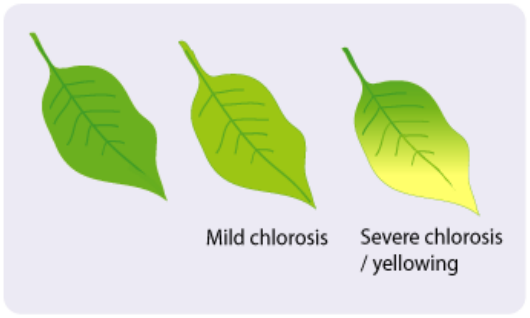
Types of symptoms



Mottling: abnormal coloration

Mosaic: If a mottle is light and creates mosaic



Chlorosis: reduced amount of chlorophyll

Vein Clearing: veins become light

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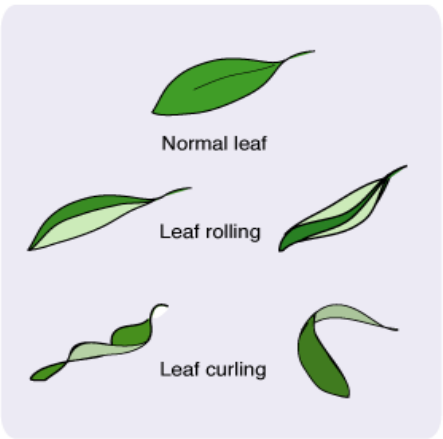
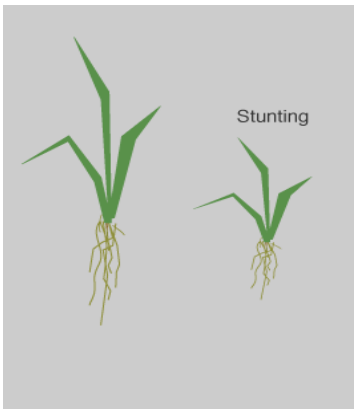


Leaf rolling and curling: Rolling is folding of leaves along their mid axes resulting in a more or less tube-like structure. When the folding is more irregular or does not result in a tube-like structure it is usually referred to as leaf curling.

Blistering



Variegation

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Mechanical inoculation of plant viruses

The virus preparation is rubbed onto the surface of the leaf in such a way as to break the surface cells without causing too much mechanical damage. This is usually accomplished by extracting sap from the infected plant, adding an abrasive, and rubbing the sap briefly onto the upper surface of one or more leaves of the test plant. The test plants should be young and horticulturally "soft"; leaves on mature and senescent plants are poorly receptive to this technique.

Note: Some plant viruses are not mechanically inoculable. Most of these are viruses are limited to the vascular tissue (usually the phloem) and, under natural conditions; they are introduced to these tissues by the feeding processes of arthropod vectors.

Materials

- Test plants, the choice of which depends on the objective of the experiment
- Virus source material: this can be either (a) plant material known or suspected of being virus infected, (b) solution of virus preparation
- Appropriate buffer: for many viruses 0.01 M phosphate buffer, pH 7.0, is satisfactory but some viruses are inactivated at this pH; if so, 0.01 M acetate buffer, pH 5.0, should be used
- "Celite" (diatomaceous earth) or carborundum powder (600 mesh) available from laboratory chemical suppliers
- Labels and a pencil, Pestle and mortar (sterilized), Cheesecloth and scissors, Gloves, Glass spatula, Squeeze bottle containing distilled water, Glasshouse or growth cabinet

Procedure

1. Thoroughly wash hands in soap and water to prevent virus carry-over.
2. Select and label test plants. If looking for local lesions, mark leaves to be inoculated by pricking the tip with a pencil point.
3. If inoculum is plant material, cut or tear into small pieces and place in the mortar.
Young systemically infected leaves usually contain the highest virus concentration
4. Add buffer (and inhibitor suppressor, if necessary) in the approximate ratio of 5 to 10 to unit weight of leaf material. Add a small amount of celite or carborundum; alternatively, the leaves to be inoculated can be dusted with celite or carborundum.
5. Grind leaf material until more or less homogeneous.
6. Squeeze the homogenate through cheesecloth; this may not be necessary for very "soft" inoculum plants that give a very watery homogenate.
7. Wearing a glove, wet forefinger with the expressed sap and wipe **gently** onto each marked leaf (or onto several leaves if wishing to propagate the virus). Alternatively, use a glass spatula.
8. Wash inoculated leaves as soon as possible with water from a squeeze bottle.
9. Place plants in a suitable glasshouse or growth cabinet.
10. Change gloves and wash hands if doing another test.