

Mechanical Inoculation of infectious plant extract onto indicator plants

1. At least two hours prior to mechanically inoculating the host range plants, place clean (no need to sterilise them) mortar and pestle at -20°C . When going from the lab to the greenhouse, maintain both the leaf samples and the mortar and pestles on ice. This is particularly critical for viruses such as CMV and tospoviruses.
2. Using a pre-chilled mortar and pestle, grind leaf (or piece of leaf) to a slurry in approximately 1 - 2 ml 0.1 M potassium phosphate (pH 7.2).
3. Add additional amounts of buffer as required to maintain the slurry (usually up to 3 ml).
4. Add approximately 40 mg of celite powder (a pinch) and mix with the ground extract.
5. Apply small amount of slurry to 2 leaves of each plant (individually) and gently rub.
6. After about 5 minutes, gently rinse inoculated leaves with water.

NB. Carborundum can be used instead of celite, however, you should be aware that carborundum has been implicated as a possible lung caseinogen. When using carborundum, it is best not to add the carborundum to the slurry but instead gently “dust” the leaves to be inoculated and then gently rub the leaves with the slurry.

Preparation of Potassium Phosphate Buffer:

1. Prepare 500 ml of 0.1M K_2HPO_4 (base) and 190 ml 0.1 M KH_2PO_4 (acid).
2. Adjust the pH of the K_2HPO_4 to 7.2 by slowly pouring in the KH_2PO_4 .
3. Autoclave and store at 4°C .

NB. DO NOT USE HCl OR ACETIC ACID TO ADJUST THE pH.