

# Sampling of Spider Mites

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## Protocol 1 – Infested Leaves

### Objective:

To collect and prepare leaf samples visibly infested with spider mites for later examination or laboratory processing.

### Materials

- Clean paper envelopes or plastic bags (zip-lock)
- Hand lens (optional, for easier viewing)
- Permanent marker and labels
- Cooler box with ice packs (for transport, optional)

### Procedure

1. **Select plants:** Identify plants showing typical spider mite symptoms — speckling, chlorosis, bronzing, or webbing on leaves.
2. **Choose leaves:** Select 3–5 leaves per plant, preferably from different positions (top, middle, lower canopy).
3. **Collect leaves:** Gently pull leaves with your fingertips to avoid dislodging mites.
4. **Inspect briefly:** Use a hand lens, if available, to confirm the presence of mites.
5. **Package samples:**
  - Place each sample (per plant or per site) in a labeled paper envelope or plastic bag.
  - Label with sample ID, date, location, host plant species, and collector name.
6. **Storage/Transport:**
  - Keep samples cool and shaded during transport (avoid direct sunlight).
  - If processing will happen within 24 hours, store the sample at 4 °C or include ice packs in the shipping box; otherwise, continue with preservation (Protocol 2).

## Protocol 2 – Preservation in Ethanol

### Objective:

To preserve spider mite specimens collected from leaves for molecular analysis.

### Materials

- Sterile tubes of 1.5–2 mL (preferred), or small, capped containers up to 50 mL.
- 70–100% grade ethanol
- Fine brush
- Labeling tape or waterproof marker
- Optional: dissecting microscope

### Procedure

1. **Transfer mites:**
    - Under a stereomicroscope or with a hand lens, gently remove mites from leaves using a fine brush.
    - Place them directly into a labeled tube containing ethanol.
  2. **Label samples clearly:**
    - Include sample ID, date, host plant, and collection site.
  3. **Storage:**
    - Store tubes at  $-20^{\circ}\text{C}$  for long-term preservation (preferred) or  $4^{\circ}\text{C}$  for short-term storage.
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