



RAPRA Protocols for Susceptibility Testing

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Protocol 5: Log Inoculation Method for Conifer and Broad-leaved Species



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Plant material:

Logs of 1.2m long x 13 – 18 cm diameter (which gives a circumference of approx. 45 – 55 cm) should be selected from trees with straight stems; cut logs should have the minimum of side branches to avoid extra wounds on the log surfaces

Method:

- Within a few hours after felling (no more than 24h) seal the log ends and any branch wounds immediately with a wound sealant such as the commercial product Aquaseal
<http://www.aquaseal.com>
- Put a band of PVC tape round each end of the log, 10cm in from each end. Within these bands, put three more tape bands around the log, equally spaced (33 cm apart). These will be the position of three zones/rings of inoculation points.
- Roughly mark out the inoculation points on each band, spacing them 10cm apart. Stagger the inoculation points so that the lesions formed in one band will not run into lesions formed in the next band.
- Inoculate the logs within 2-3 days of felling.

Inoculation method:

- For each inoculation point remove a disc of bark (down to the wood) with a sterile cork borer.
- Cut a plug from a plate of a fungal culture the same size as the bark plug and place it, mycelial side down, into the hole.
- Add a drop of sterile distilled water; replace the bark plug.
- Cover the wound site with plug of wet cotton-wool then place a square of aluminium foil over the cotton wool and secure by covering with plastic tape and staples.
- Label the covering tape with isolate code letter, using a permanent marker pen.
- Wrap bands of wounds with Parafilm, spray logs with distilled water, then bag up logs in tubular polythene bags.
- Close the open end of the bag with tape.
- Incubate logs at ca.20°C for 3-5 weeks (timing will depend on the susceptibility of the host species). If in doubt about the timing set up extra inoculation points/logs that can be destructively sampled after 3 weeks to check the progress of lesion development.



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Sampling method:

- Remove wound coverings and shave off the bark to reveal the lesion formed within the phloem tissue.
- Trace the amount of colonisation by placing a sheet of clean tracing paper over the lesion, and outlining the lesion margin on the paper, marking the inoculation point on the tracing as well.
- If required, attempt re-isolation of the inoculated fungus from the lesion area on to a suitable selective medium.
- Photocopy the tracings of each lesion (so as to have a permanent record) then carefully cut out each lesion tracing, keeping a record of the log band and isolate of fungus associated with each lesion.
- Calculate the area of each lesion by weighing the tracing and multiplying this weight by the mean weight of 1 cm² of tracing paper (the mean estimated from 5 x 1 cm² of pieces of tracing paper).
- Lesion lengths and widths can be measured on the photocopies.



Inoculum:

Isolates are cultured on carrot agar (CA) (Brasier, 1967) as stock cultures.

- Take plugs from the margins of actively growing cultures, transfer to CA and inoculated plates placed lids uppermost, in continuous day light (60-W bulbs; Daylight Company, UK suspended 30 cm above the plates) for 14 days.

References:

Brasier CM, 1967. *Physiology of reproduction in Phytophthora*. Hull, UK: University of Hull, PhD thesis.