



RAPRA Protocols for Susceptibility Testing

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Protocol 1: Detached Leaf Dip Assay for Conifer and Broadleaved Species

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Plant material:

Leaves of host species should be collected from the same plant where possible to reduce genotype effects. Plant material is obtained from mature healthy trees or healthy nursery stock:

- Conifer species - the current growth shoots, approximately 10 cm long bearing needles are used.
- Broadleaved species - fully expanded leaves should be used.
- Before inoculation, leaves are rinsed with sterile water and placed on paper towels to air dry.
- The mid-point of each shoot or leaf is marked with a permanent marker pen.
- Rhododendrons are included as positive controls in both the broadleaf and conifer experiments to confirm the pathogenicity of the isolates.

Inoculum Preparation: (See also Protocol 6)

Treatments. Plant material is divided into two groups, one for non-wounding treatment and the other for the wounded treatments.

Non-wound treatment - care is taken not to expose the cut end of the shoot or petiole to the zoospore suspension. Each shoot or leaf is dipped in the zoospore suspension, apical end first, up to the midway mark, and swirled gently for 1 min. On removal, excess liquid was allowed to drop off.

Wounded treatments - The distal end of each shoot or leaf was dipped after freshly cutting the base, and wounding the leaf. The conifers are wounded by trimming approximately 5mm off the tips of five needles on the distal part of the shoots. Two 5 mm deep V-shaped incisions are made on the broad-leaves, one on either side of the distal part of the leaves.



Incubation and re-isolation of the pathogen:

- Inoculated material is placed on raised sterile wire mesh trays in plastic storage boxes.
- The base of each box is lined with wet paper towels, creating a moist incubation chamber. The sides of the chambers are sprayed with sterile water and the chambers sealed with a layer of cling film plastic.
- Plant material is incubated at 20°C with 8 h cool white fluorescent light for 6 d.
- The chambers are damped down with sterile water daily.





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Disease estimation, data collection and re-isolation:

Broadleaved trees and conifers require slightly different assessment methods because of the differences in foliage structure. Three parameters are used to evaluate disease development six days after inoculation:

- Parameter 1 - the presence or absence of necrosis is recorded as percentage incidence necrosis. This applies to both conifer and broad-leaved hosts.
- Parameter 2 (conifer hosts) - the number of necrotic needles and the total number of needles inoculated per shoot are counted and disease severity expressed as a percentage.
- Parameter 2 (broad-leaved hosts) – Either digital images of infected leaves are scanned and disease severity is calculated by an image processing software package: Assess (APS, Minnesota, USA), and expressed as percentage necrotic surface area. Alternately, an estimation of % necrotic area can be made based on visual assessment.
- Parameter 3 - Infection is confirmed by re-isolating the pathogen from the plant tissue.

