



RAPRA Protocols for Susceptibility Testing

<http://rapra.csl.gov.uk>

Protocol 6: In vitro Production of Zoospore Inoculum



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Method:

Live, axenic zoospore suspensions are required for testing foliage susceptibility of different hosts, for investigating the inoculum potential of susceptible host leaves as well as the effects of inoculum density on disease expression, and for studying the bark infection process. Although *P. ramorum* forms sporangia naturally, the method for maximising their production under lab conditions is described here.

Inoculum:

Isolates are cultured on carrot agar (CA) (Brasier 1967, 1969) 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.
- To harvest sporangia, flood each plate with 5ml sterile water (a) and, using a flame sterilised glass rod (spreader), rub the colony surface to dislodge the sporangia into the liquid (b).
- Collect the sporangial suspension in a sterile beaker, incubate in a refrigerator at 7°C for 1hr, then return to room temperature for a further 75 min to induce zoospore release (Parke *et al.*, 2002).
- Vacuum filter the zoospore suspension through a sterile 10- μ m Millipore filter to remove the sporangia cases (c).

The zoospore concentration in the suspensions can be quantified and adjusted to requirements.

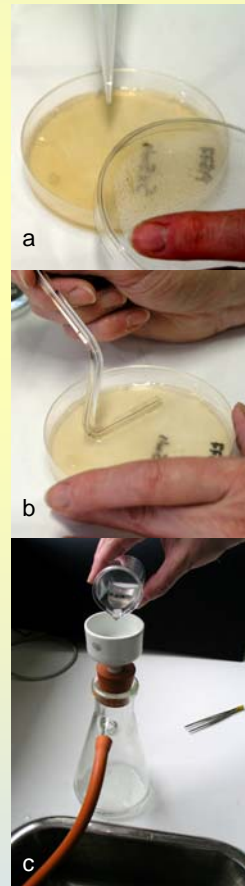
References:

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

Brasier CM, 1969. The effect of light and temperature on reproduction *in vitro* in two tropical species of *Phytophthora*. *Transactions of the British Mycological Society* **52**, 105–13.

Parke JL, Linderman RG, Hansen EM, 2002. Assessing susceptibility of Pacific Northwest nursery plants to *Phytophthora ramorum* using a detached leaf assay. In: *Sudden Oak Death Science Symposium, Monterey, California, December 2002* (abstract)

[<http://darn.ucop.edu/ihrmp/sodsymp>].





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