



Protocols for Susceptibility Testing



Protocol 10: Determination of Mating Type of *Phytophthora ramorum* Isolates by Mating Studies with Heterothallic *Phytophthora* Species

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Method:

Petri dishes of Carrot Piece Agar were prepared as follow:

- Medium was prepared with carrots that had not been sprayed with fungicides
- 15g of carrots pieces
- 22g agar
- 1000 ml distilled water

All isolates were paired on Carrot Piece Agar (CPA) with mating type A1 and A2 of four different types of heterothallic *Phytophthora* species (*P. cambivora*, *P. cinnamomi*, *P. cryptogea*, and *P. drechsleri*). From the edge of the growing colony of *P. ramorum*, a mycelium disc was cut out and placed on the plate of CPA about 1cm from the edge of the Petri dish. A similar disc was cut from the mating partner and placed on the opposite side of the plate, also about 1cm from the edge. The Petri dishes were incubated for 6 wk at 20 °C in the dark. Preliminary tests using this method had shown that most of the *P. ramorum* isolates produce oogonia within 6 wk of incubation. Gametangia developed predominantly in the surroundings of the carrot pieces. Agar discs with oogonia were cut out for size measurement under the microscope at x 500 magnification. The measurements of the oogonia were prepared from the matings with *P. cryptogea*, except for those isolates which did not produce oogonia with this *Phytophthora* species. For these isolates, oogonium sizes were calculated following mating with *P. cambivora*. Between 20-30 oogonia per isolate and pairing were measured.

References:

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