



Protocols for Susceptibility Testing

Protocol 11: Mating Studies with Phytophthora ramorum Isolates in Rhododendron Twigs



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Method:

To carry out these *in vivo* studies, c. 10 cm lengths of green, non-woody twigs of *Rhododendron* 'Cunninghams White' were prepared with one leaf on each twig. Three twigs per pairing were inoculated with *P. ramorum* by placing a mycelial disc (taken from the edge of a growing colony on Carrot Juice Agar) onto the cut ends of a twig: one isolate (BBA 9/95) was placed on one cut end, while isolate PR6 was placed at the other end.

To prevent the agar plugs from drying out, they were wrapped in wet tissue and then covered with a small plastic bag. The inoculated twigs were then incubated in a moist chamber (c.100% RH) at 20°C with a light period of 16 hours. After three days of incubation, the plastic bags, wet tissue and the agar plugs were removed. During subsequent incubation the development of infection by each isolate could be readily observed due to production of necrotic tissue. As soon as the necrosis caused by each isolate met, the twigs were surface sterilised and pieces cut from the junction were placed on plates of Carrot Piece Agar. The Petri dishes were incubated at 20°C in the dark and the production of oospores observed.

References:

Werres, S. & Ziehe, B. (2003). First studies on the pairing of *Phytophthora ramorum*. *Journal of Plant Diseases and Protection* **110**, 129-130.