Method for confirming new plant hosts of *Phytophthora ramorum*

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**Introduction**

It is important to be able to rapidly identify new plant hosts to prevent the potential spread of Sudden Oak Death. *Phytophthora ramorum* is often difficult to culture from plant species in which we have confirmed the presence of the pathogen using PCR. However, Koch’s postulates (see box) state that the organism must be cultured from diseased organisms to prove that it is the causal agent. These postulates, formulated before molecular techniques such as PCR, were available as a tool for detecting pathogens. Therefore, we propose a modified version of Koch’s postulates to confirm new hosts of *P. ramorum*.

**Case Study: Pittosporum undulatum (Pittosporaceae, Victorian Box)**

**Step 1:** Symptomatic leaves were collected (Fig 1).

**Fig 1.** *P. undulatum* (A) healthy and (B) symptomatic tree and (C) leaves sampled from the symptomatic tree.

**Step 2:** (a) Necrotic tissue was plated onto selective media; *P. ramorum* was not isolated. (b) The sample was processed using nested PCR and TaqMan PCR.

(i) Nested PCR primers Phyto1 + 4 and Phyto2 + 3 were used for first and second round respectively. *P. ramorum* DNA was detected after the second round of PCR.

(ii) TaqMan PCR primers Pram5 + 6 and probe Pram7 were used. DNA was detected at a cycle threshold of 38.6 (Fig 2).

**Fig 2.** TaqMan PCR detection of *P. ramorum* in *P. undulatum* leaf extract.

**Step 3:** The product from the nested PCR is currently being sequenced.

**Step 4:** *P. ramorum* zoospores from a culture originally isolated from a *Rhododendron* sp. was used to inoculate excised branches of *P. undulatum* (Fig 3). Symptoms of *P. ramorum* infection were recorded (Fig. 4). Twenty days after inoculation, growth of *P. ramorum* was 18.4 ±1.4 mm (± S.E.).

**Fig 3.** Infection of *P. undulatum* leaves with *P. ramorum* zoospores.

**Fig 4.** *P. undulatum* leaves with lesions caused by *P. ramorum*.

**Disadvantages**

- Currently no published information on phenotypic variation in *P. ramorum* isolates. If there is large variation in pathogenicity, we risk using an isolate that causes more/less disease than the isolate from the new host.
- *P. ramorum* DNA detected by PCR may not be from a live organism.

**Advantages**

- More rapid confirmation of new hosts.
- Molecular techniques have high sensitivity and specificity for *P. ramorum*.
- Morphological techniques for characterization of *P. ramorum* are time consuming.

**Conclusion:** This is a rapid, sensitive and specific protocol for identifying new hosts of *Phytophthora ramorum*. To date, it has been used successfully to identify 18 new hosts.