# Method for confirming new plant hosts of *Phytophthora ramorum*

**Matteo Garbelotto, Carla Wilkinson, Daniel Hüberli, Department of ESPM-ES, University of California, Berkeley and Ellen Simms, University of California Botanical Garden**

## 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*.

### Koch's Postulates

The following rules of experimental proof were developed by Koch (1882) to verify the causal agent of disease: The pathogen must be

1. found in association with disease in all plants examined,
2. isolated, grown and characterized in pure culture,
3. inoculated from pure culture onto healthy plants of the same species or cultivar on which the disease occurs and it must produce the same disease on the inoculated plants and
4. isolated in pure culture from the inoculated plants and its characteristics must be exactly like those from the original culture.

## Method for determining a new plant host

**Step 1:** Environmental Sample - collect symptomatic host tissue.

**Step 2:** (a) Culture - place tissue on selective media and isolate for *P. ramorum.*

(b) Molecular Techniques - use nested or TaqMan® PCR to test for *P. ramorum* DNA in host tissue.

**Step 3:** Sequence the DNA - using *P. ramorum* specific primers.

**Step 4:** Modified Koch's Postulates - inability to culture *P. ramorum* from an environmental sample prevents completion of steps 1 and 2 of Koch's Postulates. We propose to substitute a *P. ramorum* isolate from another host to inoculate the new host.

### 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.

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

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

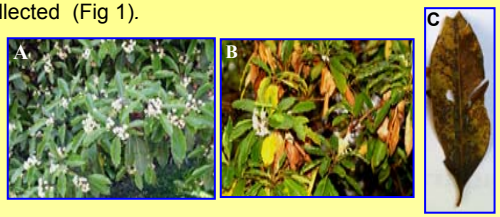


Fig 1 *P. undulatum* (A) healthy and (B) symptomatic tree and (C) leaves sampled from the symptomatic tree.

**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).

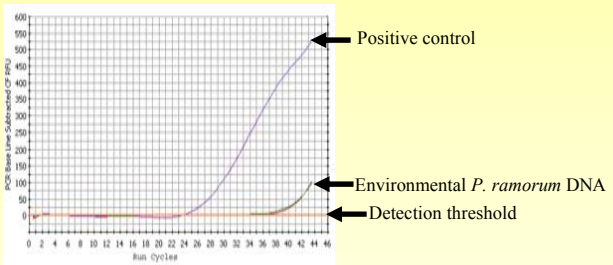


Fig 2. TaqMan PCR detection of *P. ramorum* in *P. undulatum* leaf extract.

**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*.

## Host List

The following plant species have been confirmed as hosts using this method; *Acer macrophyllum*, *Aesculus californica, Arbutus menziesii*, *Arctostaphylos manzanita, Heteromeles arbutifolia*, *Lithocarpus densiflora*, *Lonicera hispidula*, *Pseudotsuga menziesii, Quercus agrifolia*, *Q. kellogii, Q. parvula* var. *shrevei, Rhamnus californica*, three *Rhododendron* cultivars, *Rhododendron macrophyllum, Sequoia sempervirens*, *Umbellularia californica, Vaccinium ovatum* and *Pittosporum undulatum* (see case study). Eleven of these hosts were first discovered using PCR, however, we have since isolated *P. ramorum* from all but two hosts (*A. manzanita* and *P. undulatum*).