# THE NEED FOR RAPID MOLECULAR DIAGNOSTICS TO DISTINGUISH BIOTYPES OF THE MYRTLE RUST PATHOGEN (*AUSTROPUCCINIA PSIDII*)

**Rachael A. Sitz[1,2], Mee-Sook Kim[3], Phil G. Cannon[4], Acelino C. Alfenas[5], Morag Glen[6], Alistair R. McTaggart[7], Ned B. Klopfenstein[1], and Jane E. Stewart[2]**

## Background

*Austropuccinia psidii* impacts numerous forest ecosystems world-wide by causing myrtle rust disease on many myrtaceous species, including guava *(Psidium guajava),* eucalypts *(Eucalyptus spp.)*, rose apple *(Syzygium jambos)*, and 'ohi'a *(Metrosideros polymorpha).* First reported in 1884 on guava in Brazil, the rust has since been detected in tropical and subtropical areas worldwide. At least three biotypes of *A. psidii* have currently been identified (Stewart et al. 2017), including eucalypt and guava biotypes in Brazil, and the globally distributed pandemic biotype which infects multiple hosts. However, despite known genetic variation in this species and distinct invasive threats, we currently lack rapid methods to quickly distinguish among different biotypes. This becomes especially problematic as biotypes of the pathogen continue to expand their geographic ranges. Although myrtle rust is established in Florida, California, Hawaii, Puerto Rico, Australia, and New Zealand, the introduction of new biotypes to these areas poses an additional threat, which could be amplified if hybridization were to occur (McTaggart et al. 2017, Loope & La Rosa 2008). Methods currently exist to confirm the presence of specific biotypes (Machado et al. 2015, Bini et al. 2018), but we need cost and time effective tools to quickly distinguish among biotypes (current and proposed methods are compared in Table 1). Here we propose a framework for developing rapid, user-friendly diagnostic assays for distinguishing among different *A. psidii* biotypes.

***Table 1:*** *Comparison of current and proposed methods for distinguishing* Austropuccinia psidii *biotypes.*

| | **Current Method**<br>**Microsatellite Genotyping** | **Proposed Method**<br>**LAMP** |
|---|---|---|
| **Sample Collection** | Ship samples from field to lab | Identify samples in the field |
| **Sample Preparation** | DNA extraction | None |
| **Assay** | DNA amplification (multiple genomic regions, n=6 to 10)<br>DNA quantity/quality check | Isothermal incubation (~30 min) |
| **Analysis** | Send DNA for sequencing<br>Computer analysis, and genetic comparisons | Visual, real time confirmation upon sample florescence |
| **Equipment Needed** | Specialized laboratory equipment (e.g. tissuelyser or bead basher, centrifuge, heat plate, pipettors, thermocycler, gel doc or tape station, nanodrop), reagents, supplies (tips, tubes), computer software, and trained personnel | Thermos, tubes, reagents |
| **Total Time Invested** | Several months, numerous people, expensive | ~1 hour, few people, inexpensive |

*[1]USFS Rocky Mountain Research Station, Moscow, ID, USA. [2]Colorado State University Department of Bioagricultural Sciences and Pest Management, Fort Collins, CO, USA. [3]USFS Pacific Northwest Research Station, Corvallis, OR, USA. [4]USFS Forest Health Protection (FHP), Region 5, Vallejo, CA, USA. [5]Universidade Federal de Viçosa, Departamento de Fitopatologia, Viçosa, MG, BRA. [6]Tasmania Institute of Agriculture, University of Tasmania, Hobart, TAS, AUS. [7]Center for Horticultural Science, University of Queensland, Brisbane, QLD, AUS*

**Objectives**

This project aims to (1) develop rapid diagnostic assays, such as LAMP assays (Loop-Mediated Isothermal Application) to easily distinguish among the main myrtle rust biotypes; (2) further document the global distribution and host associations of each *A. psidii* biotype; and (3) provide stakeholder groups (such as quarantine officials and forest pathology researchers) with rapid diagnostic technology and training.

**Implications**

This project will develop field assays to promptly detect different biotypes of *A. psidii.* The LAMP assay will greatly reduce the labor, time, and cost needed to biotype samples and improve our ability to respond to the invasive myrtle rust pathogen. Furthermore, it will guide regulatory and management practices that address potential invasive threats.

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