ELISA and ImmunoStrip® for Detection of *Phytophthora ramorum*, *P. kernoviae*, and Other *Phytophthora* Species[1]

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Abstract

The goal of this work was to develop improved tools for the detection of *Phytophthora ramorum* and *P. kernoviae* for field and the laboratory use. ImmunoStrip® and ELISA were selected as the test formats for development. Presently, the diagnosis of sudden oak death (SOD) in the national survey of *P. ramorum* depends on the use of ELISA to pre-screen samples, and then confirms results with PCR and morphological identification. This approach has some disadvantages because the ELISA has a wide spectrum reaction with *Phytophthora* spp. and cross-reacts with *Pythium* spp. A faster and more specific serological test to detect *P. ramorum* would be very useful in the survey of this pathogen. *P. kernoviae* was first detected in rhododendron in Cornwall in 2003 during surveys for *P. ramorum*, and the pathogen has been found in New Zealand. *P. kernoviae* has not been reported in the U.S. However,

Mycelium suspensions of *P. ramorum* isolates from California, Washington, and Europe, as well as mycelium suspension of *P. kernoviae* from Europe, were injected into mice for monoclonal antibody production and in rabbits for polyclonal antibody production. The resulting antibodies were screened using a collection of *Pythium* and *Phytophthora* that included *P. kernoviae*, and *P. ramorum* (from U.S. and Europe); and healthy plants such as red pine (*Pinus resinosa*) needles, rhododendron (*Rhododendron* sp.), and oak (*Quercus*) leaves. Antibodies with highest sensitivity and specificity to *P. ramorum* and *P. kernoviae* and without healthy tissue reaction were considered candidates for immunoassay development. Although many new antibodies were produced, none of them were species-specific to *P. ramorum* or *P. kernoviae* and cross-reacted with other species of *Phytophthora*.

Several ImmunoStrip® and ELISA prototypes were developed. Each was evaluated with pure cultures of *Phytophthora* and *Pythium*, and *Phytophthora*-infected plant material. The resulting ImmunoStrips® were found to be sensitive, rapid, and easy to perform with field materials, and able to detect all *P. ramorum* and *P. kernoviae* isolates while not detecting any *Pythium* isolates. One strip prototype was not very reactive with mycelium younger than 5 weeks old; however, another prototype with a different antibodies pair detected *P. ramorum* and *P. kernoviae* in all samples tested.

Some newly developed antibodies when used in ELISA recognized *P. ramorum* and *P. kernoviae*, detected a smaller spectrum of *Phytophthora* species and did not cross-react with

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the *Pythium* species. Another set of antibodies in an ELISA format detected all *Phytophthora* species tested and recognized fewer *Pythium* species.

These results demonstrate that the tools for detection of *P. ramorum* can be significantly improved because some prototype tests recognize all *P. ramorum*, do not cross react with *Pythium*, and detect a significantly smaller spectrum of *Phytophthora* species. We are continuing this study including a comparison of the new tests with PCR especially with field samples.