

Susceptibility of Some Native Plant Species From Hawaii to *Phytophthora ramorum*¹

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Abstract

The remaining native flora of Hawaii are under continuing pressure from habitat loss and exotic, invasive organisms, including animals, plants, and pathogens. In order to assess the risk to *P. ramorum*, we inoculated seedlings of *Metrosideros polymorpha* (ohia), *Vaccinium calycinum* (ohelo), *Acacia koa* (koa), and *Leptecophylla tameiameiae* (pukiawe) with the pathogen. Two isolates were used, one from an Oregon horticultural nursery, and one from tanoak in the Oregon infested forest area. A zoospore suspension was sprayed on the intact plants. Plants were inspected periodically and symptomatic plants or plant parts were harvested and photographed. For isolation of *P. ramorum* symptomatic tissue was surface disinfested and plated in semi-selective agar. There was some symptom development in some inoculated plants for all the species tested. However, infection was confirmed by re-isolation of *P. ramorum* only in ohelo, pukiawe, and koa. Detached leaf inoculation also demonstrated susceptibility of *Vaccinium reticulatum*, another species of ohelo.

Key words: Sudden oak death, *Metrosideros polymorpha* (ohia), *Vaccinium calycinum* (ohelo), *Vaccinium reticulatum*, *Acacia koa* (koa), and *Leptecophylla tameiameiae* (pukiawe).

Materials and Methods

Metrosideros polymorpha (ohia), *Vaccinium calycinum* (ohelo), *Acacia koa* (koa), and *Leptecophylla tameiameiae* (pukiawe) were inoculated with zoospore suspensions of *Phytophthora ramorum* forest isolate 2027.1 (1.6×10^5 per ml) and nursery isolate 03-74-1 (9.5×10^4 per ml). Inoculum was applied to plants with an airbrush sprayer. Two plants of each species were sprayed with de-ionized water, 15 ml over two plants, to serve as non-inoculated controls. Five plants of each species were sprayed with about 20 ml of the zoospore suspension of either isolate 2027.1 or 03-74-1. Inoculated plants were enclosed in thin white plastic bags for three days to maintain high humidity. The plants were incubated at around 20°C, with full fluorescent light banks at 12 hour photoperiod, and inspected periodically for symptoms. Symptomatic plants or plant parts were harvested and photographed. For isolation of *P. ramorum* symptomatic tissue was surface disinfested with 10 percent household bleach, rinsed three times in de-ionized water, and plated in corn meal

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agar amended with 10 ppm natamycin, 200 ppm Na-ampicillin, and 10 ppm rifampicin (CARP).

Vaccinium reticulatum (ohelo) was tested in detached leaf assays as part of a larger experiment on the susceptibility of *Vaccinium* species and blueberry cultivars to *P. ramorum* (Parke, unpublished). Leaves were collected from greenhouse-grown *V. reticulatum* plants (accession 780.002) at the National Clonal Germplasm Repository, Corvallis, Oregon. Five leaves were dipped in each of three treatments: sterile water (non-inoculated controls), or zoospores from each of two sources of inoculum (nursery isolates and forest isolates) at 10^4 ml⁻¹. The nursery isolates (A1 mating type) were 03-74-D12A and 03-74-1. The forest isolates (A2 mating type) were 2027.1 and 4169. Leaves were incubated flat in moist chambers for 7 days at 19-21 C. *Rhododendron* "Cunningham's White" and *Vaccinium ovatum* were included as positive controls, and *V. macrocarpon* 'Bugle' was included as a negative control. The experiment was conducted twice. At six days, leaves were scanned, and digital images subjected to image analysis (Assess, APS Press, St. Paul, MN) to determine total leaf area and percent necrotic area.

Results and Discussion

There was some symptom development in some inoculated plants for all the species tested. Symptoms were observed six days after inoculation in ohelo plants 1 and 2, and in koa plants 1 and 2. Symptoms in additional plants were observed four weeks after inoculation. However, infection was confirmed by re-isolation of *P. ramorum* only in ohelo, pukiawe, and koa.

In both detached leaf trials, with both sources of inoculum, *V. reticulatum* leaves were 100 percent necrotic. Non-inoculated controls and inoculated *V. macrocarpon* 'Bugle' leaves were not necrotic. Mean percent necrotic area was 41 percent and 82 percent for inoculated *Rhododendron* 'Cunningham's White' and *V. ovatum*, respectively.

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