

Soil Moisture and Temperature Conditions Affect Survival and Sporulation Capacity of Rhododendron Leaf Disks Infested with *Phytophthora ramorum*¹

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Abstract

Soilborne inoculum (infested leaf debris which has become incorporated into the soil) may be an important contributor to the persistence of the sudden oak death pathogen *Phytophthora ramorum* in recurrently positive nurseries. To initiate new epidemics, soilborne inoculum must not only be able to survive over time, but also be capable of producing sporangia during times favorable to infection of plant material at the soil surface.

To accompany field studies of the epidemiological risk of soilborne inoculum in nurseries, laboratory assays were performed investigating how incubation of inoculum at various temperature and moisture regimes affects sporulation capacity and survival of *P. ramorum*. For all experiments, wounded rhododendron leaves were infected with *P. ramorum* zoospores and then incubated for 2 to 3 weeks. Leaf disks were punched out of the lesioned areas, which were inserted into mesh sachets and placed at the various moisture and temperature treatments. Over time we recovered sachets and placed the disks in tubes containing filtered creek water at 20°C to induce sporulation. The tubes were vortexed after 1 week and the water was filtered to capture sporangia, then the leaf disks were plated on selective media to discern how incubation conditions affected survival.

To test how incubation at different temperatures and moisture levels affects sporulation, leaf disk inoculum was packed into capsules containing soil at matric potentials of 0, -40, and -400 kPa. Capsules of each moisture level were placed in growth chambers set at an average temperature of 6.7, 14, 20, or 28°C. Six capsules per moisture level per temperature were removed at 2, 6, 12, and 18 weeks post-incubation to assess for survival and sporulation potential. Recovery was high for all but the warmest and driest treatments. Sporulation remained greatest over time for disks incubated at cooler temperatures for most treatments and was greater for treatments at non-saturated moisture levels.

To test chilling effects upon sporulation, leaf disk inoculum was incubated in saturated soil at 20°C for 3 weeks, which had the effect of reducing sporulation capacity relative to the pre-incubation sporulation and controls maintained at 4°C. Inoculum was then placed at either 4 or 20°C. Disks were retrieved and assessed for sporulation capacity for up to 168 days after the exposure of a subset of this inoculum to 4°C. Maximum sporulation from the 20°C to 4°C treatment was observed 49 days post-exposure in both trials.

To determine how prior moisture and temperature conditions affect sporulation responses to chilling, at the week-18 assessment for the experiment testing the interactions between moisture and temperature, an additional set of samples for each temperature:moisture combination was placed at 4°C. After 49 days the

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samples were retrieved and assessed for sporulation and survival. Incubation at 4°C increased sporangial production for all inoculum initially incubated at 20 and 28°C, but not 6 and 14°C regardless of moisture.

The likelihood of new epidemics developing from soilborne inoculum sources will be modulated by the time of introduction and the soil environment. Exposure to moderate temperatures and moisture regimes simulating soil conditions rapidly reduces sporulation potential from leaf material infested with *P. ramorum*; however, sporulation potential increases post-exposure to cooler temperatures, especially for inoculum incubated at 20°C. This work is in agreement with field observations that the onset of cooler temperatures in autumn and winter may initiate new *P. ramorum* infections from soilborne sources.