Importance of Rainfall and Sprinkler Irrigation in Supporting Sporulation, Spread of Inoculum in Runoff-Water, and New Infections of *Phytophthora ramorum* Under Field Conditions¹

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Abstract

If a nursery plant infected with *Phytophthora ramorum* is introduced in a non-infested area, then it is important to understand what environmental conditions could lead spread and infection of new hosts. Once an infected nursery plant is introduced in a nursery or landscape, moving water sources, such as from rain and irrigation events, could provide an important means for dispersal and infection of new hosts. We evaluated seasonal environmental factors and associated rainfall and irrigation events that could support sporulation, dispersal, and infection of new hosts under field conditions.

Ten leaves on each rhododendron and camellia in a 3.8 l pot were inoculated with P. ramorum every 3 months (once each season) from July 2006 to June 2008 and grown in field conditions. The pots of each species were grouped into six blocks with seven pots per block. Approximately every 2 weeks for 3 months after each seasonal inoculation, a 1.2 m by 1.2 m tray was placed under each block to collect the majority of run-off water that fell through and around the plant's foliage during a rainfall event. A smaller portion of run-off water was allowed to run down a small opening around the base of the plant and infiltrate into the potting soil. If there was no rainfall during the period, then the pots were sprinkler irrigated for 45 minutes in early morning. The run-off's inoculum concentration was assessed by filtering with a Milipore filter and plating the filters on PARP semi-selective media and counting P. ramorum colonies for up to 3 months after inoculation. At the end of the rainfall or irrigation event, 10 infected leaves from each block were selected and each leaf was washed on both sides with a strong stream of 100 ml distilled water. The concentration of the leaf wash was quantified by plating 1 ml subsamples directly onto a 10 cm Petri plate with PARP semi-selective media and counting P. ramorum colonies for up to 6 months after inoculation. The soil leachate, coming from the bottom of each pot, was collected in plastic bags and the inoculum concentration was assessed with pear baits for up to 3 months after inoculation. For each species, each of three 1 l subsamples of leachate was baited with one unripe pear, and resulting lesions on the pears were counted.

The disease risk was assessed in the field or in the laboratory. For the field assessment, the run-off water was collected from each species and sprayed onto three plants of the respective species. The plants were incubated in plastic covered tubs in shaded field conditions for 48 hours, removed from the tub, and periodically inspected for symptomatic leaf lesions. Beginning with the winter season of the first year, six 8 mm-diameter leaf disks of each

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species were flooded in three Petri plates containing 6 ml run-off water from each respective species. The leaf disks were periodically inspected for characteristic lesions, and infected leaf disks were counted. Six camellias inoculated in fall 2006 were evaluated for root infections about 6 months after inoculation. The number of consecutive hours that leaves were wet preceding the run-off or leaf wash sampling was measured by a leaf wetness sensor in the plant leaf canopy.

Although the highest concentrations of inoculum were found in fall (October to December) and winter (January to March) of both years, inoculum could be found in all seasons in runoff water up to the last evaluation (3 months after inoculation) and in leaf washes up to the last evaluation (6 months after inoculation) for each inoculation period. High concentrations of inoculum in run-off water and leaf washes were more commonly found after rainfall events than after irrigation events, and the concentration was positively correlated to the number of consecutive hours of leaf wetness. In general, inoculum concentration in the soil leachate was correlated with the volume and inoculum concentration of the water run-off indicating that inoculum ran through the potting soil relatively easily. However, there were also periods when the concentration of soil leachate did not significantly increase until after several weeks of the initial inoculation even though high inoculum was found in run-off water and leaf washes. Four of the six camellia pots sampled about 6 months after inoculation had roots infected with *P. ramorum*, and inoculum was detected in soil leachate even though the plants were completely defoliated after 3 months. The inoculum in the soil leachate may have been produced by sporulating infected roots. Disease on plants sprayed with run-off water occurred only in the winter of the first year after an extensive cool and rainy period. Leaf disk infection was a better measure of disease risk. Leaf disk infection was positively correlated with the concentration of inoculum in the run-off water, and leaf disk infection occurred only in the wet periods of the second year.

Rainfall events create the conditions for extended leaf wetness needed for sporulation of leaf lesions. In our study, irrigation events did not usually extend overnight leaf wetness; however, we noted that spring and fall conditions with clear cold nights could create heavy overnight dew, and leaf wetness could be extended by early morning irrigation. If dew exists in the morning, nursery operators should delay any necessary irrigation until the leaves can dry and the wet period is broken. Run-off water coming off of sporulating plants could contain sufficient inoculum to cause disease under favorable environmental conditions. Run-off water could cause secondary leaf infections on the same plant or perhaps after it flows in the nursery drainage and splashed onto other plants. Inoculum in run-off water could be splashed from the drainage onto plants or come into contact with roots and cause root infections. Run-off water that is recycled for use in the nursery could pose a risk if it is not treated with chlorine, ozone, ultra-violet light, or other sanitation treatment.