

Seasonal Symptom Expression, Laboratory Detection Success, and Sporulation Potential of *Phytophthora ramorum* on *Rhododendron* and *Camellia*¹

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Abstract

Camellias and rhododendrons are important nursery and landscape plants and are known to be highly susceptible hosts of the quarantined plant pathogen, *Phytophthora ramorum* Werres, de Cock & Man In't Veld. Nursery inspection can not always occur during optimal conditions for the disease and its detection. The goals of this research were to (1) characterize and document seasonal development of symptoms, including, lesion growth rates and host leaf abscission, (2) evaluate the USDA-APHIS approved laboratory detection methods during four seasons, and (3) assess the seasonal potential for sporangial or chlamydospore production on lesions. This paper contains the results of the first complete year of a two year field and laboratory study.

Leaves on potted *Camellia japonica* L. 'Kumasaka' plants and *Rhododendron* 'Cunningham's White' were inoculated with V8 agar plugs from *P. ramorum* cultures on 18 July 2005, 26 October 2005, 1 February 2006, and 1 May 2006, representing summer, fall, winter, and spring infections respectively. The resulting lesion growth and leaf abscission was quantified for up to 16 weeks after inoculation. Infected leaves were sampled for up to 24 weeks after inoculation. A portion of the sampled leaves were mailed by overnight courier to the California Department of Food and Agriculture Plant Pest Diagnostics Branch Laboratory where the most current federally approved methodology was used for detection (PARP semi-selective media, ELISA, and real-time and nested PCR), and detection success was evaluated. Isolations from other leaves in the sample were plated onto PARP media in a local laboratory, without shipment, and detection success was evaluated. From another portion of the sampled leaves, leaf-disks were punched from the margin of the lesions and flooded with de-ionized water. Sporangia and chlamydospores produced on the margin of the leaf-disks were identified by propagule type and counted.

Growth rates of lesions were fastest in the fall and winter and slowest in the spring. Camellia leaves abscised in significantly fewer days after inoculation in the fall and winter than in the spring and summer. Rhododendron leaves abscised infrequently after inoculation and then only in the winter. Successful laboratory detection with PARP by the State laboratory was possible with summer inoculated leaves up to 20 weeks, with fall at least up to 24 weeks, with

¹ This paper was presented at the Sudden Oak Death Third Science Symposium, March 5–9, 2007, Santa Rosa, California. It presents data but limited analysis for the first year of a 2-year study. It will become part of the completed study at the end of 2007 when data are complete and more robust analysis will be possible.

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winter up to 20 weeks, but spring only up to 4 weeks after inoculation. Laboratory detection locally (without shipment) with PARP generally mirrored this result. ELISA and PCR (nested and real time) methods were 100 percent effective in detection regardless of season or leaf age. Flooded camellia leaf-disks were capable of producing chlamydozoospores and sporangia 8 to 12 weeks after initial inoculation with the fall and winter more conducive to the production of these propagules than the other seasons. Flooded rhododendron leaf-disks produced sporangia for 12 to 20 weeks after inoculation and chlamydozoospores 4 to 20 weeks after inoculation with the fall and winter generally more conducive to the production of these propagules.

Key words: *Phytophthora ramorum*, sudden oak death, symptoms, detection, sporulation, rhododendron, camellia, nursery.

Introduction

In California, Oregon, and Washington, federal regulations require annual nursery stock inspection, leaf sampling, and laboratory analysis of host plants susceptible to *Phytophthora ramorum* for plants shipped out of state. For these regulations to be effective, agricultural inspectors must recognize disease symptoms and collect proper leaf samples, and laboratory analysis must be accurate and sensitive. Nursery inspection can not always occur during optimal conditions for the disease and its detection. If *P. ramorum* is found in a nursery and the confirmed nursery protocol is imposed, follow-up inspections and samplings could occur at nearly any time of the year. With this in mind, the goals of this research were to (1) characterize and document seasonal development of symptoms, including, lesion growth rates and host leaf abscission, (2) evaluate the United States Department of Agriculture-Animal and Plant Health Inspection Service (USDA-APHIS) approved laboratory detection methods during four seasons, and (3) assess the seasonal potential for sporangial or chlamydozoospore production on lesions. This paper contains the results of the first complete year of a two year field and laboratory study.

Materials and Methods

Ten leaves on each of 42, 3.8 L (potted) *Camellia japonica* L. 'Kumasaka' plants and 42, 3.8 L *Rhododendron* 'Cunningham's White' were inoculated with V8 agar plugs from 21 day old cultures from *P. ramorum* cultures on 18 July 2005, 26 October 2005, 1 February 2006, and 1 May 2006, representing summer, fall, winter, and spring infections respectively. The *P. ramorum* isolate used for inoculation was isolated from an infected rhododendron at a local nursery. Plants were grown under environmental conditions and with cultural practices typical of many local commercial outdoor nurseries. Plant spacing was consistent with commercial nursery practices. Plants were irrigated with overhead micro-sprinklers (Global #VS360 T), and fertilized (21-5-6 Apex®, J.R. Simplot) at a rate of 6 grams per pot every four months. All plants were placed under shade cloth (40 percent light transmission) and maintained without the use of fungicides. For each of the two species, seven plants were placed on each of six (95 cm x 82 cm x 16 cm) wooden pallets to elevate the pots well above the soil. When leaf samples were taken, they were removed randomly from each group and treated as statistical replicates.

For each seasonal inoculation and host species, the resulting uniform lesions from 30 designated leaves were digitally photographed 1, 2, 4, 8, 12, and 16 weeks after

inoculation or until the leaves abscised. The area of the lesions on each leaf was calculated with the aid of Access software (American Phytopathological Society). When available, up to 24 leaves per species were removed for laboratory detection, 1, 2, 4, 8, 12, 16, 20, and 24 weeks after inoculation (four leaves from each of six replicates). These samples were sent by overnight courier to the California State Department of Food and Agriculture Diagnostic laboratory (Sacramento, California) where the most current USDA-APHIS approved methodology was used for detection. Samples were both isolated on PARP semi-selective media and tested by ELISA (Agdia Pathoscreen Kit for Phytophthora [#PSA 92600]). All samples were tested further by either real-time or nested PCR specific for *Phytophthora ramorum*. (APHIS website:

http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/protocols.shtml.)

When available, up to 24 leaf samples per species were removed to evaluate the potential for leaf lesions to produce sporangia and chlamydo spores (4 leaves from each of 6 replicates). For each sampled leaf, up to 3 (6 mm diameter) leaf-disks were punched from the margin of the lesions. A total of 6 randomly selected leaf-disks from each replicate were placed in a Petri plate and were flooded with enough deionized water to just cover the leaf disks. Plates were incubated at 20 °C in the dark. Sporangia and chlamydo spores on the margin of the leaf-disks was identified by propagule type and counted one week after flooding.

Environmental conditions were monitored on the experimental site with a portable digital weather station (Onset Computer Co.). The average maximum, minimum, mean, temperatures and cumulative rainfall were for *summer*: 25.7 °C, 8.0 °C, 15.1 °C, and 1.9 mm respectively; for *fall*: 15.3 °C, 2.8 °C, 8.0 °C, and 257.7 mm respectively; for *winter*: 15.9 °C, 3.9 °C, 9.1 °C, and 235.4 mm respectively; for *spring*: 27.3 °C, 9.6 °C, 17.5 °C, and 5.9 mm respectively. Spring 2006 was notable for its record-breaking high temperatures and low rainfall.

Results and Discussion

Growth rates for camellia and rhododendron lesions were fastest in the fall and winter and slowest in the spring when temperatures were unusually hot (fig. 1 and 2). Leaf lesions developed as “waves” of alternating dark and tan necrotic areas. Dark brown portions surrounded by lighter halos were associated with periods when lesions were expanding rapidly during cool, wet conditions. Light tan areas were associated with periods when lesion growth slowed or stopped during hot and dry conditions. Camellia leaf abscission occurred as a result of *P. ramorum* infection. Leaf abscission occurred more quickly when lesion growth was rapid in the fall and winter. All camellia leaves had fallen by 12 weeks after inoculation. Leaf abscission occurred very slowly in the spring when lesion growth was slow. Most leaves had not abscised for up to 16 weeks after inoculation (fig. 3). Leaves often fell before the lesions became larger than 4 cm². Rhododendron leaves abscised infrequently and then only in the winter.

Under laboratory conditions, sporangia and chlamydo spore production declined as lesions aged. Flooded camellia leaf-disks were capable of producing chlamydo spores and sporangia 8 to 12 weeks after initial inoculation. More propagules were produced in camellia from leaves inoculated in fall and winter than in other seasons (fig. 4).

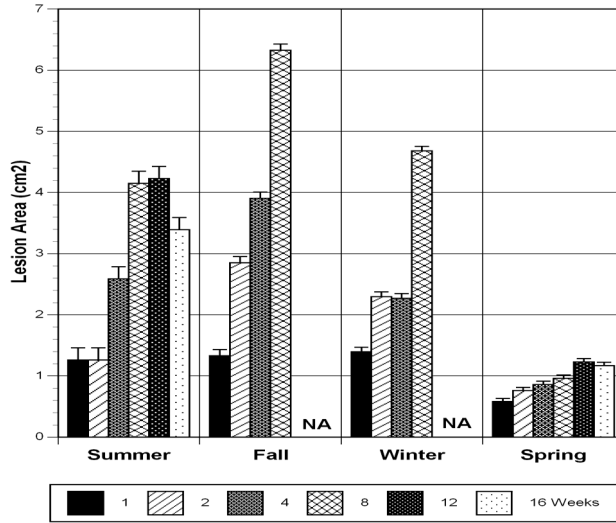


Figure 1—Mean lesion area on camellia leaves (N = 30 at first sampling) inoculated with *Phytophthora ramorum* during different seasons. Different bar patterns correspond to the number of weeks leaves were harvested after inoculation. Error bars indicate the standard error of the mean for each timepoint (NA = not available).

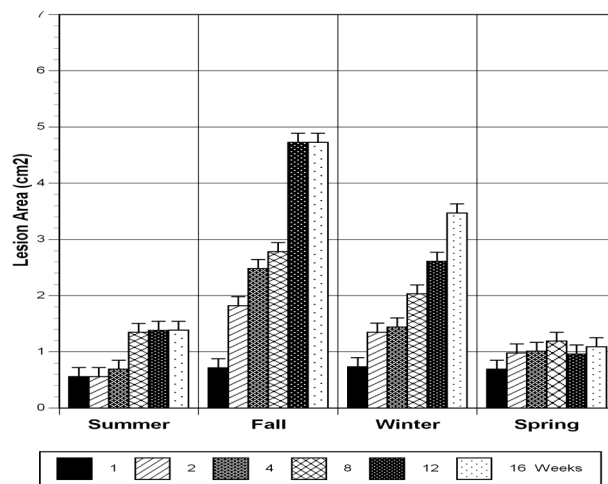


Figure 2—Mean lesion area on rhododendron leaves (N = 30 at first sampling) inoculated with *Phytophthora ramorum* during different seasons. Different bar patterns correspond to the number of weeks leaves were harvested after inoculation. Error bars indicate the standard error of the mean for each timepoint.

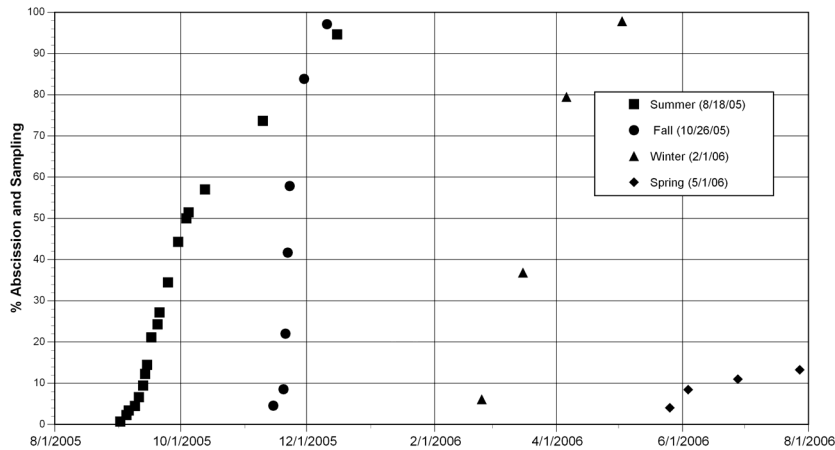


Figure 3—Percentage 420 camellia leaves (N = 420) inoculated with *Phytophthora ramorum* that had abscised or had been sampled after seasonal inoculation.

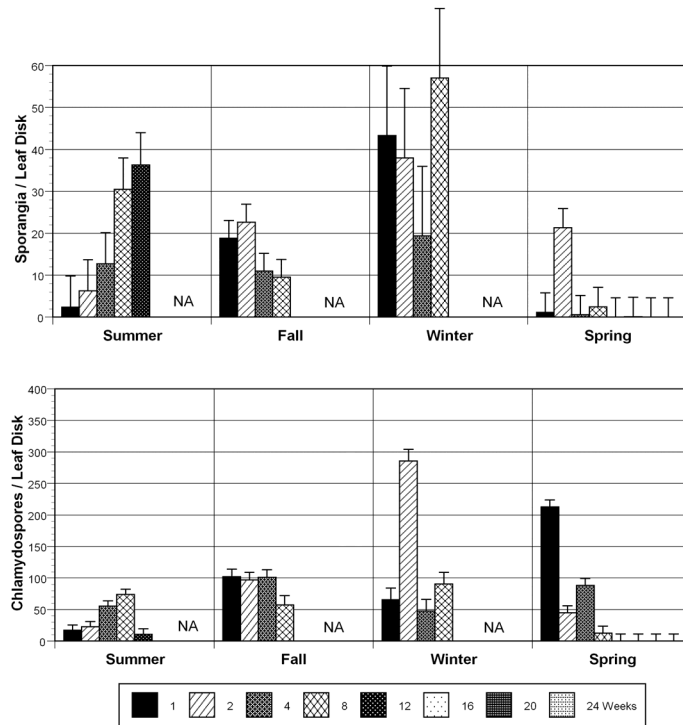


Figure 4—Mean production of sporangia and chlamydospores from *Phytophthora ramorum*-infected camellia leaf disks. Leaves were inoculated once per season and sampled weekly up to 24 weeks after inoculation. (NA = not available.) Upper figure represents sporangial counts, lower figure represents chlamydospore counts. All counts were made from the disk margin of disks flooded with deionized water.

ELISA and PCR (nested and real time) detected *P. ramorum* 100 percent of the time regardless of season and leaf age up to 16 weeks after inoculation. Successful laboratory isolations on PARP media by the state laboratory were made with summer inoculated leaves up to 20 weeks, with fall up to 24 weeks, with winter up to 20 weeks, but with spring only up to 4 weeks after inoculation. Laboratory detection locally (without shipment) with PARP generally mirrored this result (fig. 6).

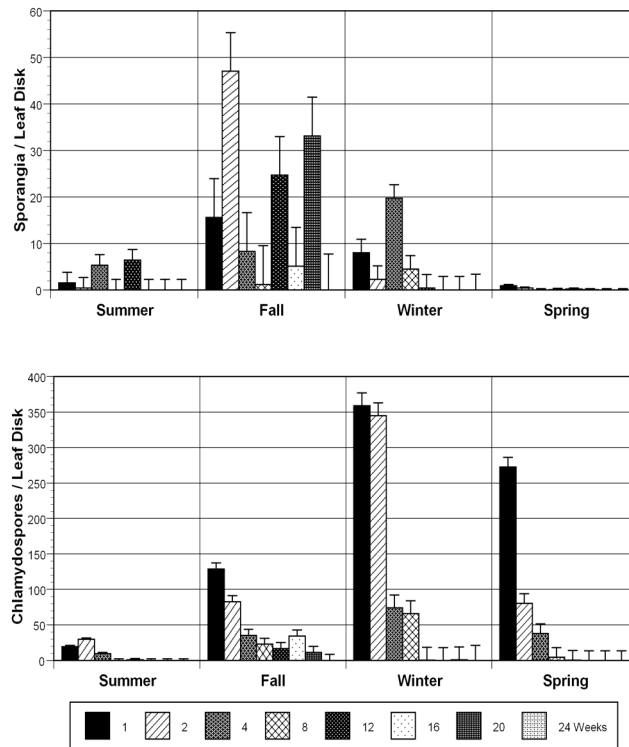


Figure 5—Mean production of sporangia and chlamydo-spores from *Phytophthora ramorum*-infected rhododendron leaf disks. Leaves were inoculated once per season and sampled weekly for up to 24 weeks after inoculation. (NA = not available.) Upper figure represents sporangial counts, lower figure represents chlamydo-spore counts. All counts were made from the disk margin of disks flooded with deionized water.

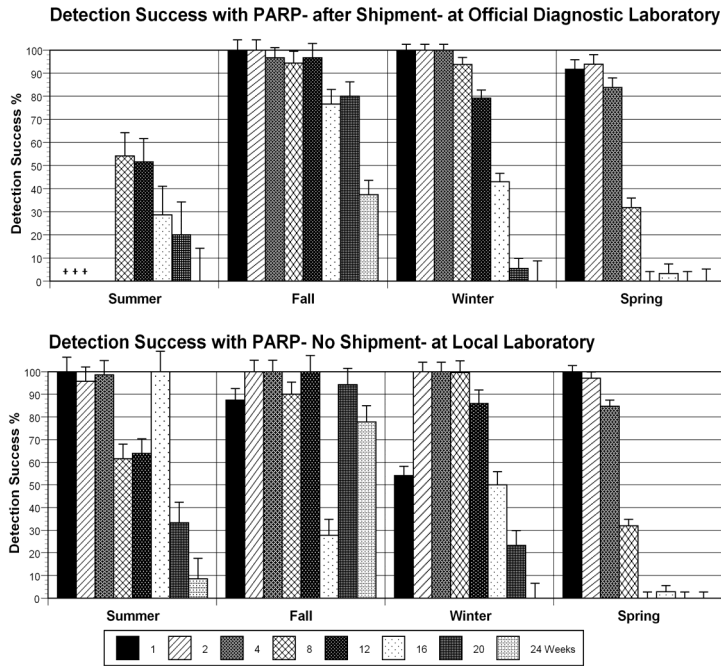


Figure 6—Mean percentage of rhododendron and camellia leaf pieces inoculated with *P. ramorum* that grew on PARP semi-selective media. Leaves from both species were inoculated once per season and then leaves (N = 24 at first sampling) were sampled once per week for up to 24 weeks after inoculation. Detection is by California Department of Food and Agriculture (CDFA) Diagnostic Laboratory (Sacramento) after courier shipment (top chart), or by local laboratory without shipment (bottom chart). Error bars represent standard error of the mean. (Detection at the first three sampling dates cultured at CDFA (top chart) were not quantified, but were all positive (+) for *P. ramorum*.)

Literature Cited

APHIS Federal Order, Posted 2/28/2007. *Phytophthora ramorum*; Quarantine and Regulations, Document #APHIS-2005-0102-0001.