

# Within-Field Spread of *Phytophthora ramorum* on Rhododendron in Nursery Settings<sup>1</sup>

Kurt Heungens,<sup>2</sup> Isabelle De Dobbelaere,<sup>2</sup> Bjorn Gehesquière,<sup>2</sup>  
Annelies Vercauteren,<sup>2</sup> and Martine Maes<sup>2</sup>

## Abstract

In Europe, *Phytophthora ramorum* has mostly been detected on rhododendron plants in nurseries. European Union (EU) phytosanitary measures state that potential host plants within a radius of 2 m of an infected plant must be destroyed, and remaining host plants within a radius of 10 m must be quarantined. Despite the lack of research on the spread characteristics of *P. ramorum* in nurseries, these distances have been generally accepted.

To test the distances over which *P. ramorum* can spread between potted rhododendron plants in a nursery setting, dispersal was monitored from individual infected plants in a mock nursery. The distance over which the disease could spread was monitored in separate experiments during spring, summer, and fall seasons. Dispersal of the pathogen was mainly to neighboring plants, and plant-to-plant contact was an important factor for successful spread. Aerial detection of *P. ramorum* with a Burkard spore sampler was consistently negative. In contrast, pathogen spread via drain water films on the non-draining growing surface took place even at a distance of several meters. Splash dispersal from water films and direct inoculation during tipping over of plants seem to play a more important role in pathogen spread than aerial dispersal.

Disease spread was also monitored during the course of 2 seasons at a commercial nursery where a large number of findings of *P. ramorum* had been made during the EU-mandated survey. Plant parts with *Phytophthora*-like symptoms were collected at regular intervals, field coordinates of samples were taken, and the pathogen was isolated and identified. Out of more than 1300 samples, *P. citricola* was the most abundant *Phytophthora* species present, but a total of 281 *P. ramorum*-positive samples were identified. The location of *P. ramorum* findings was correlated with cultivar block within the field and did not show a clear pattern of focal spread. Microsatellite-based genotyping of the *P. ramorum* isolates revealed two sets of isolates with a unique genotype. Distance in the field between isolates of the same genotype was not limited to 10 m. Presence of *P. ramorum* in drain water was confirmed.

These data again suggest that aerial dispersal of *P. ramorum* is limited, but spread via drainage water can occur over several meters on the non-draining surfaces, and is the most likely means of spread among potted plants grown under such conditions. These data have implications for both quarantine measures and practical pest management.

## Introduction

In Europe, rhododendron is the main host plant for *Phytophthora ramorum*. European

---

<sup>1</sup> A version of this paper was presented at the Fourth Sudden Oak Death Science Symposium, June 15-18, 2009, Santa Cruz, California.

<sup>2</sup> Institute for Agricultural and Fisheries Research (ILVO), Merelbeke, Belgium.  
Corresponding author: [kurt.heungens@ilvo.vlaanderen.be](mailto:kurt.heungens@ilvo.vlaanderen.be).

Union (EU) phytosanitary measures for nurseries include destruction of all host plants within a 2 m radius of an infected plant, while remaining host plants within a 10 m radius are quarantined. These distances have not yet been backed by data on in-field spread of *P. ramorum*, despite the need for measures that are both appropriate for successful pest management as well as sensitive to economic damage incurred by the grower. Studies of plant-to-plant spread in nursery settings have been limited. Tjösvald and others (2006) observed no spread beyond 30 to 50 cm, but the leaf area of the detector plants in their study was limited. Neubauer and others (2006) were not successful in observing spread of *P. ramorum* in a nursery experiment due to a rapidly spreading *P. citricola* co-infection. Werres and others (2007) observed spread from irrigation water, but their study did not focus on plant-to-plant spread. In light of the dearth of information on plant-to-plant spread in nurseries, the objective of this study was to directly and indirectly monitor the distance of plant-to-plant spread of *P. ramorum* in (mock) nursery settings and to determine the relative importance of aerial versus water-film dispersal.

## Materials and Methods

At a mock nursery where appropriate biosafety measures were taken, pathogen dispersal was monitored from a centrally placed *P. ramorum*-infected rhododendron plant (cv. Mme Masson) to up to three concentric circles of detector plants. Three to four replicate sets were installed. Frequent overhead irrigation events lead to conditions conducive for sporulation and infection. After 7 days, plants were labelled, covered with a plastic bag, and placed at 17 to 20 °C for an additional week, after which symptoms and presence of *P. ramorum* was determined. Separate experiments were conducted during spring, summer, and fall seasons. Two similar experiments (in fall and spring) were conducted with single circles of detector plants, placed either in contact, or at distances of 5 or 30 cm from the infected plant. During these experiments, aerial detection of *P. ramorum* was monitored with a Burkard spore sampler that was placed just outside the outer circle of the detector plants. Detection of *P. ramorum* in the run-off water was performed via leaf baits or via direct molecular detection (real-time PCR). In a separate experiment, a Burkard spore sampler was placed next to and downwind from infected plants in order to aerially detect *P. ramorum* spores. To evaluate infection of plants that have tipped over in a water film containing *P. ramorum*, plants were immersed for 10 seconds, 1 hour, 2 hours, or 4 hours in a zoospore suspension and symptom development was then monitored.

Presence and spread of *P. ramorum* was also monitored at a production nursery during the 2004 and 2005 growing seasons. From June to October, at 4 to 6 week intervals, all twigs and leaves with *Phytophthora*-like symptoms were collected, field coordinates of samples were taken, and the pathogen was isolated and identified. Isolates of *P. ramorum* were genotyped with EU1 polymorphic microsatellites according to Vercauteren and others (2010). Drain water was monitored for *P. ramorum* presence as described above.

## Results and Discussion

At the mock nursery, dispersal of the pathogen from the centrally-placed infected plant to the detector plants that were placed in surrounding concentric circles was

mostly limited to the first circle of plants (78, 38, and 75 percent disease incidence in the three trials). Occasional spread to the second circle (28, 2, and 8 percent disease incidence in the three trials) and third circle (0 and 7 percent disease incidence in the last two trials) was observed. The level of disease incidence was correlated with the conduciveness of the weather conditions during the given test. Detector plants in the first circle invariably showed most symptoms at the side of the plant that was in direct contact with the detector plant.

The dispersal of the pathogen to the single circle of detector plants, placed either in contact with or at distances of 5 or 30 cm from the infected plant, clearly showed that pathogen spread required direct contact between the plants. Plants in direct contact showed symptoms (25 and 69 percent disease incidence in the two trials) while plants at 5 cm only showed symptoms if contact due to wind was not prevented (6 percent disease incidence in both trials without contact prevention, 0 percent if contact prevention via wire mesh), and plants at 30 cm did not show any symptoms. Aerial detection of *P. ramorum* with a Burkard spore sampler was consistently negative, even when the sampler was placed immediately adjacent to infected plants. In contrast, pathogen detection in drain water films and run-off water was consistently positive, even at several meters from the infected plants. Tipping over of plants in zoospore-containing suspensions for 1 hour or more resulted in heavy disease symptoms. However, lighter symptoms were already present after a 10 second zoospore dip. Taken together, these experiments indicate that aerial dispersal is very limited and above-ground plant-to-plant pathogen transfer requires direct contact. Similar evidence for short distance aerial dispersal in a mock nursery setting was observed by Tjös vold and others (2006). In contrast to short distance aerial dispersal, splash dispersal from water films (on non-infiltrating growing surfaces) and direct inoculation during tipping over of plants seem to play an important role in pathogen spread.

Over 1300 samples were taken during the surveys at the commercial production nursery. Approximately 16 percent of the samples were positive for *P. ramorum*, while *P. citricola* was the dominant species (> 70 percent). Dominance of *P. citricola* was also observed in the dispersal experiments of Neubauer and others (2006). Distribution of *P. ramorum* was not linked to clear disease foci, although the incidence was higher in specific cultivar blocks and was probably linked to the susceptibility of the given cultivars. No clear spread pattern was observed. Microsatellite-based genotyping of the *P. ramorum* isolates revealed several individual genotypes, as well as two sets of isolates with a unique genotype. Distance in the field between isolates of the same genotype was not limited to 10 m, indicating that spread of the pathogen in commercial settings is not necessarily limited to the 10 m diameter quarantine area. Presence of *P. ramorum* in drain water was confirmed at the commercial nursery. These results, together with those from the mock nursery experiments, suggest that spread at the commercial nursery was most likely the result of water-film mediated dispersal of the pathogen.

These data suggest that containment measures in these types of nurseries should not focus on prevention of aerial spread, but rather prevention of water-mediated spread. Zoospores in water films can infect above-ground plant parts via splash dispersal. They may also be able to infect roots, where they can reside in a latent state. Tipped-over plants should be destroyed, or at least separated and observed.

## Literature Cited

**Neubauer, C.; Gliessmann, J. and Beltz, H. 2006.** Pathways of spread for *Phytophthora ramorum* on *Rhododendron* and *Viburnum* in container production. *Gesunde Pflanzen*. 58(3): 185–191.

**Tjösvoold, S.A.; Chambers, D.L.; Koike, S. and Fichtner, E. 2006.** Epidemiology of *Phytophthora ramorum* infecting rhododendrons under simulated nursery conditions. In: Frankel, S.J.; Shea, P.J.; Haverty, M.I., tech. coord. Gen. Tech. Rep. PSW-GTR-196. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station: 459–461.

**Vercauteren, A.; De Dobbelaere, I.; Grünwald, N.J.; Bonants, P.; Van Bockstaele, E.; Maes, M. and Heungens, K. 2010.** Clonal expansion of the Belgian *Phytophthora ramorum* populations based on new microsatellite markers. *Molecular Ecology* 19(1): 92–107.

**Werres, S.; Wagner, S. and Brand, T. 2007.** Survival of *Phytophthora ramorum* in recirculating irrigation water and subsequent infection of *Rhododendron* and *Viburnum*. *Plant Disease*. 91(8): 1034–1044.