

Susceptibility of Larch, Hemlock, Sitka Spruce, and Douglas-fir to *Phytophthora ramorum*¹

Gary Chastagner,² Kathy Riley,² and Marianne Elliott²

Introduction

The recent determination that *Phytophthora ramorum* is causing bleeding stem cankers on Japanese larch (*Larix kaempferi* (Lam.) Carrière) in the United Kingdom (Forestry Commission 2012, Webber et al. 2010), and that inoculum from this host appears to have resulted in disease and canker development on other conifers, including western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), grand fir (*Abies grandis* (Douglas ex D. Don) Lindl.), and Sitka spruce (*Picea sitchensis* (Bong.) Carrière), potentially has profound implications for the timber industry and forests in the United States Pacific Northwest (PNW). A clearer understanding of the susceptibility of these conifers to *P. ramorum* is needed to assess the risk of this occurring in the PNW.

Methods

An experiment was conducted to examine the susceptibility of new growth on European (*L. decidua* Mill.), Japanese, eastern (*L. laricina* (Du Roi) K. Koch), and western larch (*L. occidentalis* Nutt.); western and eastern hemlock (*T. canadensis* (L.) Carrière); Sitka spruce; and a coastal seed source of Douglas-fir to three genotypes (NA1, NA2, and EU1) of *P. ramorum* in 2011. In 2012, a similar experiment was conducted using only the four larch species.

Container-grown seedlings or saplings were used in all experiments. Five trees or branches of each species were inoculated with a single isolate of the three genotypes by spraying the foliage with a suspension of zoospores (10^5 /ml). Seedlings or branches that were sprayed with water alone served as the control. Trees or branches were encased in plastic bags for 5 to 6 days and placed in a biocontainment unit maintained at 19 to 20 °C with 24-hr light. In 2011, symptoms were monitored and isolations were made from all symptomatic trees 8 to 11 days after inoculation, and also repeated on certain symptomatic trees at later dates, up to 38 days after inoculation. The trees used had few, if any branches, so lesion data are based on the lesions that developed on the stem. In 2012, the trees used had an additional year of growth and branch development, so lesion length data were based on lesions that developed on both the stem and branches. Isolations were made at 11 days for trees that were almost totally dead and at 21 days for the remainder of the trees. Isolations were repeated on some trees at 55 days after inoculation.

Due to the discovery in the United Kingdom that *P. ramorum*-infected Japanese larch needles can sporulate heavily in the fall, we wanted to determine if infection could take place on larch seedlings at that time of year. We conducted an experiment during 2011 using European, Japanese, eastern, and western larch and Douglas-fir seedlings from the same nursery stock used in the spring 2011 inoculation trial that had been maintained outdoors. In early October, five trees of each species were inoculated as described above. Seedlings sprayed with water alone served as controls. After 5 days, the plastic bags were removed and the seedlings were maintained in the

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² Washington State University, Puyallup Research and Extension Center, 2602 W. Pioneer, Puyallup, WA 98371. Corresponding author: chastag@wsu.edu.

biocontainment chamber and monitored for symptom development for 8 weeks. A sampling of needles with the variety of symptoms observed were collected from both the control and inoculated trees, surface sterilized, and plated onto CARP medium.

Results

The data presented below is only for inoculated seedlings where *P. ramorum* was isolated from symptomatic tissue. No *P. ramorum* was isolated from any of the non-inoculated controls.

Symptoms observed on the seedlings inoculated in the spring ranged from those occurring on individual needles or needle bundles to shoot dieback that extended into the previous year's stems in the form of lesions. Seedlings were killed when lesions girdled the stems. In the 2011 spring inoculation trial, the number of infected seedlings varied by genotype of the pathogen and host (table 1). The four larch species had the highest number of infected seedlings. Western larch tended to have the largest lesions. No *P. ramorum* was recovered from any of the eastern hemlock, which only exhibited minor needle symptoms. Of the isolates used, the NA2 isolate infected all of the conifer species except eastern hemlock. Overall, the NA2 isolate was isolated from 27 of the 40 inoculated seedlings. In comparison, the EU1 and NA1 isolates were isolated from 14 and 10 seedlings, respectively. Although the number of seedlings that became infected varied by genotype, the average size of the lesions the isolates caused after 8 to 11 days only ranged from 5.3 cm for the NA1 and EU1 isolates to 5.7 cm for the NA2 isolate.

Table 1—Number of *Phytophthora ramorum*-positive inoculated seedlings and sizes of lesions that developed, spring 2011

Host	No. of <i>P. ramorum</i> -positive seedlings ^a				Lesion length (cm)	
	Total	NA1	NA2	EU1	Max.	Avg.
Western larch	13	4	5	4	28.5 ^b	12.6
Japanese larch	11	2	5	2	4.5	3.6
Eastern larch	10	2	5	3	4.2	2.4
European larch	9	2	5	2	5.5	3.7
Sitka spruce	6	0	4	2	4.0	2.7
Douglas-fir	3	0	2	1	2.5	2.5
Western hemlock	1	0	1	0	2.5	2.5
Eastern hemlock	0	0	0	0	0	0

^aTotal of five seedlings were inoculated with each genotype. Data was collected 8 to 11 days after inoculation.

^bProbably the result of multiple lesions coalescing.

In 2012, there was no significant (ANOVA) difference among the three isolates of *P. ramorum* with respect to the overall percentage of symptomatic bundles, number of lesions per seedling, lesion size, and mortality on the four spring-inoculated larch species (table 2).

Table 2—Effect of isolate on symptom development on four larch species, spring 2012

Isolate genotype	% sym. bundles ¹	Avg. no. of lesions/seedling ^a	Avg. lesion length (cm) ^a	% mortality ^b
NA1	35.6	4.6	6.5	35.0
NA2	45.4	4.9	7.3	35.0
EU1	52.5	4.8	7.8	40.0

^aData collected 21 days after inoculation.

^bData collected 55 days after inoculation.

Except for the limited symptom development that occurred on the eastern larch, the data from the 2012 spring inoculated trial confirmed the susceptibility of western, European, and Japanese larch to isolates of the three genotypes of *P. ramorum* used in this test (table 3). Although not significantly different, western larch tended to have the highest percentage of symptomatic bundles, number of lesions per seedling, lesion length, and mortality.

Table 3—Differences in symptom development on four spring-inoculated larch species in 2012^a

Larch species	% sym. bundles ^b	Avg. no. of lesions/seedling ^b	Avg. lesion length (cm) ^b	% mortality ^c
Western	60.1	6.0	8.3	53.3
European	44.0	4.3	6.1	46.7
Japanese	32.7	4.3	8.0	33.3
Eastern	5.3	2.7	3.2	0.0

^a Five trees of each species were inoculated with a NA1, NA2, and EU1 isolate of *P. ramorum*.

^b Data collected 21 days after inoculation.

^c Data collected 55 days after inoculation.

When the plastic bags were removed from the seedlings in the fall 2011 inoculation test, minimal symptoms (a few gray needles, yellow/brown needle tips, a faint lighter-colored needle banding, single yellow needles) were evident from any of the inoculated and control seedlings. During the 8-week incubation period, no additional symptoms developed. Isolations from symptomatic needles 8 weeks after inoculation resulted in a single positive recovery of *P. ramorum* from the proximal end and center of a single, partially gray needle from one Japanese larch seedling inoculated with the NA2 isolate.

Results from these trials indicate that all of the conifer species tested, except eastern hemlock, exhibited some level of susceptibility to *P. ramorum*. The highest recovery of *P. ramorum* and level of symptom severity occurred on the larch species, especially western larch. These experiments also indicate that the NA1, and especially the NA2, genotypes of *P. ramorum* have as much potential as the EU1 genotype to cause disease on newly emerging growth of larch. Virtually no infection occurred on any of the larch inoculated in the fall. Additional studies are needed to determine the potential sporulation of NA1, NA2, and EU1 isolates of *P. ramorum* on susceptible conifers, especially western larch.

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Literature Cited

- Forestry Commission. 2012. Pests and diseases. *Phytophthora ramorum*. www.forestry.gov.uk/pramorom. (21 January 2013).
- Webber, J.F.; Mullett, M.; Brasier, C.M. 2010. Dieback and mortality of plantation Japanese larch (*Larix kaempferi*) associated with infection by *Phytophthora ramorum*. New Disease Reports. 22: 19.