

Symptoms Associated with Inoculation of Stems on Living Douglas-fir and Grand Fir Trees with *Phytophthora ramorum*¹

Gary Chastagner,² Kathy Riley,² Katie Coats,² Marianne Elliott,² Annie DeBauw,² and Norm Dart³

Abstract

To obtain a better understanding of the potential risk of infection and colonization of living Douglas-fir (*Pseudotsuga menziesii*) and grand fir (*Abies grandis*) stems, the stems on over 150 trees of each species were inoculated at a Christmas tree farm near Los Gatos, California. This study had the following objectives: 1) Determine if inoculation timing affects the infection of living Douglas-fir and grand fir stems, 2) Characterize symptom development associated with inoculated stems, and 3) Determine what stem tissues were colonized by *P. ramorum*.

Trees were inoculated during fall 2007, late winter 2008, and summer 2008 by using a cork borer to remove a bark plug from the stem on opposite sides of each tree. On each host, a mycelial plug of an NA1 isolate of *P. ramorum* from that host was then placed in one hole and a plug of plain agar media was placed in the other hole. The bark plugs were replaced, sealed with Vaseline, and covered with duct tape. The average stem diameter at the point of inoculation was 5.9 cm (Douglas-fir) and 6.2 cm (grand fir). Field data on symptom development have been taken at approximately 4-month intervals since inoculation. The colonization of stem tissue was evaluated by periodically dissecting a representative subset of inoculated stems. Bark and woody tissues were surface sterilized for 30 seconds in a 1:9 dilution of bleach and plated onto CARP selective medium. Surface sterilized bark and woody tissues were also subjected to qPCR testing.

Four months after inoculation of the Douglas-fir, there was no difference in the percentage of trees that had cankers regardless of whether they were inoculated in the fall or winter (fall – 90.2 percent and winter – 90.5 percent). Most of the fall- and winter-inoculated trees exhibited extensive resin flow. Branch flagging in the area of the cankers was evident on a few trees. On the fall-inoculated trees, there was a slight increase in canker length between March and July. By July, the average canker length on the winter-inoculated trees was about half the size of the cankers on the fall-inoculated trees (4.8 cm vs. 11.6 cm). The longest canker on the fall- or winter-inoculated trees was 47 cm long and cankers had girdled 12.5 percent of the fall-inoculated trees. One of these girdled trees died during spring 2009. No cankers were observed on any of the check plugs.

The size of cankers on the grand fir stems were much smaller than those that developed on the Douglas-fir. Four months after inoculation, about twice as many of the trees inoculated in the winter (76.8 percent) had visible cankers compared to those inoculated in the fall (34.0 percent). Although some cracking of the bark was observed in the canker area, very little resin

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

² Washington State University, Puyallup Research and Extension Center, 2606 W. Pioneer, Puyallup, WA 98371.

³ Virginia Department of Agriculture and Consumer Services, Richmond, VA 23219.

Corresponding author: chastag@wsu.edu.

flow was observed. On the fall-inoculated trees, the average canker length was still only about 2.0 cm 1 year after inoculation. On the winter-inoculated trees, the average length of cankers in July 2009 was 4.9 cm; 1 year after inoculation (March 2009), canker lengths had increased slightly to 6.4 cm. The largest canker on any of the inoculated trees was 25.7 cm long. None of the cankers have girdled any of the grand fir trees and no cankers were observed on any of the check plugs.

No cankers developed on any of the trees that were inoculated during the summer. In addition, limited expansion of the cankers on trees that were inoculated in the fall or winter has occurred since July 2008.

Isolation from bark and wood has resulted in very limited recovery of *P. ramorum*. The pathogen was recovered from 1.4 percent of 1,780 isolations from Douglas-fir and 0.2 percent of 1,418 isolations from grand fir. qPCR positives from surface sterilized tissues were restricted to symptomatic bark and the woody tissue just beneath the bark or a thin layer of discolored tissue between the 2007 and 2008 wood below cankers. Histological studies are in progress to determine what tissues are colonized and what pathogen structures are present in those tissues.

Acknowledgments

This research was financially supported by the U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station, and the Pacific Northwest Christmas Tree Association. The assistance of Robbie Criswell and Ann Northrup is gratefully acknowledged.