Preliminary Observations of Heat Treatment to Control *Phytophthora ramorum* in Infected Wood Species: An Extended Abstract[1]

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Abstract

Identification of appropriate phytosanitary treatments that can be used for certifying solid wood packing material movement from areas infested or threatened by actionable plant pests and pathogens into uninfested areas is important. Heat treatment has been used on commodities to control fungal diseases and insect infestations for many years. The restricted use of methyl bromide has revived the interest on heat disinfestation for commodities. The purpose of this research is to determine the efficacy of heat treatment to control *Phytophthora ramorum* in wood species. Artificially inoculated and naturally *P. ramorum*-infected tanoak rounds and boards were heat-treated in a dry kiln set at the temperatures 50, 56, 60, and 65°C for 30, 45, and 60 minute durations. Core temperature, heating time, wet bulb, and dry bulb temperatures were monitored. *Phytophthora. ramoru*m was detected in wood using baiting and plating techniques before and after the treatment. Preliminary data showed that the required phytosanitary temperature of 56°C for 30 minutes might not be adequate to kill *P. ramorum* in wood. However, laboratory techniques have been redefined for subsequent tests using the temperatures of 50, 56, 62, and 68°C for 15, 30, and 60 minute durations.

Key words: *Phytophthora ramorum*, sudden oak death, heat treatment, tanoak.

Introduction

The potential to spread plant diseases by moving infected plants and plant materials from one location to another is well documented (Erwin and Ribeiro 1996, Davidson and others 2003, Shelly and others2005). Although kiln-dried lumber would likely experience temperatures lethal to *P. ramorum*, wood that is not kiln-dried could carry the organism. Current regulations for *P. ramorum* require the wood of hosts to be certified as debarked before they may be moved interstate. Research has suggested that *P. ramorum* could survive the recommended control measures such as removing the bark and air drying thus not completely mitigating the risk. There is a need for better understanding of the current recommended *P. ramorum* treatment methods as well as the identification of treatment alternatives for logs, bark, wood, and fire wood that are efficacious for *P. ramorum*. The restricted use of methyl bromide has revived the interest on heat disinfestation at 56˚C for 30 minutes for commodities. The

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purpose of this research is to determine the efficacy of heat treatment to control *P. ramorum* in infected wood that is not intended to be kiln-dried.

Objectives

This study was designed to determine the relationship between oven temperature, wood temperature, and *P. ramorum* survivability in infected wood.

Materials and Methods

Tanoak (*Lithocarpus densiflorus*), a hardwood host for *P. ramorum* with known commercial potential, was chosen as the specimen material for this project (Shelly and others 2006a, 2006b). The specimens were collected from the California Department of Forestry's (CDF) Soquel Demonstration Forest in Soquel, California. Twenty one trees with symptoms of sudden oak death disease were identified during a survey of the forest. Samples from suspect trees were collected and sent for PCR analysis to the California Dept. of Food and Agriculture (CDFA) Diagnostic lab, Sacramento and cultured at the University of California at Berkeley (UCB), Forest Products Laboratory (tables 1, 2). The 15 trees that tested positive for *P. ramorum* (ranging in diameter from 12 to 30 inches = 30.48 to 76.20 cm)) were cut down and three to four; eight-inch (20.32 cm) thick rounds were cut from the *P. ramorum* infected zones of each tree. In addition an equal-sized sample of non infected tanoak trees were also sampled for lab inoculation. Half of the field sample rounds, and lab inoculated samples were debarked prior to testing, the other half kept the bark. Short lumber sized samples (1-inch x 8- inch x 12-inch = 2.54 x 20.32 x 30.48 cm) were also inoculated and heat treated. All inoculations were placed at three depths in each specimen (at the surface, 15 mm deep, 30 mm deep and 50 mm deep). The field samples (rounds) and inoculated wood samples were heated in an 8.2 m^3 laboratory dry kiln. Heating tests were conducted at four temperatures (50, 56, 60, 65°C) and three time durations (30, 45, and 60 minutes) keeping the relative humidity constant at about 20 percent.Temperature was measured at each depth and the time duration measurements included both length of time the chamber was at the stated temperature and the length of time the wood was at the stated temperature. After treatment, samples were removed from the *P. ramorum* infected areas of each round and cultured to determine if the treatment was effective in killing the organism.

Table 1—PCR results of artificially inoculated-tanoak rounds in the laboratory (rounds* were collected from a sudden oak death free area)

	PCR Analysis	
Lab ID Round	*Real Time*	*Nested*
1	**+**	
2	**+**	
3		**+**
4		**+**
5		**+**
6		**+**
7	**-**	
8	**-**	
9		**+**
10	**+**	

*Laboratory samples were inoculated with *P. ramorum* infested shavings placed at 15, 30, and 50 mm deep in wood.

Table 2—PCR and culture results of naturally infested tanoak rounds collected from the Soquel Demonstration Forest in Santa Cruz, CA[1]

	PCR Analysis		
Tree ID	*Real-Time*	*Nested*	*Culture*
601	+		+
602	-		
603	+		
604	-		
605	+		
606		+	
607	+		
608	+		+
609	+		
610	+		
611	-		
612		-	
613	+		
614		-	
615	+		
616			+
617			+
618			+
619			+
620	+		
621	-		

[1] All samples were analyzed (Nested and RT-PCR) and cultured prior to heat treatment in a 8.2 m^3 experimental dry kiln.

Results and Discussion

Results from preliminary data are inconclusive and show low detection of *P. ramorum* in controls, pre-heat treated, and post-heat treated samples. Positive *P. ramorum* was found in 4 of the 12 tests, but only in 16.6 percent of the samples (table 3). This low detection of the pathogen in samples might be attributed to the delay between collection and processing of the samples, an uneven distribution of *P. ramorum* in collected samples, and the recognized difficulty of proving the presence of active *P. ramorum*. The isolation of one active *P. ramorum* sample treated at 56°C for 30 minutes suggests that this required phytosanitary treatment might not be adequate to kill *P. ramorum* in wood. Subsequent tests will be conducted with artificially inoculated boards in the laboratory and bay laurel/pears/rhododendron leaves will be used to maximize the detection. The chlamydospore germination will also be monitored.

Table 3—Experimental heating conditions used in this study[1]

	Time, minutes		
Temperature, ˚C	*30*	*45*	*60*
50	**+**	**+**	**-**
56	**+**	**-**	**-**
60	**-**	**-**	**-**
65	**-**	**-**	**-**

[1]Temperatures listed are target temperatures and differ slightly from actual temperatures attained. Positive *P. ramorum* was determined by wood tissue culture and/or chlamidospore germination.Samples were two rounds with bark, two rounds with no bark, and two boards used in each temperature x duration combination. Control samples were positive for *P. ramorum.*

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