

Elimination of *Phytophthora ramorum* in the Tunnel Composting Process¹

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Introduction

Phytophthora ramorum is the causal agent of sudden oak death, which has a devastating effect on a wide range of host plants (Werres and others 2001, Rizzo and others 2002). In contrast to infected trees, which seem to be a dead-end host, *Rhododendron* and *Umbellularia californica* seem to play a key role in the spread of this pathogen, probably by acting as main inoculum source (Swiecki and Bernardt 2002, Garbelotto and others 2003). Partly due to its aggressiveness, the pathogen is placed on the E.U.-Alert list. Phytosanitary regulations on such pathogens require destruction of infected plant material. Several composting or heat treatment techniques have been tested recently to determine the effect on the survival of *P. ramorum* (Garbelotto 2003, Harnik and others 2004). In the present research, the use of tunnel composting for the elimination of *P. ramorum* in infected plant material was investigated *in situ*.

Tunnel composting is a closed system composting method, and therefore, suitable for composting plant material infected with quarantine organisms, such as *P. ramorum*. The essence of this method is that the plant debris is composted under strict temperature and moisture conditions, using hot circulating air, which is blown through a perforated floor and the plant material. The actual composting process can be divided into two phases: pre-composting and after-composting. The pre-composting phase consists of a pasteurisation step, in which the debris is heated up to a minimum of 60° C (140° F) for at least 10 hours, followed by a drying step, which takes approximately four days. The actual composting takes place during the after-composting phase in which the material is kept at 48° C (118° F). This part of the

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process takes at least 12 days. The composting is followed by a filtration step. Particles larger than 15 mm are removed from the end product and incinerated or composted again.

Methods

The effect of the above mentioned pasteurisation step on the survival of *P. ramorum* was investigated. For this experiment, plant material was collected from wildtype *Rhododendron catawbiense* showing *P. ramorum*-like symptoms. The material was divided in 20 shoot samples, as well as 20 samples of leaves. Within a sample, each individual piece of material was cut in half to obtain two sets of 'identical' subsamples. To prove the viability of the pathogen, one set of subsamples was tested in the presence of *P. ramorum* using a leaf-baiting test, followed by a Taqman ITS-PCR assay for the detection of the pathogen.

The remaining subsamples were placed in small nylon nets, which were hidden in large coarse woven plastic nets among approximately 15 litres of *Rhododendron* plant debris. The nets containing the plant debris and the test material were placed in a filled tunnel-composting chamber. After 10 hours at 60° C, the recovered samples were tested in the described baiting test, again followed by Taqman ITS-PCR.

Results and Discussion

The results of the bait and the Taqman PCR, which was conducted on the first, non-composted set of subsamples, showed that *P. ramorum* was present in 37 samples (18 leaf and 19 shoot samples). Three samples did not show presence of viable *P. ramorum*. Their corresponding 'identical' subsamples in the second set were not composted, but acted as a negative control in the baiting test on the composted material.

After the pasteurisation, 27 samples (13 leaf and 14 shoot samples) were recovered, the other ten samples were lost in the process. None of the recovered samples showed presence of viable *P. ramorum*. Our conclusion is that *P. ramorum* is eliminated in the pasteurisation step of the tunnel-composting process.

Garbelotto (2003) found that *P. ramorum* infected material was sanitized after two weeks exposure to 55° C. Recently, Harnik and others (2004) demonstrated that *P. ramorum* survives at least one week at 55° C in infected leafs of *U. californica*. In our present study, we concluded that a five degree (Centigrade) higher temperature

shortens the exposure time needed. This is supported in a recent experiment by Harnik and others (2004) using infected California bay laurel leaves. They showed that a short period at 60° C, in combination with 22 hours in a vacuum, is sufficient to eliminate the pathogen. The main advantage of the tunnel composting pasteurisation step is that the eradication of *P. ramorum* is completed in only 10 hours, which makes tunnel composting a highly effective option for the elimination of *P. ramorum* in infected plant material.

Table 1—Results of the baiting test on leaf (a) and shoot (b) material, before and after pasteurisation at 60°C for 10 hours.(+) *P. ramorum* present, (-) *P. ramorum* absent, (nt) not tested/not recovered from the composting installation.

a) Leaf materia

	Sample Number																				Tot
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
Before composting	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	18/20
Post-treatment	-	-	-	-	-	-	-	-	-	-	-	-	nt	-	nt	nt	nt	nt	nt	nt	0/13
b) Shoot material																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Tot
Before composting	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	19/20
After 10h at 60°C	-	-	nt	-	-	-	-	-	-	-	-	-	-	-	-	nt	nt	nt	nt	nt	0/14

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