

Aerial Application of Agri-Fos[®] to Prevent Sudden Oak Death in Oregon Tanoak Forests¹

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Abstract

We have been testing the practicality and efficacy of aerial application of Agri-Fos[®] for control of sudden oak death (SOD) in the Oregon tanoak (*Lithocarpus densiflorus*) forest. Helicopter application to forest stands has been compared with bole injection and ground-based spray application to seedlings and stump sprouts. We bio-assayed for Agri-Fos[®] uptake using twig and seedling inoculation with zoospores and bole wound inoculation. Experiments are continuing. Preliminary results indicate small but significant reductions in growth of *Phytophthora ramorum* in Agri-Fos[®] sprayed trees, comparable to the results obtained from bole injection.

Introduction

Phosphonate (Agri-Fos[®]) has been shown to be effective in limiting development of *Phytophthora ramorum* when sprayed directly onto or injected into individual tree boles. There have been no tests, however, of its application and uptake under forest conditions, or of its efficacy as a disease protectant in landscape scale application on tanoak (*Lithocarpus densiflorus*). Proper governmental registration and permission for use of phosphonate in the forest requires demonstration of efficacy. We are conducting trials in Oregon to demonstrate the feasibility of aerial application from helicopter, phytotoxicity at various spray concentrations, and the uptake and translocation of the chemical within treated trees. We are also testing the efficacy of Agri-Fos[®] as a protectant fungicide by spraying tanoak seedlings and then exposing them to natural inoculum of *P. ramorum* in the field. Results presented here are preliminary and do not represent the complete data set. Final results and analysis will follow completion of the final assays in November 2009.

Methods

Three methods of Agri-Fos[®] application are being compared: helicopter application to mature tanoak forest trees; backpack pressurized spray to simulate the helicopter application to tanoak seedlings and sprouts; and bole injection of mature tanoak trees. The aerial spray application rates were: no treatment; 3 gal Agri-Fos[®] 400/20 gal water/acre; and 6 gal Agri-Fos[®] 400/20 gal water/acre. The seedling application rates were the same as the aerial rates except the 6 gal per acre rate was replaced by 0.1 gal

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Agri-Fos[®] 400/20 gal water/acre. Pentra-Bark[®] was added to all spray mixes. Tanoak trees were injected with Agri-Fos[®] 400 at the label rate of 10 ml injection fluid (1 part Agri-Fos[®]:2 parts water) per 15 cm trunk circumference.

Five different biological assays are being used to measure Agri-Fos[®] uptake:

1. Twig assay: Ten treated trees per treatment plot were felled and 20, 25 cm long shoot tips (twigs) from the outer canopy were collected from each and transported to the laboratory. Cut ends of shoot tips from forest trees or sprout clumps are immersed in a 1 cm deep zoospore suspension then incubated 3 weeks. Resultant lesion lengths are measured.
2. *In situ* bole inoculation with *P. gonapodyides*: Intact tanoak trees are wound inoculated in the field. Lesion area is measured after 5 weeks. *P. gonapodyides* was used in field inoculations instead of *P. ramorum* due to quarantine regulations.
3. “Log” bolt inoculation with *P. ramorum*: Trees are felled and 1 m bolts cut and returned to the laboratory. Logs are wound inoculated in the laboratory, and lesion area measured after 5 weeks.
4. Artificial inoculation seedling assay: Treated seedlings are challenge inoculated with a zoospore and sporangium suspension of *P. ramorum*, and incubated in a growth chamber until symptoms develop.
5. Treated seedlings are exposed to natural inoculum beneath infected tanoak trees in Curry County, Oregon.

Treatment areas for helicopter spray treatments were selected in extensive stands of tanoak north of the SOD infestation in Curry County, Oregon. Treatments were applied by contract helicopter November 2007, May 2008, December 2008, and May 2009 (table 1). At each application time, we sprayed three blocks, each consisting of three 10 acre treatment plots.

Table 1—Aerial application of Agri-Fos[®] to tanoak forests: tests, treatments, and assays

Test	Spray Dates	Treatments	Area Treated	Assay	Assay Date
1	Nov 07	0, 3, and 6 gal /Acre	3, 10 Acre Blocks/Treatment	Branch dip In situ <i>P. g</i>	May 08
2	May 08	0, 3, and 6 gal /Acre	3, 10 Acre Blocks/Treatment	Log- <i>P.ram</i> Branch dip In situ <i>P. g</i>	Jan 09 Nov 09
3	Nov 08 and May 09	0, 3, and 6 gal /Acre at each time	3, 10 Acre Blocks/Treatment	Branch dip In situ <i>P. g</i>	June 09 Nov 09

Results

Aerial Application

The twig assay (fig. 1), the *in situ* assay with *P. gonapodyides* (fig. 2), and the log assay with *P. ramorum* (fig. 3), all indicated an Agri-Fos[®] effect in sprayed trees 6 months or 18 months after treatment. Lesion length differences with the twig assay were usually small, but significant. The *in situ* and log assays gave larger and more consistent differences in lesion area between treatments. There was no consistent difference between the two fungicide doses.

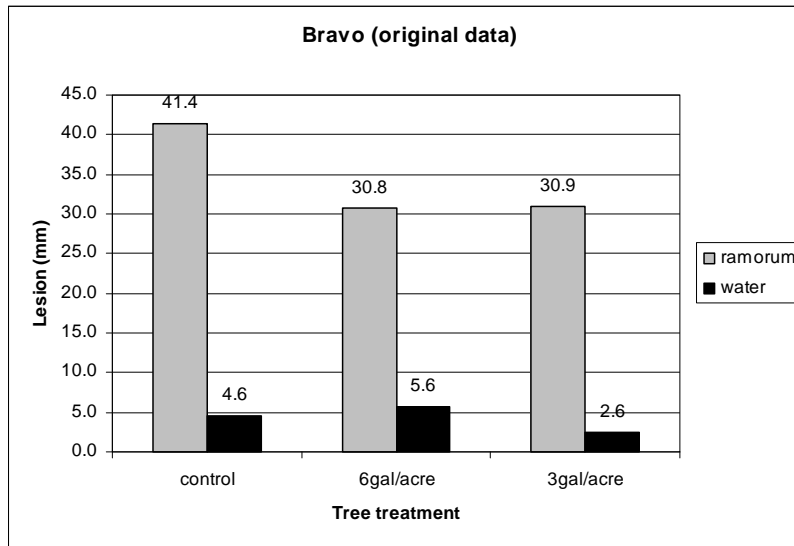


Figure 1—Aerial application of Agri-Fos®: twig assay. Data from one plot in the fall 2008 application.

Means and 95.0 Percent LSD Intervals

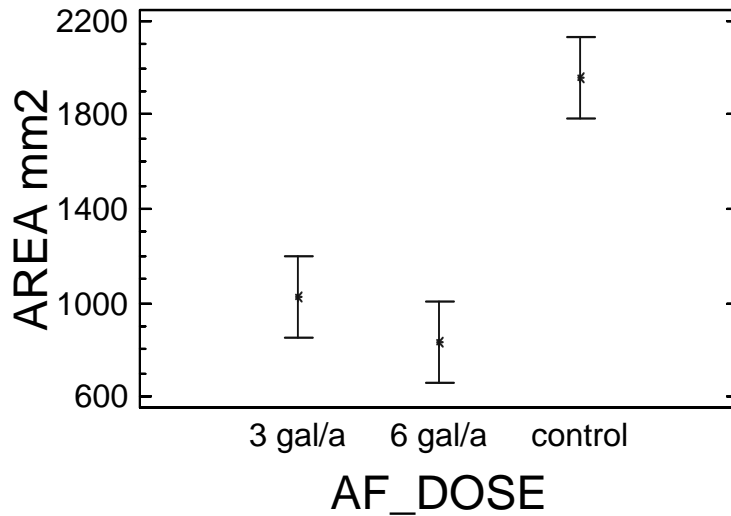


Figure 2—Aerial application of Agri-Fos®: *in situ* bole inoculation assay with *P. gonapodyides*. Average lesion area for all sites after November 2007 application.

Results from double application of Agri-Fos® (treatment three, sprayed November 2008 and May 2009) will not be available until final assays are completed fall of 2009. At that time the overall project will be evaluated and results reported.

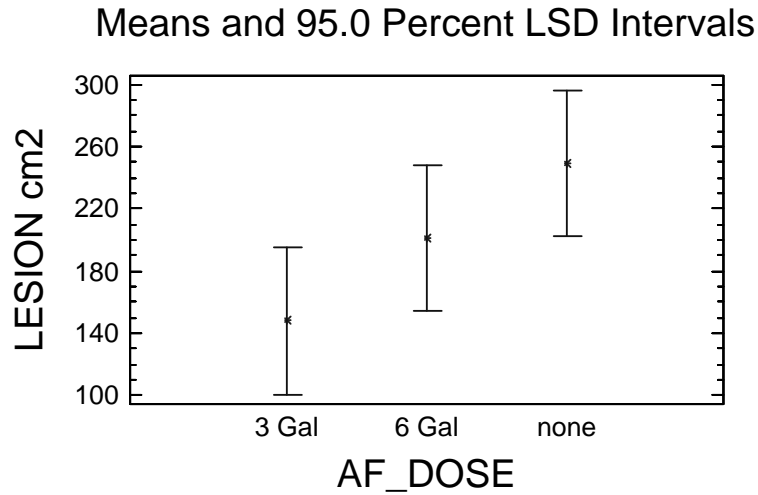


Figure 3—Aerial application of Agri-Fos® (AF): log inoculation assay with *P. ramorum*. Lesion area data from all sites after the May 2008 application.

Bole Injection

Ten Agri-Fos® injected trees were paired with control trees in both 2007 and 2008 trials. The shoot dip assay showed slightly smaller lesion length in the injected trees compared to controls. Bole inoculation with *P. gonapodyides* as well as log inoculation with *P. ramorum* showed similar but larger differences between injected and control trees (fig. 4 and 5; table 2).

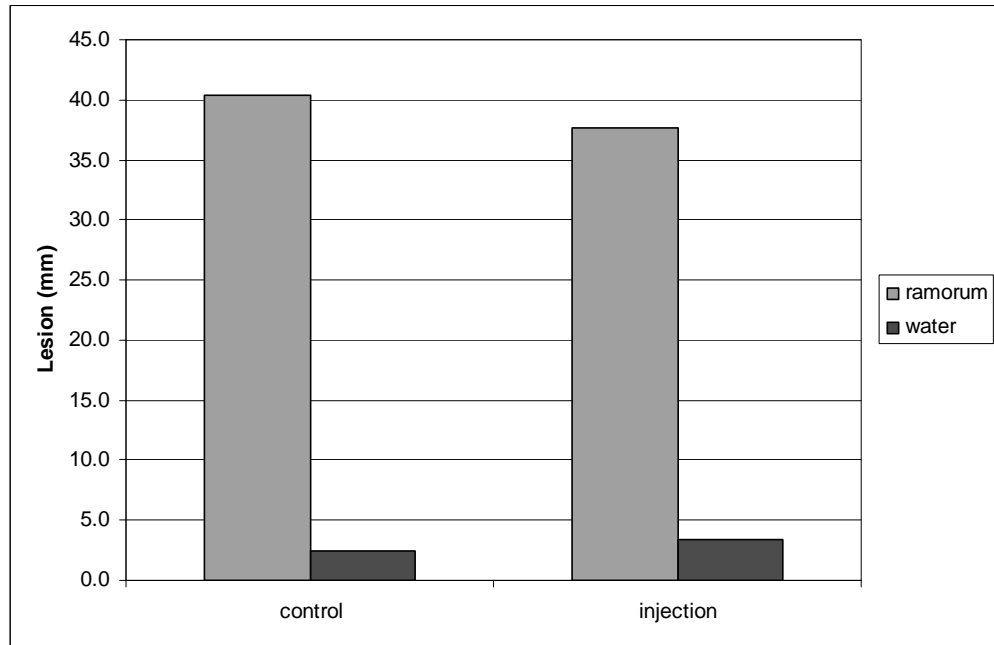


Figure 4—Tree injection with Agri-Fos®: twig assay from the 2007 trial.

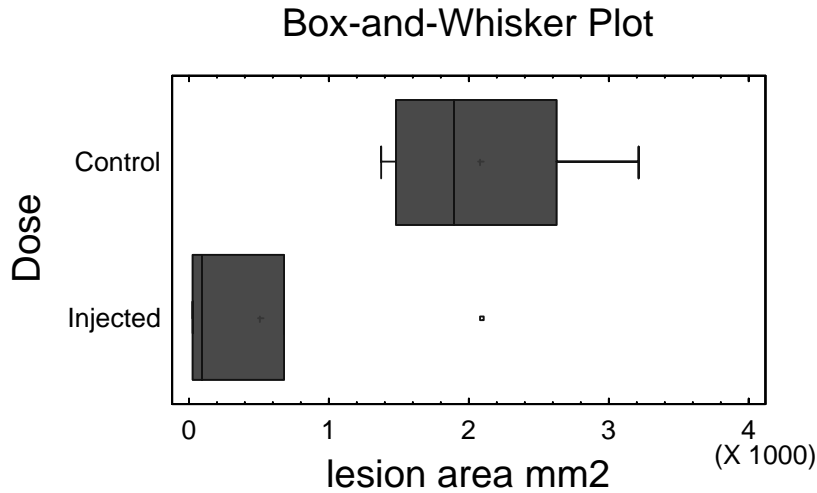


Figure 5—Tree injection with Agri-Fos®: *in situ* bole inoculation assay with *P. gonapodyides*. 2008 trial.

Table 2—Tanoak bole injection with Agri-Fos®: summary of tests and assays

Assay	Injection Date			
	NOV 2007		MAY 2008	
	Agri-Fos®	Control	Agri-Fos®	Control
Twig (mm)	37.6	40.4	45.8	47.6
Bole Pg (cm ²)	5.0	20.8	0.4	24
Log Pg (cm ²)	x	x	29	63
Log Pr (cm ²)	x	x	82	206

Stump-Sprout Foliar Spray Treatments

There were no obvious symptoms of phytotoxicity resulting from the spray treatments. There were significant differences ($p = 0.00$) in sprout response to challenge inoculation; lesion length on sprouts sprayed at the high dose (6 gal/acre) averaged 44 mm, and control, unsprayed sprouts averaged 78 mm.

Box-and-Whisker Plot

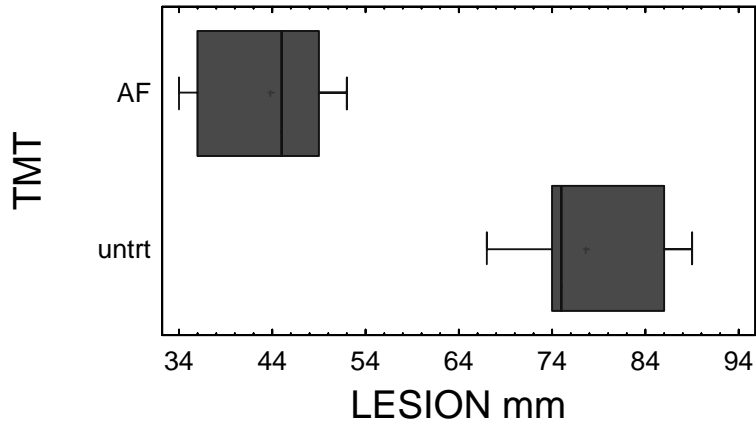


Figure 6—Stump sprout spray with Agri-Fos® (AF) compared to untreated control (untrt): twig assay. Data from 2008 trial.

Seedling Spray Treatments

Seedlings were sprayed in October 2008 and in April 2009, with one set of seedlings sprayed in both October and again in April, then challenge-inoculated with *P. ramorum* in the lab or exposed to natural inoculum in the field. In October, only incidence of infection was recorded. In April, both the percent seedlings infected and the extent of stem infection (proportion of total stem length) that was infected were recorded (fig. 7 and 8). Agri-Fos® did not prevent infection by *P. ramorum* in any treatment, and the effect of Agri-Fos® dose on incidence and extent of infection was variable between trials.

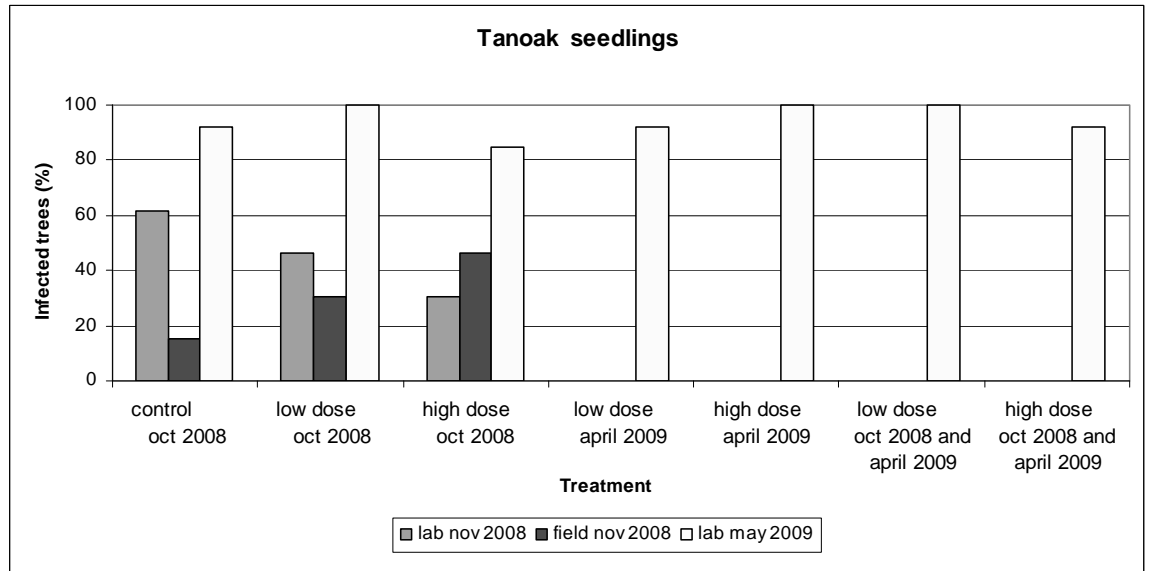


Figure 7—Incidence of infection (percent infected) of tanoak seedlings sprayed with Agri-Fos® at low (0.1 gal/acre) or high (3 gal/acre equivalent) dose, + Pentra-Bark® surfactant, or unsprayed. Seedlings were artificially inoculated with *P. ramorum* by

zoospore spray, or transported to the field for natural infection beneath infected tanoak trees.

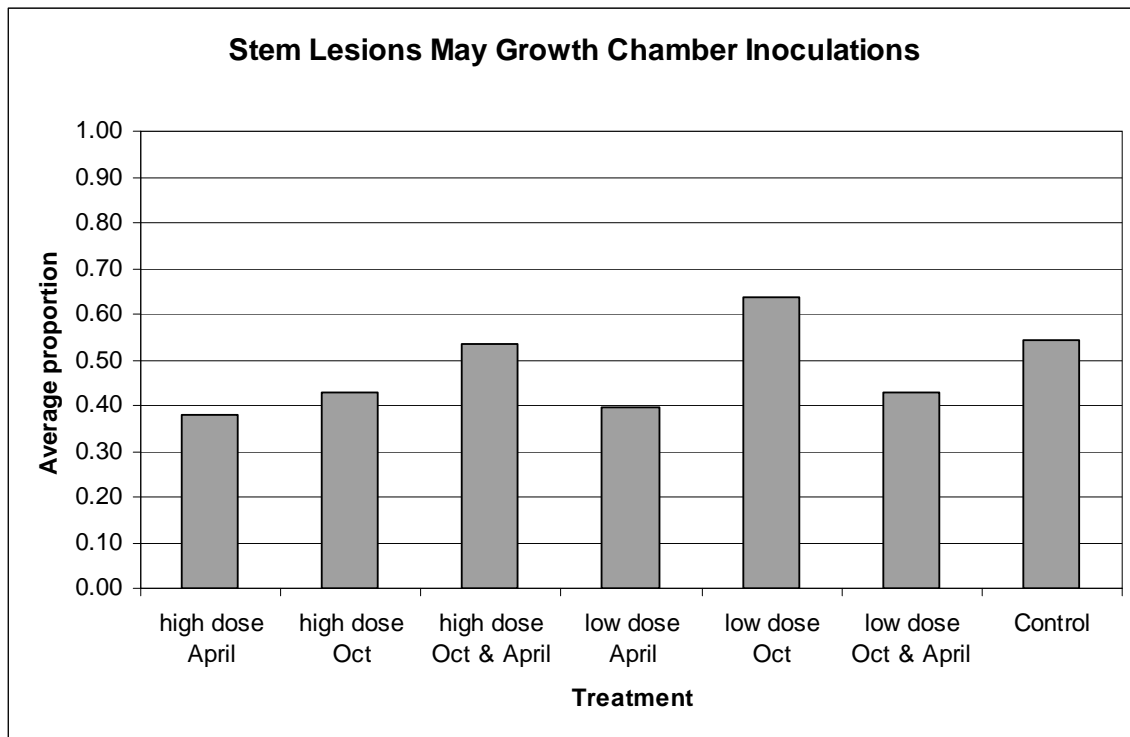


Figure 8—Extent of infection (percent of stem length infected) of tanoak seedlings sprayed with Agri-Fos[®] at low or high dose, or unsprayed in April 2009. Seedlings were artificially inoculated with *P. ramorum* by zoospore spray or transported to the field for natural infection beneath infected tanoak trees.

Discussion

Interpretation of our Agri-Fos[®] trials is limited by two factors: lack of a realistic assay for Agri-Fos[®] uptake of sprayed plants, and inability to test aerial application against natural infection. We are trying to protect tanoak forest trees from initial infection, which occurs through leaves and twigs in the tree crown. Our twig bioassays of the upper crown, however, show only very small differences in twig lesion lengths between sprayed and unsprayed trees, although our large sample sizes mean that even small differences are often statistically significant. In order to get consistent infection of the twigs, which vary widely in condition, we use a high zoospore concentration (10^4 to 10^5 /ml), perhaps overwhelming the Agri-Fos[®] effect. Wound inoculation of tree boles in the field or in the lab with *P. gonapodyides* or *P. ramorum*, gives larger, more consistently significant treatment differences, demonstrating clearly the uptake of chemical into stems with results comparable to those seen with stem injection. Again, however, Agri-Fos[®] treatment does not prevent infection entirely, and there has as yet been no demonstration that tree life can be extended appreciably by injection.

Perhaps the small differences in infection we measured by challenge inoculation are sufficient to significantly reduce natural infection of trees in the field. We cannot test aerial application of Agri-Fos[®] against natural infection of trees, however, because of the ongoing disease control program in Oregon. We cannot leave untreated control plots for comparison with Agri-Fos[®] treatment. Furthermore, the overall infection rate in the Oregon tanoak forest is very low, necessitating large treatment areas to show differences.

Our aerial application rates are comparable to those used in Western Australia. The bole injection rate, calculated on a per acre basis, is also comparable to our aerial rate (4.15 gal Agri-Fos[®]/acre). Total helicopter application costs (chemicals, helicopter time, and pilot) in Oregon have been \$153/acre for the 3 gal rate and \$219/acre for the 6 gal rate.

The seedling results are discouraging. We saw no consistent reduction in infection levels to either artificial inoculation or exposure to natural inoculum, in either trial. The low dose was selected to be equivalent to the standard nursery application rate, and the high seedling dose was the same as the lower of the doses used in the helicopter application. Our assays were not as refined as for the lesion area or twig assays, and we did not have as many replications. Evidently, however, Agri-Fos[®] does not protect seedlings from *P. ramorum* under these circumstances.

Further complications to the large scale use of Agri-Fos[®] arise from ownership patterns and land management objectives in southwest Oregon. Industrial landowners have little incentive to protect tanoak that they plan to kill anyway to favor growth of Douglas-fir. Small landowners and rural residential developments do not offer the large treatment areas necessary to make aerial spray applications practical. The conservation management objectives of the federal forest management agencies and large non-profit land conservancies are theoretically more amenable to use of Agri-Fos[®] to protect stands of tanoak. In these cases, however, cultural and sometimes legal constraints to fungicide use must be overcome.