



## Phosphite Injections and Bark Application of Phosphite + Pentrabark™ Control Sudden Oak Death in Coast Live Oak

M. Garbelotto, D.J. Schmidt, and T.Y. Harnik

**Abstract.** In each of seven fully controlled experiments, potted California, U.S. coast live oak trees were artificially inoculated with *Phytophthora ramorum*, the agent of a tree disease commonly referred to as sudden oak death. Phosphites were applied to trees using a range of application approaches either as preventive or therapeutic treatments. Soil drenches and bark application of phosphites were ineffective; foliar application of phosphites amended with surfactants were effective only at times and always caused phytotoxicity. On the other hand, injections of phosphites and bark applications of phosphites + the organosilicate surfactant Pentrabark™ (Agrichem, Medina, OH, U.S.) were consistently effective in suppressing bark colonization by this pathogen without causing phytotoxicity. This is the first study describing the use of a chemical treatment amended with an organosilicate surfactant for topical bark applications.

**Key Words.** Chemical treatments; disease control; forest disease; forest management; *Phytophthora ramorum*; *Quercus agrifolia*; SOD.

The use of phosphite fungicides has become common practice in some agricultural or orchard crops severely affected by *Phytophthora* diseases. In particular, citrus and avocado orchards have been treated with such compounds for decades in Africa, Asia, Australia, and even the United States and Europe (Guest et al. 1995; Erwin and Ribeiro 1996; Hardy et al. 2001). The active ingredient in such fungicides is phosphonic acid, and although its mode of action is complex and still not completely understood, most researchers agree the effect of phosphonic acid is the induction of defense mechanisms in the plant rather than direct antimicrobial action. The effects of phosphite treatments include enhanced lignification, increased cell wall thickness, and enhanced production of secondary plant metabolites, some of which are known to possess antimicrobial properties (Guest and Grant 1991). Phosphites are applied as extremely soluble neutralized salts of phosphonic acid. The compounds first move acropetally as a salt in the outer xylem into the leaves and then basipetally as phosphonic acid through the cambium in the trunk and roots. Efficacy of the compounds is thus maximized on breaking down of the salt into cations and phosphonic acid occurring in the leaves. Some researchers place a group of phosphite-like compounds characterized by a further direct effect on microbial growth into a different category called phosphonates (Hardy et al. 2001). In this study, we compared the effect of both types of compounds and we refer to both as phosphites.

Because of their mode of action, aimed at enhancing the defense mechanism of treated hosts, rather than at directly arresting or killing the microbial disease agent, phosphites are excellent candidates not only for agricultural treatments, but also for control of soilborne pathogens in natural ecosystems. Secondary effects, in fact, are mostly on the plant itself (phytotoxicity, changes in flowering or fertility, potential changes in mycorrhization rate) without any consequence to the existing forest microbial community. Finally, phosphites are excellent candidates for treatment in natural ecosystems because they have extremely low toxicity to invertebrates, aquatic organisms, or animals, including humans. Phosphite applications have been extensive in Australian wildlands invaded by the aggressive exotic soilborne pathogen *Phytophthora cinnamomi* (Hardy et al. 2001), and some successful treatment attempts are reported for oaks infected by the same pathogen in southern Europe (Fernandez-Escobar et al. 1991). Phosphites are normally applied as injections on trees and as foliar sprays on herbaceous shrubs. The most common side effect, phytotoxicity, varies depending on the plant species.

Sudden oak death (SOD) is a devastating forest disease caused by the newly described pathogen *Phytophthora ramorum* (Garbelotto et al. 2001; Rizzo et al. 2002). Although its origins are still unknown, the pathogen is present in three distinct areas: in coastal forests of California and southern Oregon, in European plant nurseries, and in nurseries of the

west Coast of the United States and Canada. It is a generalist pathogen that causes foliar blotches and extensive branch dieback on tens of plant species both in the wild and in nurseries. In California forests, this pathogen produces abundant infectious airborne propagules on the leaves of bay laurel (*Umbellularia californica*, Lauraceae) and on the petioles and leaves of tanoak (*Lithocarpus densiflorus*, Fagaceae), whereas it sporulates less abundantly on the leaves and twigs of several species. The disease on such hosts can vary from an aggressive dieback, resulting in death of the infected plant, to a modest foliar blotch or blight. During periods characterized by wet conditions and mild temperatures, the stems of several oak species and of the related tanoak may be infected. Stem infections almost invariably end with a lethal girdling of the entire plant as the pathogen thrives in the sugar-rich cambial layer and sometimes can reach the outer xylem of infected plants, which are incapable of walling off cambial infection. Such lethal trunk cankers do not allow for sporulation of the pathogen and are thus not involved in its dispersal. For spread of the disease to occur at the landscape and regional scale, infectious foliar hosts need to be present. Long-distance movement of the pathogen is linked to the trade of infected nursery plants as clearly shown by Ivors et al. (2006). Although related to the soilborne *P. cinnamomi*, *P. ramorum*, whose life cycle is dominated by an aboveground (e.g., aerial) phase, has a unique biology and epidemiology among forest *Phytophthora* species.

Girdled oaks and tanoaks can survive up to 2 years after girdling. It takes from 2 months to 1 year for the pathogen to infect and girdle an adult tree. Although variation in susceptibility to SOD has been reported for the two most severely affected hosts, coast live oak (Dodd et al. 2005) and tanoak (Hayden and Garbelotto 2005), it is unclear whether the less susceptible trees are capable of surviving the infection or whether they may experience a relatively more prolonged, but equally lethal, disease. From an ecological perspective, tanoaks are the most severely affected trees with an average of 30% mortality in the areas infested by the pathogen and with the loss of entire adult populations in certain areas (Maloney et al. 2005). Average mortality is lower for coast live oaks, and trees away from infectious hosts such as bay laurels or tanoaks, until now, appear unaffected (Swiecki and Bernhardt 2002; Davidson et al. 2005).

Despite the intermediate susceptibility to infection of oaks, urban development in California has favored mixed evergreen forests characterized by a significant component of both bay laurel (infectious host) and coast live oak (terminal host). For this reason, SOD has seriously impacted many coastal communities of California, infecting and killing tens of thousands of oak trees at the urban-wild interface. Many of these trees are integral components of residential properties and are commonly located near roads, homes, camping

sites, and recreational areas. Infected trees are prone to be windthrown even before they are dead attributable to the enhanced activity of subsequent wood decay agents once the tree is girdled. The development of a chemical treatment appears as a necessary tool to slow the potential extinction of the very susceptible tanoak and to protect valuable oak trees from infection and death.

This article reports on a series of studies in which we tested the efficacy of phosphites to control SOD on coast live oaks. The aims of such studies specifically were: 1) to determine the efficacy of phosphite treatments in coast live oak against *P. ramorum*, including potential unwanted phytotoxicity; 2) to compare the efficacy of different application methods, including a thoroughly novel approach; 3) to compare the efficacy of preventive versus early and late therapeutic treatments; and 4) to compare the efficacy of different phosphites.

## MATERIALS AND METHODS

### Experimental Setup

Coast live oak trees in 15 gal (3.9 L) pots were tested at three experimental sites, two in Marin county and one in Alameda county. Trees were drip-irrigated with well, unchlorinated water and kept under a shade cloth intercepting 50% of solar radiation to recreate conditions favorable to infection by *P. ramorum*. Trees were 2 to 4 m (6.6 to 13.2 ft) tall with calipers ranging between 2 and 8 cm (0.8 and 3.2 in). Trees were in part purchased by the University of California and in part donated by Valley Crest Tree Company (Calabasas, CA) and were placed at the study sites at least 1 month before the beginning of each trial. At the very end of each experiment, to ensure safe disposal of inoculated wood, portions of the stem artificially inoculated with *P. ramorum* were cut off and autoclaved. Similarly, before being discarded, soil from each pot was tested for the presence of the pathogen by standard baiting techniques using D'Anjou or Bartlett pears (Erwin and Ribeiro 1996). Soil was never found to be infested by the pathogen and thus was discarded without autoclaving.

### Inoculation Techniques

Three isolates of *P. ramorum*, namely 0-4, 0-7, and Pr217, were used in trials 1 through 4. Because no significant differences of the tested treatments were observed among isolates (see "Results"), only Pr217 was used in all other trials and all results are presented ignoring pathogen isolate as a variable. The identity of the isolates was confirmed by microscopic observation of the characteristic large terminal and lateral chlamydospores produced by *P. ramorum* and by DNA sequencing of the ITS region of the nuclear ribosomal operon as described in Rizzo et al. (2002). All three isolates

were obtained by isolation in California forests from infected oaks or tanoaks.

Isolates were first inoculated on bay leaves, and then margins of the resulting lesions were subcultured first on corn meal-based P<sub>10</sub>ARP followed by V8 agar. Plugs measuring 8 mm (0.32 in) in diameter were then taken from the margins of the resulting colonies and placed in the outer xylem of oak trees after removal of the bark using a sterile 10 mm (0.4 in) diameter cork borer. The bark was then replaced on top of the inoculum plug and the inoculation area sealed with grafting wax, Parafilm™ (Alcan Inc., Neenah, WI), and aluminum tape. Inoculations were performed on the stem 1 m (3.3 ft) from the root collar.

### Lesion Evaluation

At the end of each trial (12 to 33 weeks long; see Table 1), the bark of all trees was carefully peeled off and the size of lesions was measured. Longitudinal growth along the stem was determined by overlaying a flexible, graduated ruler on top of the lesion. Care was taken to measure the entire length of the lesion by progressively peeling deeper into the bark and the outer xylem of trees in the trial. This was a necessary requirement, because *P. ramorum* tends to grow in a serpentine fashion in and out between the cambium and the outer xylem. The length of the longitudinal lesion acropetally toward the tree top was measured independently of the basipetal lesion toward the root collar. The extremities of each lesion were plated on PARP media to ensure lesions were caused by the inoculated pathogen. Each culture resulted in the growth of a *P. ramorum* colony. In each case, the pathogen was identified by microscopic observation of the growing colonies.

### Treatments

Seven different experiments were performed to evaluate the therapeutic and preventive efficacy of phosphite treatments. Table 1 summarizes treatment types and products used. The following products were tested: NutriPhyte (Chemical Dynamics Inc., Plant City, FL), Agrifos (Agrichem Manufacturing Industries Pty. Ltd., Loganholme, Queensland, Australia), Phostrol (Nufarm Americas Inc., Burr Ridge, IL), Aliette (wetable powder from Rhone-Poulenc for foliar and soil applications, TreeTech for injections), Vital (Luxembourg-Pamol Inc., Memphis, TN), and SuperSODAway (University of California Berkeley, not commercially available). Concentration of active ingredients was 6% to 9.5% for injections (6% for Phostrol and Phytoguard; 8% for NutriPhyte, Agrifos, and SuperSODAway; 9.5% for Aliette), 0.5% for foliar or soil drenches, and 18% for bark application of Agrifos + Pentrabark™ (Agrichem, Medina, OH). Surfactants were added to phosphites as described by label or as recommended

by the manufacturer. Breakthru® (Western Farm Services Inc, Fresno, CA) was mixed to the foliar spray at a final concentration of 0.05%; Pentrabark™ was tested at concentrations of 7.5% and 2.5%. Tree plots were divided in blocks, each containing one replicate of each treatment. Positioning of treatment within the block was randomly selected. Analyses were done as for a completely randomized design, because there was no treatment replication within the block. Treatments were avoided in the period between late December and the end of January, when temperatures are relatively cold. When possible, they were administered in October or November or between March and May when trees are physiologically active and can translocate and process phosphites. Therapeutic treatments were administered 64 to 250 hr after the inoculum had been placed in the stems. Preventive treatments were always administered 1 week before inoculation.

A single injection per tree, 10 mL (0.3 fl oz) in volume, was administered by drilling a hole above the root collar using a bit slightly smaller than the tip of the tree injector. Depth of injection was variable depending on tree size, but all injections remained in the outer layer of rings of the xylem. The tip of the plastic injector (Marley® injector, Quest Products, Louisburg, KS) is conical and a seal is formed between the injector and the tree simply by slightly pressing the injector into the drill hole. Holes are normally drilled, not perpendicular to the stem, but at a slight angle to ease flow of the solution. Positive pressure by means of a piston or spring mechanism is constantly applied until the product is completely absorbed.

Foliar treatments were applied by either mixing a wettable powder (weight:volume dilution) or by diluting a concentrated product to the desired concentration. The solution was then sprayed using a backpack sprayer until runoff (approximately 500 mL [15 fl oz] per tree). Soil drenches are applied by mixing the solution as indicated and by watering each tree with 2 L (0.52 gal) of solution.

Topical bark applications were performed by spraying the same solution as in the foliar treatments on the bark of trees. Bark applications of Pentrabark™ + Agrifos® were performed by mixing different percentages of Pentrabark™ with a 1:1 solution of AgriFos® and water.

### Statistical Analyses

All statistical analyses were performed using the program JMP (SAS Institute Inc., Cary, NC). The variable measured to assess efficacy of treatments was the longitudinal growth of the pathogen along the stem in both directions from the inoculation point. Data sets for each trial were independently tested for lack of normality and unequal variances among treatments using the Shapiro-Wilk and the O'Brien tests, respectively. When the data from a trial violated either one of

**Table 1. List and date of individual experiments, including all treatments tested for each trial and untreated controls.<sup>z</sup>**

Expt.	Treatment and dates	N	Avg. lesion length	SD	P	Phytotoxicity
<b>1</b>	<b>Seven treatments; begin 5/15/2001 end 8/15/2001</b>	<b>105</b>			<b>&lt;0.001</b>	
1	Aliette drench therap.	15	70.8	18.1		No
1	Aliette foliar therap.	15	59.5	10.4		Yes
1	Aliette injection therap.	15	49.7	10.5	<0.05	No
1	Nutriphyte drench therap.	15	67.1	17.4		No
1	Nutriphyte foliar therap.	15	60.4	13.5		Yes
1	Nutriphyte injection therap.	15	41.3	10.2	<0.01	No
1	Untreated	15	66.0	16.9		No
<b>2</b>	<b>Three treatments; begin 11/14/2001 end 2/29/2002</b>	<b>45</b>			<b>&lt;0.001</b>	
2	Aliette bark therap.	15	77.4	32.3		No
2	Nutriphyte injection therap.	15	27.9	14.9	<0.001	No
2	Untreated	15	77.8	37.4		No
<b>3</b>	<b>Three treatments; begin 11/7/2001 end 3/15/2002</b>	<b>45</b>			<b>&lt;0.05</b>	
3	Nutriphyte injection therap.	15	50.8	17.8	<0.05	No
3	SuperSODAway injection therap.	15	45.0	18.4	<0.05	No
3	Untreated	15	77.6	25.9		No
<b>4</b>	<b>Eight treatments; begin 12/7/2001 end 4/10/2002</b>	<b>135</b>			<b>&lt;0.0001</b>	
4	Nutriphyte injection therap.	15	37.7	13.2	<0.0001	No
4	Phostrol injection therap.	15	42.2	17.7	<0.001	No
4	Phytoguard injection therap.	15	37.3	12.9	<0.0001	No
4	Aliette injection therap.	15	42.5	13.9	<0.001	No
4	Nutriphyte foliar therap.	15	55.6	18.7		Yes
4	Nutriphyte drench therap.	15	68.9	38.5		No
4	Nutriphyte injection prev.	15	8.3	0.7	<0.0001	No
4	Untreated	30	93.6	60.8		No
<b>5</b>	<b>Six treatments; begin 12/7/2001 end 7/15/2002</b>	<b>105</b>			<b>&lt;0.001</b>	
5	Phostrol injection prev.	15	67.7	123	<0.0001	No
5	Phytoguard injection prev.	15	47.3	59.7	<0.001	No
5	Aliette injection prev.	15	43.9	42.8	<0.001	No
5	Nutriphyte injection prev.	15	30	38	<0.0001	No
5	Nutriphyte foliar prev.	15	75	41.8		Yes
5	Untreated	30	169	193		
<b>6</b>	<b>Three treatments; begin 4/15/2002 end 7/12/2002</b>	<b>27</b>			<b>&lt;0.0001</b>	
6	Nutriphyte injection prev.	9	39.2	7.3	<0.0001	No
6	Nutriphyte foliar prev.	9	93.6	46.9		Yes
6	Untreated	9	100	15.5		
<b>7</b>	<b>11 treatments; begin 7/16/2002 end 10/22/2002</b>	<b>50</b>			<b>&lt;0.0001</b>	
7	Agrifos + Pentrabark (2.5%) preventive trunk appl.	5	13.6	2.6	<0.0001	No
7	Agrifos + Pentrabark (7.5%) preventive trunk appl.	5	13.4	3.4	<0.0001	No
7	Agrifos injection prev.	5	14	3.6	<0.0001	No
7	Aliette foliar prev.	5	15.3	5.8	<0.0001	Yes
7	Aliette injection prev.	5	17.1	6.9	<0.0001	No
7	Nutriphyte injection prev.	5	16.9	7.9	<0.0001	No
7	Phostrol injection prev.	5	13.9	2.4	<0.0001	No
7	Phostrol foliar prev.	5	26.6	14.8		Yes
7	SuperSODAway inj. prev.	5	10.7	1.8	<0.0001	No
7	Vital injection prev.	5	13.6	5.8	<0.0001	No
7	Untreated	5	51.7	9.8		No

<sup>z</sup>Number of trees (N) used in each experiments and for each treatment are shown, followed by average length of lesion, standard deviation (SD), statistical significance (P), and phytotoxicity as foliar scorch and dieback.

these basic assumptions required by analysis of variance (ANOVA), the entire data set for that trial was log-transformed. The actual means and standard deviation values, and not the transformed ones, are reported in Table 1. *P* values obtained using transformed data sets when necessary are reported instead. Statistical significance was never affected by the transformation of data sets required to meet the basic assumptions of ANOVA.

Linear lesion measurements were compared among treatments using ANOVA, excluding measurements from uninfected negative controls. Treatments were then individually compared with the controls (e.g., pathogen-inoculated but chemically untreated trees) using Dunnett's test.

The average efficacy of therapeutic injection treatments (ET) was calculated for each experiment as follows:  $ET = LT/LPC$ , where *LT* = average length of the lesion of treated trees and *LPC* = average length of the lesion of untreated inoculated trees. ET was regressed against hours since inoculation of the pathogen using the least significant difference model in JMP.

### RESULTS

Table 1 summarizes results of all experiments. Results from Expts. 4 and 7 are also depicted in Figures 1 and 2. Therapeutic phosphite injections were effective in reducing the growth of *P. ramorum* in artificially inoculated potted coast live oak trees as determined by the size of lesions. Lesions were not only visually measured, but their nature was always verified by successful culturing of the pathogen from their margins. In all experiments, average lesion size in

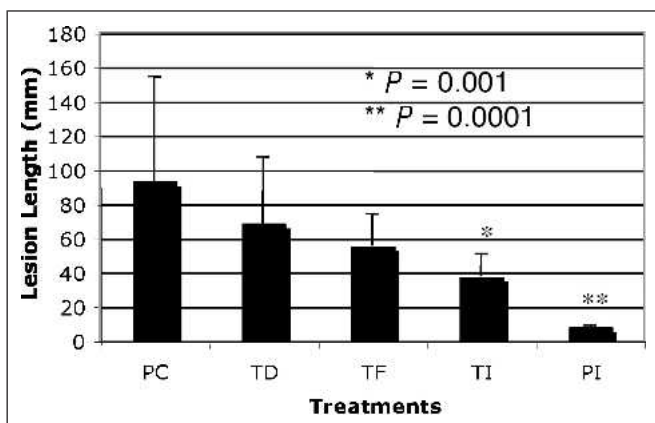


Figure 1. Length of lesions (Y axis) caused by artificial inoculations of *Phytophthora ramorum* in untreated and phosphite-treated potted coast live oak trees in Expt. 4. Length of lesion in untreated trees (PC) is compared with that in trees treated by means of therapeutic drench (TD), therapeutic foliar spray (TF), therapeutic injection (TI), and preventive injection (PI).

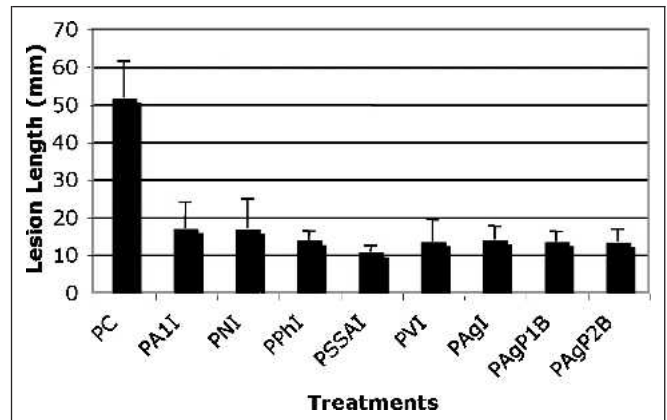


Figure 2. Results from Expt. 7 comparing efficacy of several preventive phosphite treatments. Treatments were as follows: PC = untreated trees; PennsylvaniaI = preventive Alette injection; PNI = preventive Nutriphyte injection; PPhI = preventive Phostrol injection; PSSAI = preventive SuperSODAWay injection; PVI = preventive Vital injection; PennsylvaniaI = preventive Agrifos injection; PennsylvaniaP1B = preventive Agrifos + Pentrabark 2.5% bark application; PennsylvaniaP2B = preventive Agrifos + Pentrabark 7.5% bark application. All treatments were effective at  $P < 0.0001$ .

trees injected with phosphites was smaller than the average lesion size of untreated trees. All phosphite products tested were effective in slowing the pathogen's growth in treated trees. All three pathogenic isolates in Expts. 1 through 4 were equally suppressed by the phosphite treatments, as indicated by the lack of significant differences in lesion sizes caused by

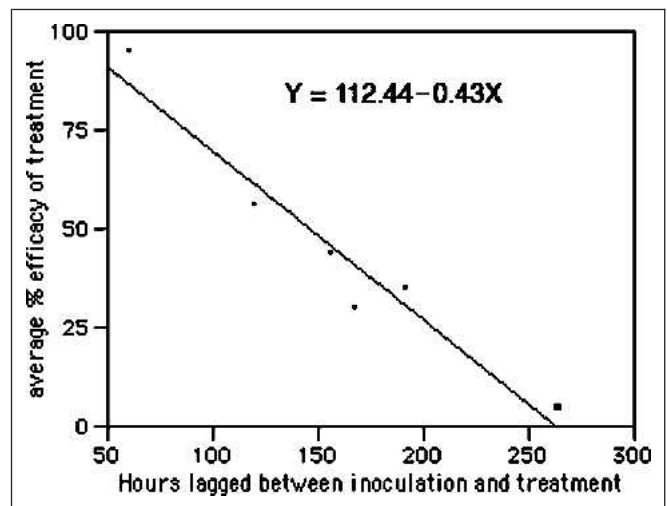


Figure 3. Regression analysis between average percentage of disease control (Y axis) and hours elapsed between pathogen inoculation and treatment (X axis).

the three isolates in treated trees. Results of the comparative analyses among isolates for Expts. 1 through 4 were, respectively, as follows ( $df = 2$  for all four trials):  $F_{ratio} = 1.7$  and  $P = 0.19$ ;  $F_{ratio} = 0.46$  and  $P = 0.64$ ;  $F_{ratio} = 0.47$  and  $P = 0.62$ ; and  $F_{ratio} = 0.49$  and  $P = 0.61$ .

Figure 3 indicates that a delay in treatment resulted in a decrease of average efficacy in slowing the growth of the pathogen. An inverse correlation was found ( $Y = 112.44 - 0.43X$ ,  $R^2 = 0.94$ ,  $P = 0.0013$ ) between efficacy of treatment and time of treatment since inoculation. Average efficacy of treatment was 95%, 56%, 44%, 30%, 35%, and 5% for injections administered at 60, 120, 156, 168, 192, and 264 hr after inoculation. No phytotoxicity was ever observed with injection treatments at the rates of active ingredient used for this study. Therapeutic foliar sprays slowed growth of the pathogen as evidenced by the smaller lesion size in trees whose crowns were sprayed with phosphites, but the reduction was limited. The effect on trees was inconsistent as indicated by the large variation in response among trees, and there was no strong statistical difference between lesion size of treated versus untreated trees. Mild to severe phytotoxicity with abundant leaf scorching was noticed in all but one experiment. Preventive injection treatments were always successful in consistently and durably controlling disease.

Preventive foliar treatments resulted in a temporary reduction in pathogen growth as evidenced by smaller lesion sizes. In Expt. 6 after 2 weeks, lesion size was 0% and 31% of untreated controls for preventive injection and foliar treatments, respectively. Four weeks after treatment, lesion size was 6% and 62% of untreated controls for preventive injections and foliar treatments, respectively. In Expt. 6, all positive effects of foliar treatments disappeared after 8 weeks, whereas injections were still effectively reducing growth of the pathogen (Table 1). Eight months after treatment (Expt. 5), there remained a significant reduction in lesion size of trees that had been injected with phosphites. Phytotoxicity on coast live oak foliage was observed after preventive foliar treatments with phosphite + surfactant solutions.

Topical applications of phosphites and phosphites + surfactant did not cause any significant reduction in lesion size associated with growth of the pathogen. Expt. 2 shows that lesion size in trees whose bark had been treated therapeutically was indistinguishable from lesion size of untreated trees. However, when an organosilicate surfactant (Pentrabark™) was mixed with the phosphite solution, topical application on the bark was as successful as preventive injections, and lesion sizes in treated trees were indistinguishable from the negative control. Soil drenches failed to produce any significant results as shown by results of Expts. 1 and 4 (Table 1; Figure 1).

## DISCUSSION

Administration of phosphites to coast live oak potted trees resulted in significant reduction, or even arrest, of lesion growth caused by *P. ramorum* in planta. It has been observed that trees affected by the typical terminal sudden oak death symptom, e.g., sudden necrosis of the entire crown, are girdled or almost completely girdled along the entire circumference of the trunk. In most cases, the girdling occurs  $\approx 1$  m ( $\approx 3.3$  ft) above the root collar, but girdling cankers have been reported at all heights on the main stem. We believe that plants affected by smaller lesions may survive for longer periods of time. When trees' growth rate surpasses growth rate of the pathogen, healing of the lesion should occur. Phosphite treatments may lead to an increase of percentage of healing cankers attributable to their dual ability to slow the growth of the pathogen while enhancing growth of the plant host and compartmentalization of lesions caused by *P. ramorum*.

Not all application methods were successful. Soil drenches and topical bark applications of phosphites without additives yielded no significant reduction of disease levels. Foliar applications resulted in a trend toward smaller lesions, but results were inconsistent among trials, and mild to extreme leaf scorching and twig dieback was noted, indicating phytotoxicity was a side effect of such treatment. Furthermore, data from Expt. 6 indicated the beneficial effects of foliar treatments are short-lived and are lost after only 8 weeks (Table 1). Preventive injections and bark application of phosphites amended with the organosilicate Pentrabark™ were the most effective and consistent treatments. Injections were shown to be effective up to 8 months after application (Table 1). In all trials, lesions in trees treated according to either one of the two successful methods described were indistinguishable from lesions of negative controls, i.e., trees that were wounded but only mock-inoculated with a plug of sterile agar.

No phytotoxicity was ever observed associated with either of the two effective treatment approaches, but it should be highlighted that if the solution for bark application is applied on plant leaves, it will completely burn the vegetative tissue. If valuable plants are around the tree, we suggest protecting them with a nylon sheet or a plastic tarp.

Injections on adult trees should be done at a frequency of 15 cm (6 in) across the circumference. Colored plastic screw-cap plugs can be inserted in the hole drilled for injection. Because no product is dispersed in the environment, injections are extremely appropriate for public areas. On the other hand, injections require trained professionals and cannot be performed on all trees. Injections can be easily performed on young cylindrical trees, but as trees grow older, imperfections such as knots, irregular grain, cavitation, and embedded branch stubs may result in failed injection. Injections were

observed to fail when they were applied directly into or below such imperfections. Injections need to enter the outer xylem of the tree (last three rings), because these are the rings actively involved in acropetal translocation of fluids into the leaves. Because translocation in coast live oaks occurs at high pressure, positive injection systems, in which pressure is constantly applied until the product is absorbed, are likely to be the most effective. It is best to inject phosphites during clear warm days when plants are known to be physiologically more translocative. Rainy or cold days should be avoided, because physiologically inactive plants will absorb the products more slowly.

The data clearly indicated that the best options to control SOD were provided either by preventive treatments or by treatment of trees only recently infected. Unfortunately, because infection in adult trees can remain latent for several weeks or months, estimating the time since infection may be difficult or impossible. Hence, we strongly recommend preventive treatment of trees at risk of becoming infected.

A combined understanding of the epidemiology of SOD and of the temporal dynamics of treatments' efficacy is essential for best planning of chemical treatments (Rizzo and Garbelotto 2003). We have shown that phosphite treatments can be effective as long as 8 months. It is likely phosphite treatments will be effective for 2 to 6 years as reported for other tree species (Hardy et al. 2001), but until the length of efficacy of treatments is clearly studied, we recommend at least one yearly treatment. Three to 6 weeks from application may be necessary to obtain maximum control in adult trees. Oak infections appear to have a peak of infection in May through June and potentially another peak in February through March, especially in the milder coastal areas. In such areas, two treatments may be recommended each year: one in November and one in March each year. In areas characterized by colder winters, a fall treatment in November followed by a spring treatment in March may be recommended for the first year followed by a single treatment in March for subsequent years. If a particularly wet year is predicted, two treatments should always be prescribed everywhere, because conditions for infection may be extremely favorable.

Besides chemical treatments, it is necessary to embrace other approaches to slow the spread of this exotic disease in California and elsewhere. Ensure all plant material is planted is certified as "free of pathogen" and do not move any plant material within the infested area or between infested and uninfested areas. Infested plant material should be locally disposed of by burning or composting. Debris exclusively made up of wood can be chipped and locally broadcasted in the dry season (rather than piled and covered by a tarp) to ensure rapid drying. It has been shown, in fact, that the patho-

gen does not survive in the woody debris of oaks and tanoaks if dry (Davidson et al. 2005; Swain et al. 2006). Finally, all infectious hosts such as bay laurels, tanoaks, rhododendron spp., camellias, and the lower branches of redwood trees should be removed at least from an area 10 m (33 ft) in radius around the most valuable oak trees. Inoculum reduction appears to be one of the best options to curtail infection on coast live oak, a plant species that does not carry the pathogen in an infectious form, i.e., oak-to-oak contagion has not been reported or observed. The presence of tolerance or partial resistance to SOD is currently being investigated and may be another tool to control this devastating forest disease.

In this study, we developed and tested a relatively novel application method, i.e., bark application of a fungicide in combination with an organosilicate surfactant. This application is user- and environment-friendly while remaining relatively inexpensive. We believe this application may allow for treatments of large number of trees without the need of drilling into the plant trunk. This application may enhance our outlook on chemical treatments of forest trees and widen it from a purely landscape tree perspective. Preventive treatments of selected trees by application of phosphite + Pentra-bark™ in a forest setting may represent a possible way of protecting natural populations of trees endangered by this introduced pathogen.

**Acknowledgments.** This study was performed thanks to a competitive grant funded by the USDA Forest Service Pacific Southwestern Station and by a grant from the Gordon and Betty Moore Foundation, both to MG. We are grateful to Valley Crest Tree Company for donating 50% of the trees used in these experiments and to the Sunnyside Nursery and to Lucasfilm Ltd. for allowing placement of study plots within their properties in Marin County, CA.

#### LITERATURE CITED

- Davidson, J.M., A.C. Wickland, H. Patterson, K. Falk, and D.M. Rizzo. 2005. Transmission of *Phytophthora ramorum* in mixed-evergreen forests of California. *Phytopathology* 95:587–596.
- Dodd, R.S., D. Hüberli, V. Douhovnikoff, T.Y. Harnik, Z. Afzal-Rafii, and M. Garbelotto. 2005. Is variation in susceptibility to *Phytophthora ramorum* correlated with population genetic structure in coast live oak (*Quercus agrifolia*)? *The New Phytologist* 165:203–214.
- Erwin, D.C., and O.K. Ribeiro. 1996. *Phytophthora* Diseases Worldwide. APS Press, American Phytopathological Society, St. Paul, MN.
- Fernandez-Escobar, F.J.B.R., M. Gallego, and M. Benlloch.

1999. Treatment of oak decline using pressurized injection capsules of antifungal materials. *European Journal of Forest Pathology* 29:29–38.
- Garbelotto, M., P. Svihra, and D.M. Rizzo. 2001. Sudden oak death syndrome fells three oak species. *California Agriculture* 55:9–19.
- Guest, D.I., and B.R. Grant. 1991. The complex action of phosphonates as antifungal agents. *Biological Reviews of the Cambridge Philosophical Society* 66:159–187.
- Guest, D.I., K.C. Pegg, and A.W. Whaley. 1995. Control of Phytophthora diseases of trees crops using trunk-injected phosphonates. *Horticultural Reviews* 17: 299–330.
- Hardy, G., S. Barrett, and B.L. Shearer. 2001. The future of phosphite as a fungicide to control the soilborne pathogen *Phytophthora cinnamomi* in natural ecosystems. *Australasian Plant Pathology* 30:133–139.
- Hayden, K., and M. Garbelotto. 2005. Resistance to *Phytophthora ramorum* in tanoak. Second Sudden Oak Death Symposium, Monterey, CA, January 18–21, 2005.
- Ivors, K., M. Garbelotto, I. De Vries, C. Ruyter-Spira, B. Te Hekkert, N. Rosensweig, and P. Bonanat. 2006. Microsatellite markers identify three lineages of *Phytophthora ramorum* in US nurseries, yet single lineages in US forest and European nursery populations. *Molecular Ecology* 15:1493–1505.
- Maloney, P.E., S.C. Lynch, S.F. Kane, C.E. Jensen, and D.M. Rizzo. 2005. Establishment of an emerging generalist pathogen in redwood forest communities. *Journal of Ecology* 93:899–905.
- Rizzo, D.M., and M. Garbelotto. 2003. Sudden oak death: Endangering California and Oregon forest ecosystems. *Frontiers in Ecology and the Environment* 1: 197–204.
- Rizzo, D.M., M. Garbelotto, J.M. Davidson, G.W. Slaughter, and S.T. Koike. 2002. *Phytophthora ramorum* as the cause of extensive mortality of *Quercus* spp. and *Lithocarpus densiflorus* in California. *Plant Disease* 86: 205–214.
- Swain, S., T. Harnik, M. Mejia-Chang, K. Hayden, W. Bakx, C. Creque, and M. Garbelotto. 2006. Composting is an effective treatment option for sanitization of *Phytophthora ramorum*-infected plant material. *Journal of Applied Microbiology* 101:815–827.
- Swiecki, T.J., and E. Bernhardt. 2002. Evaluation of stem water potential and other tree and stand variables as risk factors for *Phytophthora ramorum* canker development in coast live oak, pp. 787–798. In Proc. Symp. California Oak Woodlands, 5th. Standiford, R., McCreary, D., and Purcell, K.L., eds. 2002. USDA For. Serv., Gen. Tech. PSW-GTR-184. Pacific Southwest Res. Stn., Albany, CA.
- M. Garblotto (corresponding author)  
 Department of Environmental Science Policy and Management  
 Ecosystem Sciences Division  
 137 Mulford Hall  
 University of California  
 Berkeley, CA 94720, U.S.  
 matteo@nature.berkeley.edu
- D.J. Schmidt  
 Department of Environmental Science Policy and Management  
 Ecosystem Sciences Division  
 137 Mulford Hall  
 University of California  
 Berkeley, CA 94720, U.S.
- T.Y. Harnik  
 Department of Environmental Science Policy and Management  
 Ecosystem Sciences Division  
 137 Mulford Hall  
 University of California  
 Berkeley, CA 94720, U.S.
- Résumé.** Dans chacune des sept expériences pleinement contrôlées, des chêne verts de Californie empotés ont été inoculés artificiellement avec *Phytophthora ramorum*, l'agent de la maladie communément appelée de la mort subite du chêne. Des phosphites ont été appliqués aux arbres au moyen de différentes approches d'application, toutes étant des traitements préventifs ou curatifs. Les applications de phosphites par mouillage du sol et directement sur l'écorce se sont avérées inefficaces; celles par application foliaire amendées de surfactants ont été efficaces seulement par moments et ont toujours causées des phytotoxicités. D'un autre côté, les injections de phosphites et les applications de phosphites sur l'écorce avec le surfactant d'organosilicate Pentrabark™ ont été efficaces de manière intéressante en supprimant la colonisation de l'écorce par cette maladie, et ce sans causer de phytotoxicité. Ceci est la première étude décrivant l'utilisation d'un traitement chimique avec un surfactant d'organosilicate pour des traitements sur l'écorce.
- Zusammenfassung.** In jedem von sieben voll kontrollierten Experimenten wurden getopfte Lebensichen mit *Phytophthora ramorum* inokuliert, dem Erreger einer Baumkrankheit, die landläufig als plötzliches Eichensterben bekannt ist. Die Phosphite wurden mit einer Reihe von Applikationsmöglichkeiten (preventiv und/oder kurativ) auf die Bäume appliziert. Gräben im Erdreich und Rindenapplikationen waren ineffektiv, Blattapplikationen, angereichert mit Netzmittel waren nur zu bestimmten Zeiten effektiv und verursachten immer Phytotoxizität. Auf der anderen Seite waren Phosphit-Injektionen und Rindenapplikationen+Pentrabark (organosilikatreiches Netzmittel) anhaltend effektiv in der Unterdrückung von Rindenkolonisation durch des Pathogen und verursachte keine Phytotoxizität. Das ist die erste beschreibende Studie für die



Anwendung von chemischen Mitteln, die mit organosilikatreichen Netzmitteln angereichert wurden, beim Einsatz als Rindenapplikation.

**Resumen.** En siete experimentos controlados, árboles de encino en contenedor fueron artificialmente inoculados con *Phytophthora ramorum*, el agente de la enfermedad comúnmente referida como Muerte Súbita del Encino. Se aplicaron fosfitos a los árboles usando un rango de aplicaciones, bien sea como tratamientos preventivos o terapéuticos. La aplicación de fosfitos con zanjas al suelo y a la

corteza resultaron inefectivas; la aplicación foliar de fosfitos mejorada con surfactantes fueron efectivas algunas veces, y siempre causaron fitotoxicidad. Por otro lado, las inyecciones de fosfitos y las aplicaciones a la corteza de fosfitos+el surfactante órgano-silicato Pentrabark™ fueron consistentemente efectivas en suprimir la colonización de la corteza por este patógeno, sin causar fitotoxicidad. Este es el primer estudio que describe el uso de un tratamiento químico mejorado con un surfactante órgano-silicato para aplicaciones tópicas en la corteza.