Phytophthora ramorum and Sudden Oak Death in California: IV. Preliminary Studies on Chemical Control[1]

Matteo Garbelotto,[2] David M. Rizzo,[3] and Lawrence Marais[4]

Abstract

Chemical applications may provide one means of control for *Phytophthora ramorum*, the cause of Sudden Oak Death (SOD). Such controls have been effective with other *Phytophthora* species in landscape and orchard situations. We have initiated laboratory and field studies to test the efficacy of a number of products previously reported to control *Phytophthora*. "In vitro" tests have determined that many of the standard chemical controls (e.g., metalaxyl, copper sulfate, phosphoric acid) are effective against *P. ramorum*. Field and green house studies include experiments to treat trees and saplings already infected with the pathogen as well as treatments to protect trees from infection. Application of chemicals has been by drench, injection, and topical application directly to the bark. Although preliminary results are positive, it should be pointed out that use of chemical controls will be limited to urban situations and specimen trees. It is unlikely that chemical controls will be of practical use in California wildland situations.

Introduction

The genus *Phytophthora* includes several plant pathogens, and chemical control of *Phytophthora spp*. is a common practice for agricultural crops (Erwin and Ribeiro 1996, Guest and others 1995). A wide array of compounds is commercially available, often with active ingredients and formulations that may be specific to one or a few species of the pathogen and to one type of application (e.g., foliar sprays vs. injections). Rate of active ingredient, time of application, phytotoxicity, and the potential for the rise of resistance to the chemicals in individuals of the pathogen, are all important considerations when planning a chemical application (Garbelotto and others 2001). In the case of natural ecosystems, the use of chemical control is, for obvious reasons, even more problematic. Nevertheless, chemical control may be an important tool in specific situations, for instance if trying to eradicate a pathogen from limited infestation foci. Chemical control can also be important as part of an integrated management approach, when trying to protect individual or small groups

[1] An abbreviated version of this paper was presented at the Fifth Symposium on Oak Woodlands: Oaks in California's Changing Landscape, October 22-25, 2001, San Diego, California.

[2]Cooperative Extension Specialist, Department of Environmental Science, Policy, and Management, Ecosystems Science Division, University of California, Berkeley, CA 94720 (e-mail address: matteo@nature.berkeley.edu)

[3]Associate Professor, Department of Plant Pathology, University of California, Davis, CA 95616

[4] Extension Specialist/Plant Pathologist, Department of Plant Pathology, University of California, Riverside, CA 92521

of trees. There are at least two known successful examples of chemical treatments of *Phytophthora* in forest ecosystems (Fernandez-Escobar and others 1999, Hardy 1999).

Phytophthora ramorum is a newly described species (Werres and others 2001), and the likely cause of a lethal disease of at least three species of California oaks (*Quercus* spp.), and tanoak (*Lithocarpus densiflora*) (Garbelotto and others 2001, Rizzo and others 2002). No information is available on the susceptibility of this pathogen to compounds used for the control of other *Phytophthora* species, and on the efficacy of chemical treatments in controlling the canker disease this pathogen causes.

In this paper, we describe the first series of "in vitro" tests aimed at determining the sensitivity of *P. ramorum* to several chemical compounds. We also describe an experiment on potted saplings in which chemical injections were used to control the growth of *P. ramorum*.

Materials and Methods

In Vitro Tests

Chemical compounds listed in *table 1* were used in these trials. For each compound, the 1X concentration was based on amounts recommended by the manufacturer for woody ornamentals or orchard trees. Each compound was tested at the following concentrations: 10X, 1X, 1:10X, 1:50X, 1:100X, 1:200X, 1:500X. Standard 10 percent V8 growth medium (Erwin and Ribeiro 1996) was amended with varying amounts of each compound, in order to obtain the concentrations listed above. V8 agar without any additions was used as a control treatment. Both the amended and unamended media were poured in 6 cm-diameter Petri dishes, and left to solidify overnight.

Table 1—*Chemical compounds and their concentrations that were used in tests for control of* Phytophthora ramorum.

Active ingredient (A. I.)	1X concentration (percent A.I.)
Metalaxyl	0.012
Phosetyl-Al	3.200
Phosphorous acid	2.000
Propiconazole	0.017
Copper Sulfate Pentahydrate	0.100

Three isolates of *P. ramorum* were employed: Pr-1 (coast live oak, Marin CO.), Pr-2 (tanoak, Marin CO.), and Pr-4 (tanoak, Marin CO.). Plugs from actively growing edges of colonies were then transferred at the center of each Petri dish. For each isolate and compound concentration, five (trial a) and three (trial b) replicates were performed, so that at a total of 15 (trial a) plates were used for each compound concentration tested.

Plates were inoculated and placed in an incubator at 22°C. Size of each colony was outlined with a marker at 2, 4, 7, and 11 days. The minimum and maximum diameters of each colony at each measuring time were averaged and used for the analysis. For this paper only the average colony diameter at 11 days was used in the analysis. Analysis of variance (ANOVA) was used to compare the efficacy of each

chemical at 1X concentration, a Tukey-Kramer analysis was performed to compare different compounds.

Potted Saplings Experiment

A total of 80 saplings of coast live oak (*Q. agrifolia*) with calipers ranging from 3 to 6 cm, and heights between 3 and 5 m were used in the experiment. The saplings, grown in 15-gallon containers, were covered with a 50 percent shade cloth, and drip irrigated daily. On August 10, 2000, 75 saplings were inoculated on their East facing side with *P. ramorum*. One of five isolates was inoculated on each sapling in the following way. A cork borer (diameter 1.2 cm) was used to cut the bark at 1 m from the root collar; a 0.8 cm plug of agar obtained from the edge of a colony was placed in contact with the cambium, the bark plug was replaced on top agar and sealed with grafting wax. All inoculation points were wrapped in foil. On October 31, a second inoculation was performed at 10 cm from root collar, and facing North on each sapling as described above. Three days after the second inoculation, the chemical treatments, including a plain water control, were administered. Each tree was injected with 10 ml of either water, 0.1 percent copper sulfate pentahydrate, 5 percent phosetyl-Al, 11 percent metalaxyl, or 9 percent phosphorous acid. Each treatment included 15 saplings. A total of 5 saplings were mock inoculated by placing a plug of sterile agar in the wound.

On March 3, 2001, the trial was terminated. The bark around each inoculation point was carefully removed until the canker necrosis in the cambium was visible. For each inoculation 4 measurements were taken starting from the center of the inoculation plug: upwards towards the crown, downwards towards the roots, clockwise across the stem, and counterclockwise across the stem. Four isolations were performed by plating on pimaricin-ampicillin-rifampicin-Pentachloronitrobenzene (PARP) medium (Erwin and Ribeiro 1996) a chip from the further edge of the canker in each of the four directions. ANOVA was performed to compare lesion size across treatments. In this paper, we present only analyses performed on the sum of longitudinal and transversal growth values.

Results and Discussion

In Vitro Tests

Figure 1 summarizes the results of the "in vitro" tests. While several compounds resulted to be actively reducing or totally inhibiting the growth of *P. ramorum* in culture, not all compounds were equally efficacious as indicated by the variation in ED_{50} levels, and by different efficacy as indicated by ANOVA performed on data from 1X concentrations *(fig. 2)*. While some compounds like metalaxyl are effective even at minimal concentrations, others like the copper sulfate formulation used in this trial lost efficacy quite rapidly with decreasing concentration. In the case of trunk topical treatments, where chemical action is expected to occur by contact (prevention or eradication of infections), chemical concentration is a minor issue, if within the range of environmental and human safety. In the case of foliar applications, soil drenches, or injections, effective concentration is an extremely important issue. Although some chemicals may be effective against the pathogen at certain concentrations, these same chemical concentrations may be phytotoxic to the plant.

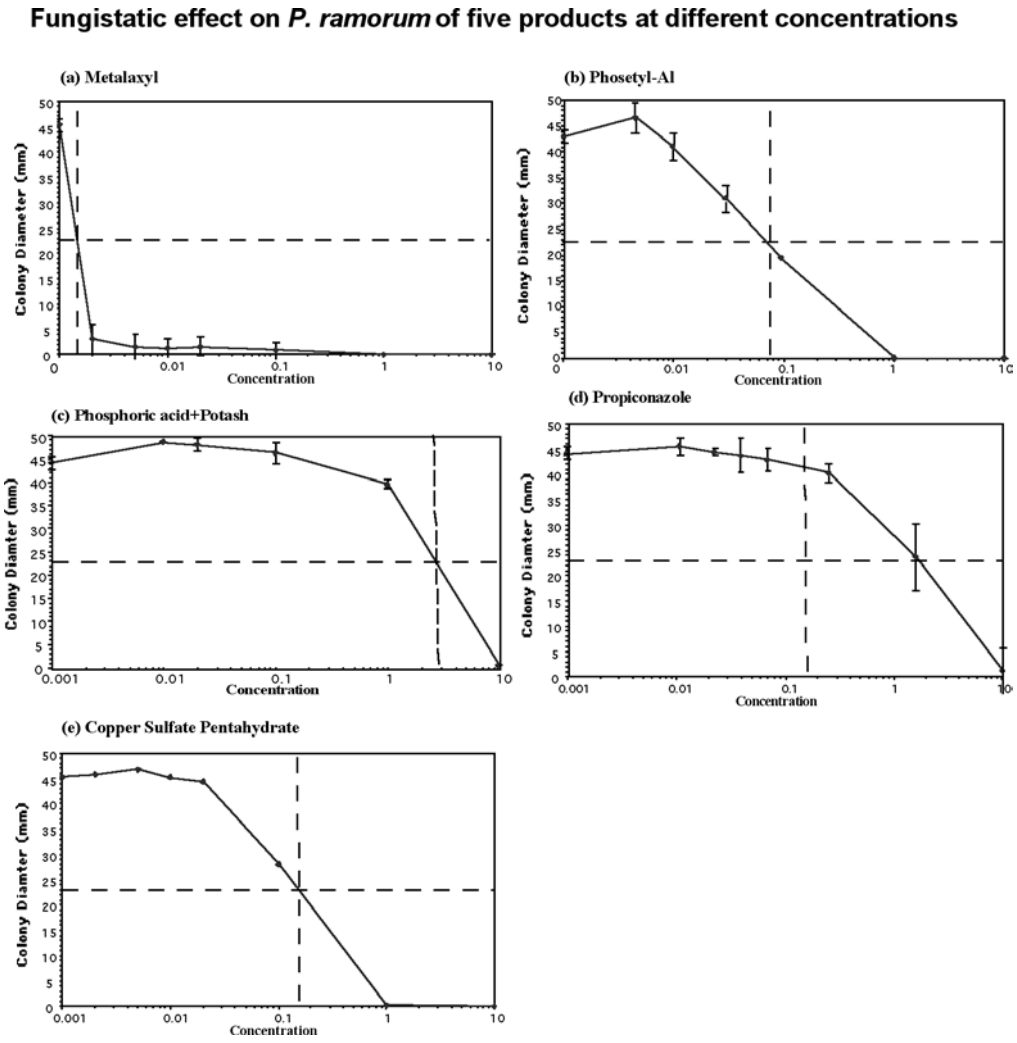

Figure 1—Mean colony diameter of *P. ramorum* 11 days after inoculating Petri dishes filled with growth medium amended with different concentrations of each chemical compound used in this trial is shown on the Y axis. Data are shown individually for each compound (panels a to e). A solid line connects the control on the Y axis (e.g., mean colony diameter of *P. ramorum* on unamended growth medium) and mean diameter values of *Phytophthora* colonies at each tested concentration. Standard deviations are shown as horizontal solid bars.The X axis is in logarithmic scale. For each compound, an approximate ED_{50} value (e.g. the concentration needed to obtain a 50 percent reduction of colony size), is indicated by the intersection of the vertical dashed line and the X axis.

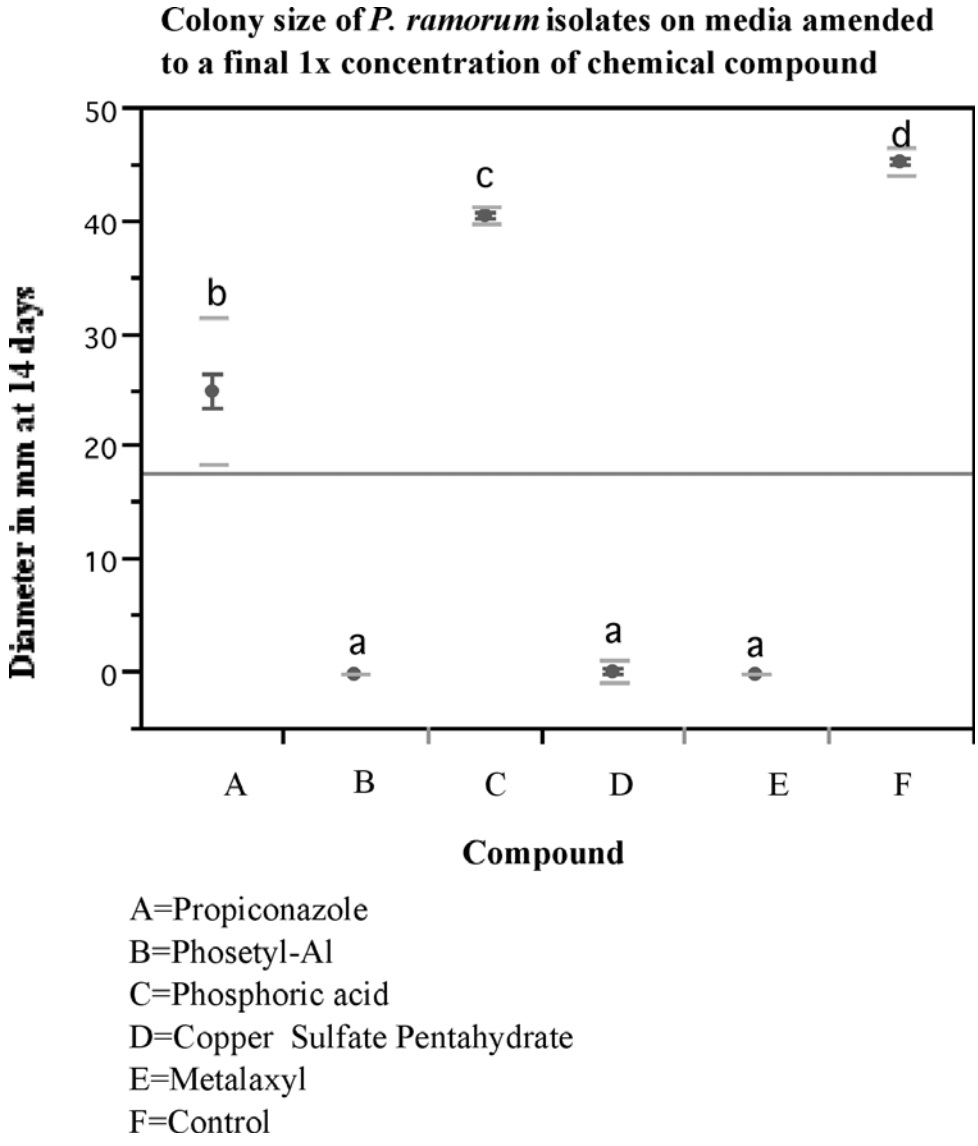

Figure 2—ANOVA results of mean colony diameter of *P. ramorum* grown on media amended with chemicals up to a 1X concentration (see *table 1* and text). Letters identify treatment grouping as indicated by a Tukey-Kramer multiple range test with alpha set at 0.05. (*P=0.0001)*.

It should be noted that some compounds, for instance phosetyl-Al, phosphites, and phosphonates are known to be a lot more effective "in planta," where the active ingredient of these chemicals, phosphorous acid, is released. For these and similar compounds, results from "in vitro" testing have limited value. It should also be noted that the mechanisms of action of each compound are different and need to be taken into account when selecting a treatment. For instance, while metalaxyl was undoubtedly the most powerful compound against *P. ramorum*, it is also known that other *Phytophthora* species can develop resistance to metalaxyl through sexual recombination. The potential for the selection of metalaxyl-resistant strains suggests a minimal use of this chemical until more is known about the biology of the pathogen.

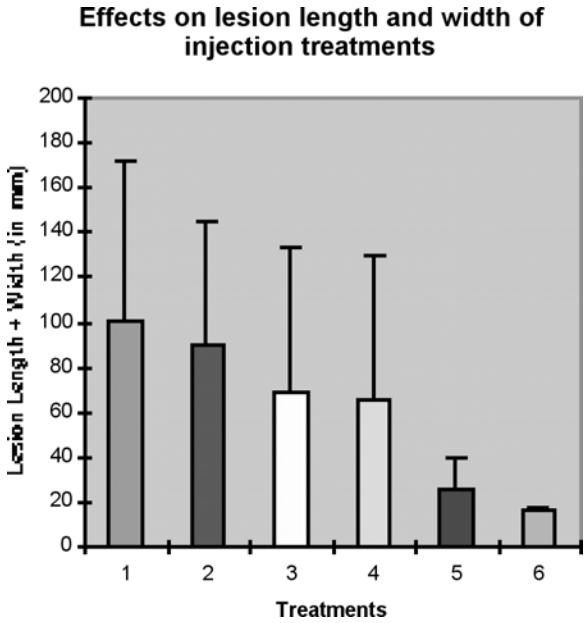

Figure 3—Lesion length+width of inoculated *P. ramorum* on *Q. agrifolia* saplings after chemical treatments. Pathogen inoculations occurred in the fall of 2000, and treatments were performed 72 hours after inoculation. 1=untreated, 2=copper sulfate (picrocubic), 3=metalaxyl, 4=phosetyl-Al, 5=phosphorous acid (phosphite), 6=mock inoculated. Both Tukey-Kramer and pairwise comparisons indicated that at alpha set at 0.05 no statistical significance was found between untreated saplings and the copper sulfate treatment. All other treatment were statistically different from the water treatment. The most effective chemical was the phosphite. Mean lesion size in saplings treated with this compound were not significantly different from lesions created by wounding only without pathogen inoculation.

Results from our "in vitro" tests provide us with several potentially valid options for chemical treatments. Copper compounds may be used for preventive coating of the trunks, phosphorous acid and metalaxyl may be used for curative treatments. Further testing in planta is required before the actual efficacy of each treatment can be evaluated.

Potted Saplings Experiment

At least three chemical compounds were effective in reducing pathogen growth rate. Saplings injected with phosetyl-Al, metalaxyl, and phosphorous acid had significantly smaller cankers than saplings untreated or injected with copper sulfate pentahydrate. Statistical significance was at the 5 percent level for cankers treated 72 hours after inoculation (DF=79, Fratio=3.97, P=0.003) *(fig. 3)*. For 10-week old cankers, identical trends were observed but statistical significance was obtained only at the 24 percent level, once the mock inoculated saplings were excluded from the analysis *(fig. 4)*. The reason for lack of strong statistical significance was clearly linked to the fact that growth rate of the pathogen inoculated in the summer slowed down significantly after a few weeks, and may have come to a complete halt. The slow-down or halting of fungal growth may not have allowed for a stronger

differentiation among treatments. This interpretation is supported by the observation that in control saplings (e.g. saplings that were inoculated, but went untreated), 5-month old lesion were significantly (P=0.08) smaller than 2.5-month lesion (t-test=1.8, DF=28). Success of pathogen isolations from older cankers was also significantly lower (data not shown) than isolations from younger cankers. These results may indicate that either older inoculations were negatively affected by a hot spell in the early fall (temperature reached 40° C), or that saplings only allow for a maximum size of canker development, reached in this case in about 2.5 months for both inoculation trials.

Further studies need to verify whether canker size is positively correlated with disease development. Only when that correlation will be verified, the full beneficial potential of these chemical treatments may be understood. Further studies are also needed to determine potential negative collateral effects of these treatments, as well as optimal rates of active ingredients, ideal time and methods of application.

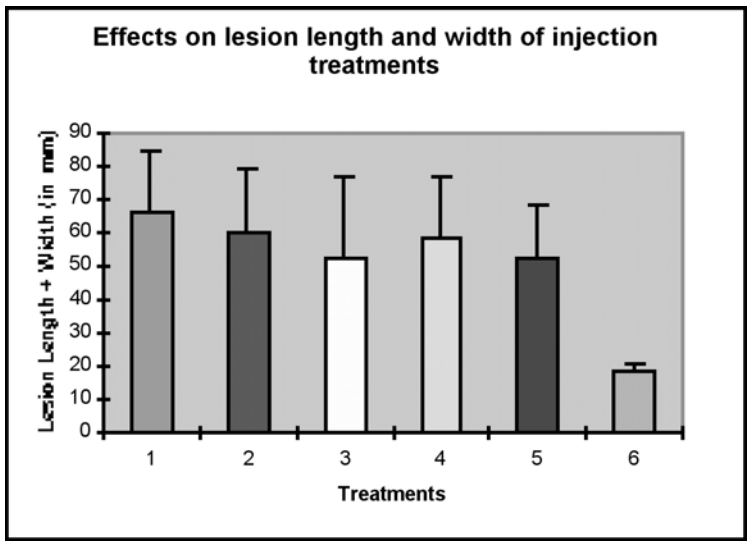

Figure 4—Lesion length+width of inoculated *P. ramorum* on *Q. agrifolia* saplings after chemical treatments. Pathogen inoculations occurred in the summer of 2000, and treatments were performed 11 weeks after inoculation. 1=untreated, 2=copper sulfate pentahydrate, 3=metalaxyl, 4=phosetyl-Al, 5=phosphorous acid (phosphite), 6=mock inoculated.

References

Erwin, D.C.; Ribeiro, O.K. 1996. ***Phytophthora* diseases worldwide.** St. Paul, Minnesota: APS Press, American Phytopathological Society.

Fernandez-Escobar, F.J.B.R.; Gallego, M.; Benlloch, M. 1999. **Treatment of oak decline using pressurized injection capsules of antifungal materials.** European Journal of Forest Pathology 29: 29-38.

Garbelotto, M.; Svihra, P.; Rizzo, D.M. 2001. **Sudden oak death syndrome fells three oak species.** California Agriculture 55(1): 9 - 19.

Guest, D.I.; Pegg, K.C.; Whiley, A.W. 1995. **Control of *Phytophthora* diseases of tree crops using trunk-injected phophonates.** Horticultural Review 17: 299-330.

Hardy, G. 1999. **Phosphite and its potential to control *P. cinnamomi* in natural plant communities and adjacent rehabilitated minesites in Western Australia.** In: Hansen E.; Sutton W., eds. *Phytophthora* diseases of forest trees, Corvallis, Oregon: Forest Research Laboratory, Oregon State University; 82-86.

Rizzo, D.M.; Garbelotto, M.; Davidson, J.M.; Slaughter, G.W.; Koike, S.T. 2002. ***Phytophthora ramorum* as the cause of extensive mortality of *Quercus* spp. and *Lithocarpus densiflorus* in California**. Plant Disease 86: 205-214.

Werres, S.; Marwitz, R.; Man In'T Veld, W.A.; De Cock, A.W.A.M.; Bonants, P.J.M.; De Weerdt, M.; Themann, K.; Ilieva, E.; Baayen, R. P. ***Phytophthora ramorum* sp. nov., a new pathogen on *Rhododendron* and *Viburnum*.** Mycological Research 105: 1155-1165.