A report on a comprehensive series of experiments, both in *vitro* and in *planta*, to develop treatments for *Phytophthora ramorum*, the cause of Sudden Oak Death.

Prepared by Matteo Garbelotto[1,]

[1]Department of Environmental Science, Policy, and Management. Ecosystem Sciences Division, 151 Hilgard Hall, University of Caliornia, Berkeley, CA 94720
email: matteo@nature.berkeley.edu ; Voice: 510 410 7058

Introduction

Sudden Oak Death is probably the most significant emergent epidemic of forest trees reported in the U.S. since Dutch Elm Disease. Although it was first observed around the San Francisco Bay Area in 1994, the causal agent was discovered only in 2000. The pathogen, at the time of the discovery, was a still unknown and undescribed oomycete belonging to the genus *Phytophthora*. It has now been called *Phytopthora ramorum*. *P. ramorum* affects hosts in two major different ways: on red oaks and tanoaks it girdles the cambium of main stems, while on a wide range of other hosts it simply causes leaf spots , leaf blight and occasionally twig and branch dieback. Although these hosts play a very important epidemiological role, their survival is rarely put at risk by infection *by P. ramorum*. This report is a comprehensive analysis of potential for treatment of *P. ramorum* on hosts that are lethally affected by it; namely oaks and tanoaks. The report is based on over thirty independent trials -some *in vitro*, but most *in planta*, focusing on potential chemistries for control, and on optimal application methods. Products already registered for the control of other *Phytophthora* species were tested following label prescriptions, or trying novel application methods. Innovative products and approaches were experimented in depth . Ad our knowledge on this new pathosystem expanded, experiments were designed to fully address the potential for control of any successful treatment approach. In particular knowledge on the genetic diversity of *P. ramorum* in California was specifically addressed, as well as the confounding effect of potential variability in susceptibility among hosts. Chemical treatments in fact, have to be focused on the majority of the affected population: resistant individuals may survive even without such treatments, and extremely susceptible individuals may succumb, even when treated. While no information is available for tanoaks, we have recently reported the finding of significant variability in susceptibility to *P. ramorum* among coast live oaks, and factored this knowledge in our testing. The most successful treatment(s) was tested on all known 12 genotypes of the pathogen, and the discovery of a wide range of susceptibility, allowed for an understanding of extremely unusual results with a few individuals. The report is organized in three portions:

I-   *In vitro* experiments
II-   *In planta* experiments: potted trees
III-   *In planta* experiments: woodland trees

Materials and Methods

In vitro experiments

Although a detailed description of methods can be obtained by contacting the author, a simple outline of experimental protocols is summarized here. In vitro experiments were undertaken by the common "poison plate" approach. According to this approach molten but cooled agar is mixed with varying concentrations of products or active ingredients (a.i.). Mycelial growth rates and zoopsore germination rates are then scored by either

measuring colony size at fixed intervals or total number of germinating zoospores. At least three isolates of the pathogen and five replicates per isolate were employed for these experiments. Controls were performed by using unamended media. Translation of results from *in vitro* settings to natural conditions is never straightforward. In most cases positive results in the extremely artificial setting of a petri dish (the "poison" plate) are impossible to replicate *in vivo*. Conversely, some treatments do not directly affect the icrobe but need to be mediated by the plant. In these cases, *in planta* experiments will outperform result obtained in vitro. Nevertheless in vitro experiments are extremely useful in determining efficacious rates of a.i.to be used   and constitute a first necessary stem in the discovery of treatments for a new disease such as Sudden Oak Death.

In planta experiments
In planta experiments presented here were all conducted by using the "underbark" inoculation method. .According to this method, a small section of the bark is removed and a plug of inoculum is placed on top of the cambium. The bark is then carefully replaced and the inoculation point sealed with cheesecloth and/or grafting wax. This method is strongly biased in favor of the pathogen for two obvious reasons: 1- the barriers provided by the bark are bypassed by the wounding process and, 2- the amount of inoculum is incredibly high and unrealistic.   The hypothesized process of natural infection in fact involves infection by zoospores. These are much less resilient and short-lived infection structures than mycelial masses including large number of sporangia and chlamydospores. The strong bias in favor of the pathogen imposed by our method, suggests any significant results in favor of the tested treatments, is likely to be even more significant in more "natural" situations.

Potted trees experiments
All potted trees experiments were designed in randomized blocks. Each contiguous block had equal representation of both all treatments tested and of all isolates employed in the tests. At first, three different P. ramorum isolates were used. These isolates were confirmed to be different genotypes by DNA AFLP fingerprinting analysis. Because, it was later determined that a single clonally reproducing genotypes is responsible for over 85% of the infections in California, further experiments were done using only such isolate. In most experiments a treatments consisted of 15 replicates. Positive controls consisted of inoculations without any treatment, and negative controls consisted in wounding saplings and inserting a plug of sterile agar, rather than a plug of mycelial inoculum. Trees are between2 and 6 cm in caliper and between 3 and 5 meter tall. All potted trees experiments were conducted on coast live oak. One inoculation per tree was used in most experiments, but in some experiments multiple inoculations with the same isolate were performed at different times on the same tree, to study durability of treatments. The three inoculations were never directly on top of one another, but rather spiraled around the stem. Inoculations per tree were also randomized, to ensure position on the stem (low, medium, high) would not affect the results. Three experimental plots are permanently set up to accommodate large number of potted trees: two in Marin County and one in Alameda county

Size of cankers resulting from the inoculations was the main variable measured. Rather than area, maximum extension of cankers in four orthogonal directions was measured; this measurement was defined as NSEW. Maximum linear spread is probably the most significant measurement, as it can be hypothesized that any lesion, even thin, can effectively girdle a tree. Effectiveness of the treatments was evaluated by comparing size of cankers in treated and untreated trees. Canker size is generally considered an excellent proxy of pathogen success or aggressiveness. Most trees in fact appear to die as the result of girdling cankers: any treatment capable of slowing down canker growth is likely to prolong the life of the infected tree. In other similar pathosystems: e.g. the *Phytophthora cinnamomi-Quercus agrifolia* one, infected trees can survive for decades, due to the slow-spreading nature, and the elongated shape of the cankers. In the case of SOD, it is unclear how much resistance may be available, and most cankers appear to develop around the tree circumference in a relatively rapid fashion. Treatments may effectively slow the process of canker formation, or at times even prevent it, as it has been shown fort *P. cinnamomi* on orchard trees worldwide and even in wildland situations. Viability of the pathogen was assessed by isolating it on selective PARP media from the four cardinal directions of each canker.

Treatments

Treatments were of two major types: curative and preventive. The first were performed by treating the trees after they were inoculated, the latter by first treating the tree and then inoculating it. Products were applied differently, based on label specifications of products already available for other similar diseases, or on experimental testing of new products. The application methods tested in these trials were the following:

> a)- Injections in the outer sapwood using at least two injection methods, both using positive pressure during the injection to ensure uptake of the product
> b)–Soil Drenches
> c)- Foliar sprays of products with or without spreader-stickers
> d)- Topical applications on cankers and bark of products
> b)- Basal bark applications of products with a carrier.

Injection volume was standardized to 10 ml per injection, containing varying amounts of active ingredients. Number of injections was either decided based on canopy area, diameter of the drip line, or trunk circumference for more columnar trees. In general one injection every 25-35 cm of the tree trunk circumference was the norm.

Soil drenches were easily performed by watering the tree pots (15 gallon-pots) with the appropriate dilution of each desired product. Foliar sprays were applied with a professional sprayer, and an average of 500 ml per tree was applied to the canopy. In order to avoid dripping of the foliar sprays on the soil, potted trees were laid down on the side and the product was applied on the entire crown. Trees were left to dry before being lifted upright and placed back in the experimental parcel.

Topical applications were performed using brushed , and bark application by spraying the product on the bark of the main stem, until the first branch scaffold. On mature trees bark applications were done up to a maximum of 3 m from root collar.

Phytotoxicity of the treatments was determined by assessing changes in vigor of the tree crown. Color of the foliage, amount of twig and branch dieback, and amount of burnt or scorched foliage was recorded at the beginning and at the end of the experiment. Most experiments on potted trees lasted about 4 months.

Trials in woodlands
Experiments in woodlands (both coast live oak and tanoak) were similar to the ones described above for potted trees. Only phosphites were used in these trials. Both injections and foliar sprays were tried on tanoak saplings, while only injections were tested on mature tanoak, and both injections and basal bark application of pshosphite+carrier were performed on mature coast live oaks. Only preventive trials were fully completed. Tanoak trials were performed in Santa Cruz County, coast live oak trials were perfomed in Marin County

Phosphites need to be translocated by the plant and broken down into phosphorous acid, in order to be effective. Our trials aimed at determining not only overall efficacy of the treatments, but also the time necessary for this process to occur in adult trees. In potted trees, a few days are enough for translocation and break-down of the phsophites, but a much longer time was expected to be necessary for larger trees.

In the case of woodland tree trial, we have been operating under constraint on number of available trees for experimentations. Rather than a completely randomized design, a paired-sample design was followed. Pairs (one treatment and control) or triplets (two treatments and control) of trees were carefully selected. These trees were generally growing next to each other, and had similar leaf morphology and architecture.

Statistical analyses
All statistical analyses were performed using the program JMP (SAS), the data has not been transformed, but is directly analyzed. In some cases corrections for unequal variances have not been performed yet. T-tests, ANOVA, and paired t-tests were employed. When necessary, non-parametric statistics were used, and in the case of multiple comparisons, treatments were individually compared to the controls (e.g. untreated trees or uninoculated trees) using Dunnett's test. Because of the large number of treatments in some of the experiments, when an obvious reduction in canker size was observed with a particular treatment, student's t tests were performed between the control and the treatment, blocking out other unrelated treatments that were tested in the same experiments. This is an acceptable practice when the hypothesis asked is whether a treatment has a significant effect in contrast to untreated trees. This practice is unacceptable if a comparison among treatments is sought.

The experiments

The following is a list of the experimental trials on treatments for *P. ramorum*. For each trial I include:

> 1-Subjects (potted trees, in vitro, adult trees)
> 2-Location and staring and ending date
> 3-Treatments tested (including treatment size, rate, and mode of application)
> 4- Data on durability of treatments if available
> 5-Treatments that were effective and their level of significance (*P*), treatments that were not effective
> 6- File name or appendix with statistics and graphics if available

In vitro experiments

This complex series of experiments started in 2000 and is still ongoing. Effect by contact of a number of fungicides and biopesticides was tested by the "poison plate" method. For a list of used products and results refer to the two files below:

> a)- **Effect of chemical and biological control agents on *Phytophthoraramorum* growth in *in vitro* trials**
> *TamarY. Harnik, Monica Meija-Chang, Matteo Garbelotto*
>
> b)- *Phytophthora ramorum* **and Sudden Oak Death in California: IV. Preliminary Studies on Chemical Control**
> *Matteo Garbelotto, David M. Rizzo, and Lawrence Marais.*
> *Forest Service general Technical Report*

Results show that most compounds known to be effective against other Phytophthora species ,including metalaxyl, are also effective against P. ramorum. The sensitivity of zoospores to most tested compounds was higher than that of the mycelia.

Potted tree experiments

Experiment one (preliminary)

To determine whether injections of chemicals may slow down P. ramorum canker formation.

- *Quercus agrifolia* in 15 gallon pots
- Petaluma (Sonoma County), October 31st 2000- March 3rd-2001
- Treatments:

- o Phosphite injection (1/tree; 10 ml; 8% a.i.;Nutriphite 0-28-26)
- o Al-fosetyl injection (1/tree; 10 ml; 8% a.i.;Aliette injectable)
- o Metalaxyl injection (1/tree: 10 ml, 11% a.i., Subdue, NOT in label)
- o Copper sulfate injection (picrocubic copper) (1/tree, 6% a.i., Phyton 27)

Treatments were administered 3 and 21 days after inoculation. Phosphite injection was effective ($P<0.05$) in the first treatment. No treatment was effective after 21 days. This preliminary experiment is already published (Garbelotto, Rizzo, and Marais, 2002). Why were the treatments not effective after 21 days? A possible explanation may be due to an artifact of the experimental set-up. It is possible that cankers in potted trees with limited stem size may grow rapidly and then slow down their growth   Obviously, treatments after canker size has already peaked will appear to be ineffective. In a later experiment we have demonstrated (data not shown but available upon request) that most canker growth, although variable in each experiment, mostly occurs in the few days after inoculation. Efficacy of treatments should thus be tested in that initial period of pathogen growth.

Experiment two

To determine whether injections, foliar applications, and soil drenches may effectively slow down the growth of *P. ramorum*.

- *Quercus agrifolia* in 15 gallon pots
- Petaluma (Sonoma County), May –August 2001
    - o Phosphite injection (1/tree; 10 ml; 8% a.i.;Nutriphite)
    - o Al-fosetyl injection (1/tree; 10 ml; 9.5% a.i.;Aliette injectable)
    - o Metalaxyl injection (1/tree: 10 ml, 0.07% a.i., SubdueMaxx, NOT in label)
    - o Al-fosetyl foliar (500 ml/tree, 0.65% a.i. Aliette wettable powder)
    - o Ali-fosetyl drench (2l/tree, 0.65% a.i. Aliette wettable powder)
    - o Phosphite drench (2l/tree, 0.65% a.i. Nutriphite)
    - o Phosphite foliar (500ml/tree, 0.65% a.i. Nutriphite)
    - o Metalaxyl drench (2l/tree, 0.007%, Subdue maxx)

Treatments were administered a week after inoculation. Phosphite injection was effective ($P<0.05$), when comparing length of cankers on inoculated trees. Significant increase in scorched leaves was observed with the phosphite (Nutriphite) foliar treatments. Results are summarized in Appendix 1.

Experiment three

To determine whether injections, soil drenches, and topical bark applications may effectively slow down the growth of *P. ramorum*.

- *Quercus agrifolia* in 15 gallon pots
- Fairfax (Marin County), 11,14.01 –2, 29,02

- o Phosphite injection (1/tree; 10 ml; 8% a.i.;Nutriphite 0-28-26)
- o Al-fosetyl topical (full strength application as paste on bark, NOT in label)
- o Metalaxyl injection (1/tree: 10 ml, 11%, SubdueMaxx, NOT in label)
- o Several application of Greenbox
- o Metalaxyl drench (2l/tree, 0.007%, Subdue maxx)

Treatments were administered a week after inoculation. Phosphite injection was effective ($P<0.05$). The second best treatment was injection of metalaxyl. Metalxyl drenches were ineffective. See Appendix 2.

Experiment four

To confirm phosphite injections as an effective therapeutic treatment

- - *Quercus agrifolia* in 15 gallon pots
- - U.C. Berkeley (Alameda County), Dec 2001 –April 2002
   - o Phosphite injection (1/tree; 10 ml; 8% a.i.;Nutriphite)
   - o Phosphite injection (1/tree: 10 ml, 8% a.i. SuperSODaway was manufactured in the laboratory using equal volumes of high grade phosphoric acid and potassium hydroxide, until pH is neutral

Treatments were administered a week after trees were inoculated with the pathogen. Both treatments were effective ($P=0.05$). See Appendix 3.
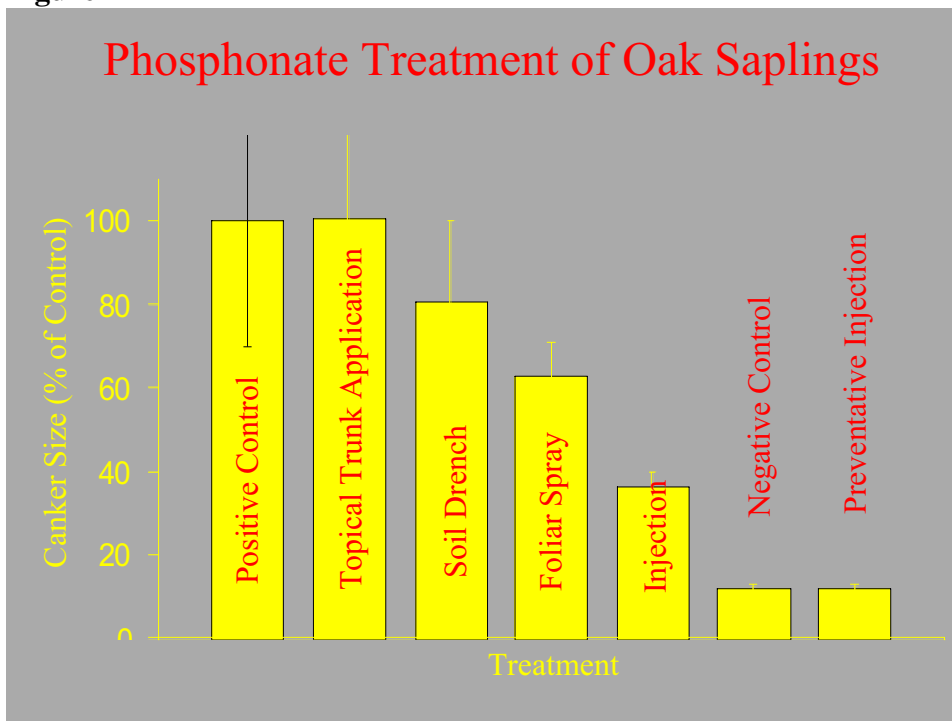
Experiment five

Phosphite therapeutic (curative) treatments were compared to one another. In a parallel test, preventative phosphite injections were tested

- - *Quercus agrifolia* in 15 gallon pots
- - Lucas Valley (Marin County), Nov2001 –March 2002
   - o Curative Phosphite injection (1/tree; 10 ml; 8% a.i.;Nutriphite 0-28-26)
   - o Curative Phosphite injection (1/tree; 10 ml; 9.5% a.i.;Aliette injectable)
   - o Curative Phosphite injection (1/tree; 10 ml; 6% a.i.;Phytoguard)
   - o Curative Phosphite injection (1/tree; 10 ml 6% a.i.;Phostrol)
   - o Curative Phosphite foliar application (500 ml/tree; 0.5% a.i.;Nutriphite 0-28-26)
   - o Curative Phosphite drench (2L/tree; 10 ml; 0.5% a.i.;Nutriphite 0-28-26)
   - o Curative Phosphite topical bark application (100ml/tree; 13% a.i.;Nutriphite 0-28-26)
   - o Preventative Phosphite injection (1/tree; 10 ml; 8% a.i.;Nutriphite 0-28-26)

Treatments were administered a week after inoculation. Phosphite injection treatments effectively reduced canker size *($P<0.05$)*. Phosphite drenches, foliar, and topical bark

applications were not effective curative treatments, although a positive response was noted in the foliar applications. The best treatment was the preventative phosphite injection (injected one week before inoculation). Size of cankers in preventatively injected trees was identical to canker sizes in negative control. See Figure 1 and Appendix 4.

Figure 1

Phosphonate Treatment of Oak Saplings

Experiment six

A second inoculation was performed on most treatments from Experiment five. In most cases debarking to look at cankers had been limited due to small size of the lesions. New inoculation was performed not above the previous one , but at a 90 ^{0} angle from it. Trees were re-inoculated but not treated a second time. Original chemical treatment occurred in December 2001, trees re-inoculated in April 2002, evaluation in July 2002

-   *Quercus agrifolia* in 15 gallon pots

- Lucas Valley (Marin County), Apr –July 2002
    o Curative Phosphite injection (1/tree; 10 ml; 8% a.i.;Nutriphite)
    o Curative Phosphite injection (1/tree; 10 ml; 9.5% a.i.;Aliette injectable)
    o Curative Phosphite injection (1/tree; 10 ml; 6% a.i.;Phytoguard)
    o Curative Phosphite injection (1/tree; 10 ml; 6% a.i.;Phostrol)
    o Curative Phosphite foliar application (500 ml/tree; 0.5% a.i.;Nutriphite)
    o Preventative Phosphite injection (1/tree; 10 ml; 8% a.i.;Nutriphite)

After 8 months, and in spite of the previous inoculation, all phosphite treatments were still significantly curtailing canker size. There were differences in efficacy among therapeutic phosphite treatments. The preventative phosphite injection was also still effective. Foliar applications were ineffective, despite the positive trend (without significance at the 0.05 alpha level) shown in Experiment 5. See Appendix five
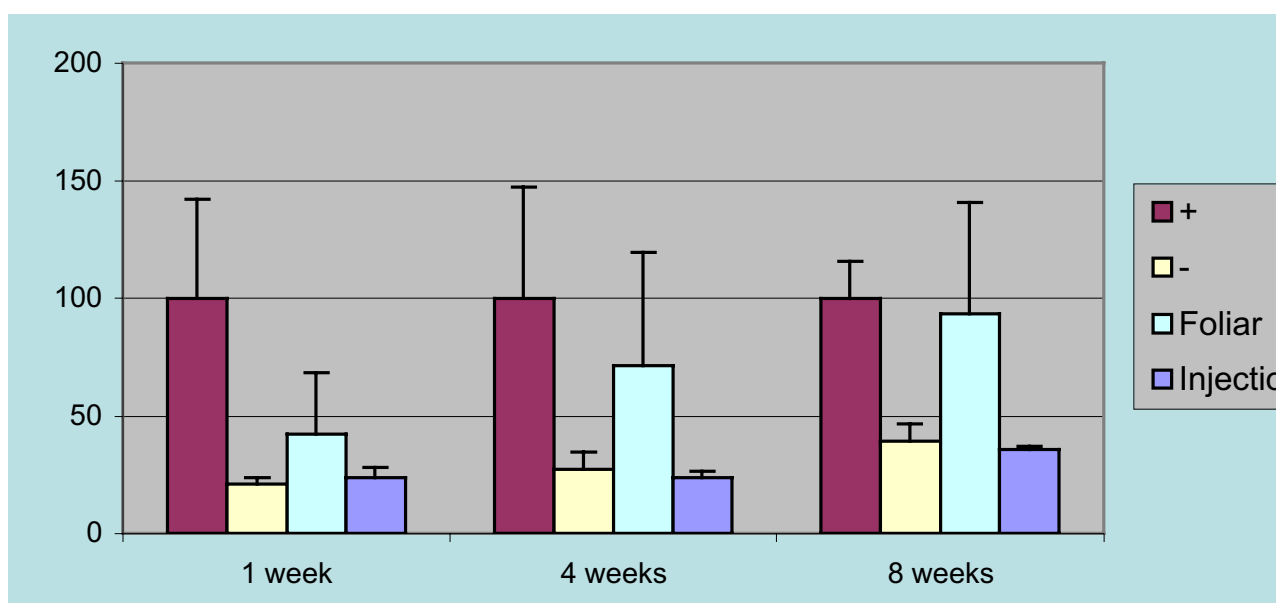
Experiment seven.

Efficacy and durability of phosphite preventative treatments were studied. Each tree was inoculated at three different times.

- *Quercus agrifolia* in 15 gallon pots
- U.C. Berkeley (Alameda County), Apr –July 2002
    o Preventative Phosphite injection (1/tree; 10 ml; 8% a.i.;Nutriphite 0-28-26)
    o Preventative Phosphite foliar application (500 ml/tree; 0.5% a.i.;Nutriphite 0-28-26)

Preventative injections were effective and durable, preventative foliar applications were moderately effective, but not durable. See Appendix 6 and figure 2.

Figure 2. Effectiveness of phosphite injections and foliar treatments at one, four, and eight weeks from treatments. Size of untreated cankers represents 100%. For actual canker sizes, see Appendix 6.

Preventative injections were as effective at eight weeks as they were at one week. In all three cases, lesion size of negative controls was undistinguishable from lesion size on preventatively injected trees. Foliar treatments had only a marginal effect that was rapidly lost after one week.

Experiment eight.

A variety of treatments using different chemistry and application methods wee used as a preventive rather than a curative tool. Some treatments that had not succeeded post inoculation were reassessed in this experiment.

Treatments: 7/16/02
Inoculated: 1 Wk: 7/24/02
                3 Wk: 8/7/02
                6 Wk: 8/28/02

- *Quercus agrifolia* in 15 gallon pots
- U.C. Berkeley (Alameda County), 7/16/02-10/22/02
-
    Injection: Phostrol, Nutriphite, SuperSODaway, Emerson NC04GE, Phyton
    27, Agrifos 400, Vital, LP-AM400 (Agrifos), Aliette, Greenbox, Subdue
    (rates and modes as described above)

    Foliar: Phostrol, Phyton 27 (as described above)

    Bark appl.: Agrifos 75T (75% Agrifos, 45.8 % a.i., 25% Organosilicate
              carrier) applied in a 1:1 dilution on bark (200 ml per tree)
              Agrifos 25T (25% Agrifos, 45.8%a.i., 75% Organosilicate carrier),
              applied ina 1;1 dilution on bark (approx. 200 ml per tree).

Results indicated phosphite injections were extremely effective and durable (effective even at 6 weeks), all other tested products were not effective. A foliar application of phosphites was also effective, but resulted in some phytotoxicity (data not shown). Bark applications of phosphite+organosilicate carrier were as effective as injections. In Experiment five, we had show n that bark application of simple phosphites were not effective. The presence of the coupled organosilicate allows for the phosphite to be carried through the bark . This application is quite revolutionary, as it allows to avoid the injection process. See Figure 4 and Appendix 7 for detailed data information.
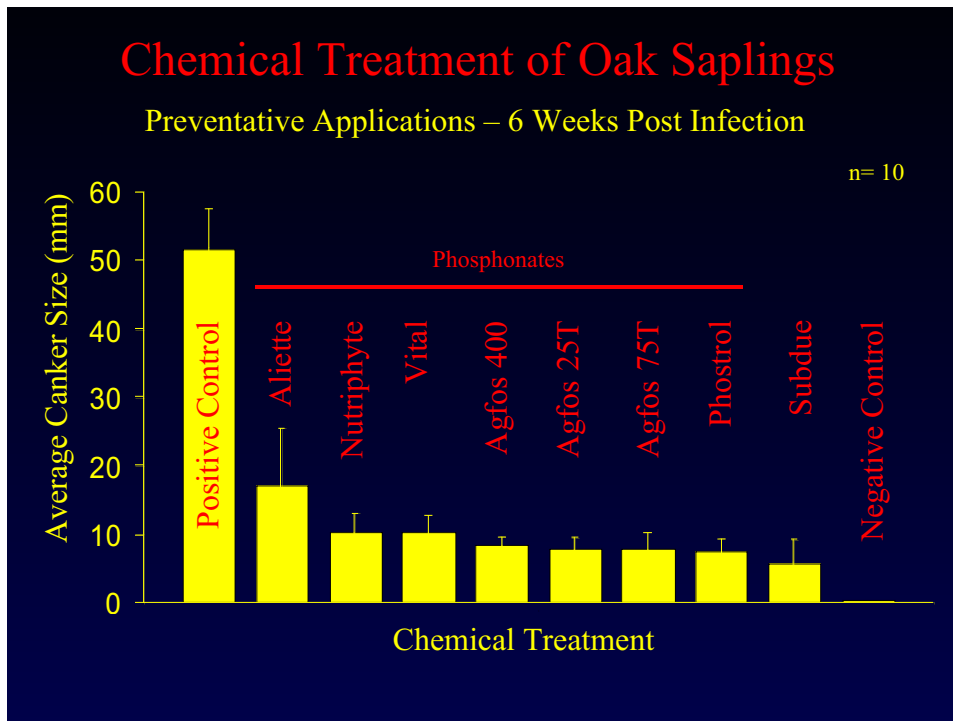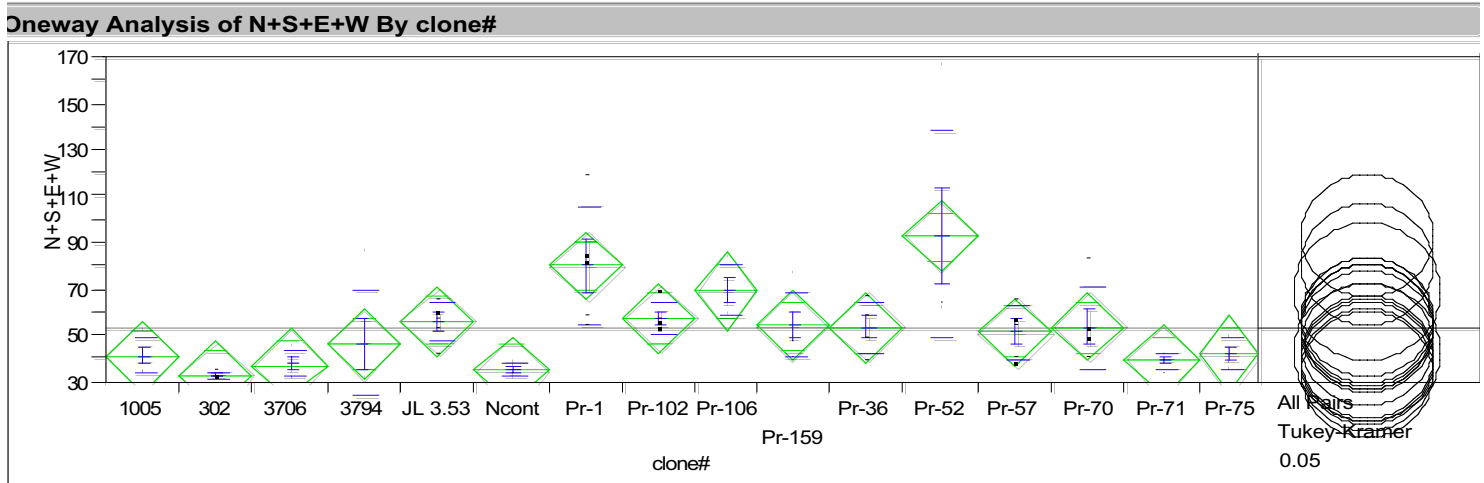
Figure 3

Experiment nine

Preventive phosphite injection was tested on all known genotypes of *P. ramorum* from North America available to us. Each *P. ramorum* isolate was inoculated on ten trees. Five of these were then treated and five were left untreated.

- *Quercus agrifolia* in 15 gallon pots
- U.C. Berkeley (Alameda County), Oct 2002-March 2003
    o Preventative Phosphite injection (1/tree; 10 ml; 8% a.i.;Agrifos)

Figure 4 exemplifies the variability in growth of different P. ramorum clones on *Q. agrifolia.* . Phosphite preventive injections were overall successful in reducing pathogen growth ($P<0.0001$), when all isolates were taken into account. For more details see Appendix 8.

Figure 4. Comparative growth rate of *P. ramorum* isolates on potted *Q. agrifolia* trees.

Y axis= the sum of the maximum linear growth (mm) longitudinally and transversally on the tree stem.

Experiments in woodlands

Experiment ten

Tanoak (*Lithocarpus densiflora*) saplings were preventively treated both with phosphite injections and phosphite foliar applications. Saplings were each inoculated three times at one, three, and six week after treatment. Inoculations were spiraled around stem, and randomly assigned to each inoculation time
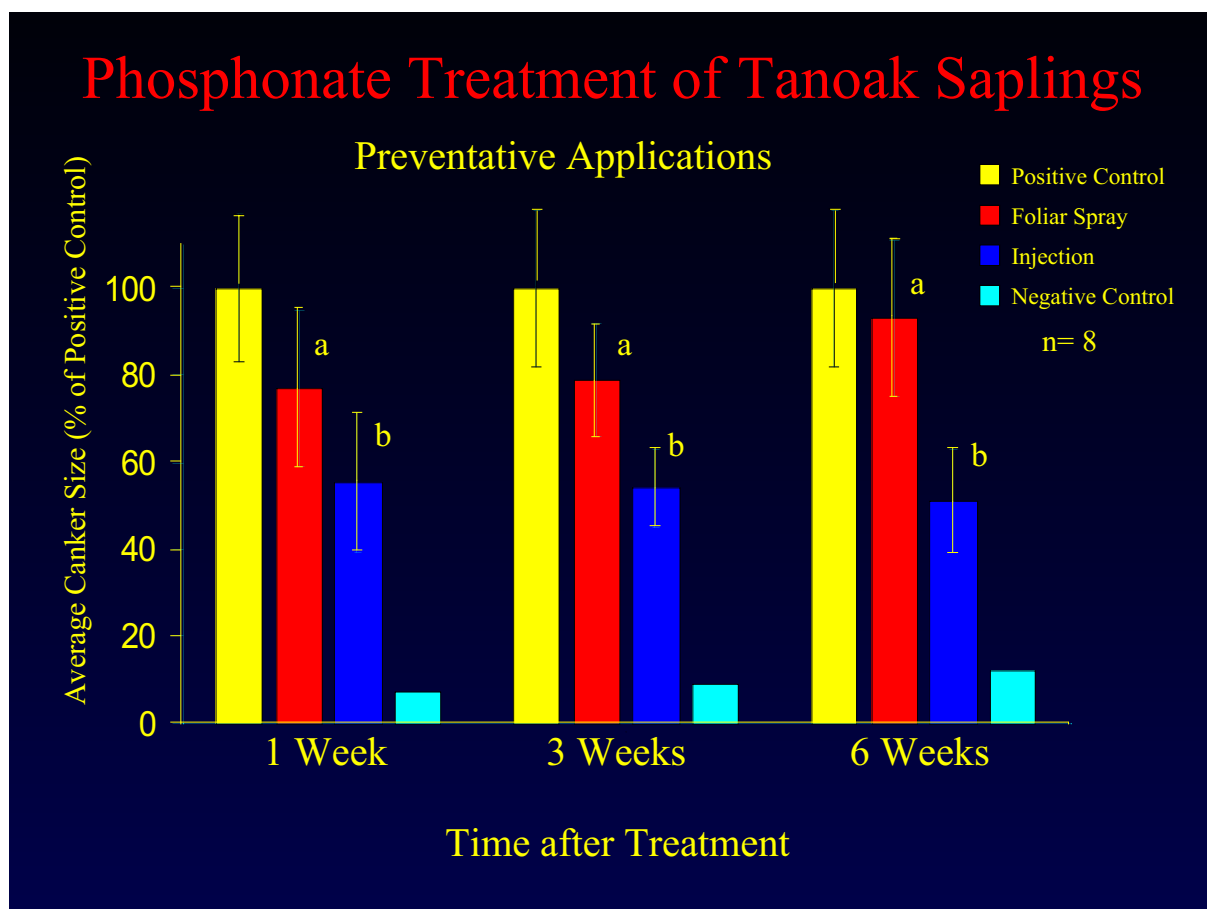
> Santa Cruz County, 5/17/02-7/23/02
> Tanoak saplings (2-4 cm, caliper, 3-5 m tall)
> Preventive phosphite injections (1 per sapling, 8% a.i., Nutriphite)
> Preventive foliar sprays (500 ml per tree, 0.6% a.i., Nutriphite)

Canker size was significantly smaller in phosphite injected tanoaks (alpha level=0.05). Effect was durable and after 6 weeks, treatments were still effective. Foliar sprays had a moderate effect, that was rapidly lost. Figure 5 summarizes the results. Detailed analyses in Appendix 9.

Figure 5. Canker size in phosphite-treated and untreated tanoak saplings. Inoculations were performed one, three, and six weeks after treatment.

Experiment eleven

To test the efficacy of preventive phosphite injections on adult tanoaks.

Santa Cruz County, 5.10.02-7.17.02
1 injection for every 15-20 cm of circumference, 10 ml, 8% a. i.,
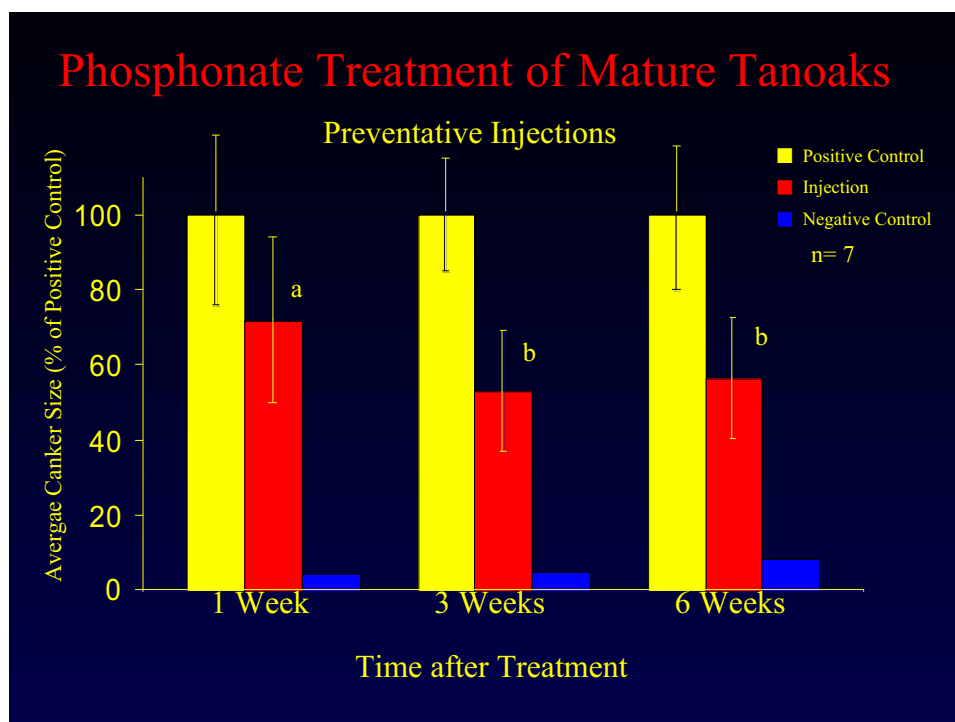Nutriphite
Inoculated one, three, and six weeks after injecting
Although supposed to be a paired test, trees were not picked correctly
and was treated as a completely randomized test
Mean tree diameter was 162 mm (range 127-185)

ANOVA on all inoculations indicates that treatment was significant in reducing canker size (P=0.038). Because of lack of enough replication, highest significance for any inoculation time is P=0.07 for the three week inoculation.   Trends in Figure 5 suggest that in adult trees at least 3 weeks are required for translocation and metabolization of the administered phosphite. Data in Appendix 10.

Figure 6

Experiment twelve

A replication of Experiment eleven, with more careful paired-tree design. Improved injection technique. Based on results from the previous experiment, trees were inoculated after 4 weeks, to allow for translocation and metabolization of the phopshite

Adult tanoaks
Santa Cruz County,
 Injected December 2002, inoculated in January 03, ended in April 03
Phosphite injection, Agrifos 400, Sidewinder injection system
1 injection for every 15-20 cm of circumference, 10 ml, 8% a. i.)

Preventative injections effectively reduced canker size on inoculated trees (P=.0.035) No phytotoxicity was observed. Data summary and analyses in Appendix eleven.

Experiment thirteen

Top test the efficacy of preventative phosphite injections on Coast live oak (*Qercus agrifolia*) adult trees.

> *Q. agrifolia* adult trees, mean diameter: 214 mm (range 137-283)
> Marin CO.
> Treated: Nutriphite injection 5/14/02
>   Inoculated: O-463
>   1 Wk: 5/21/02
>   3 Wk: 6/4/02
>   6 Wk: 6/25/02
> Exp ended: 7/31/02
> Sidewinder injection system, 1 injection every 15-20 cm of trunk circumference
> Paired test design, three areas in same general location.

Small scale experiments on oaks like this one are always rather difficult to run. The amount of variability in susceptibility in oaks makes it easy to run into outliers. The problem was compounded by the fact that at the time the experiment was run, we had not considered that a necessary lag period may be necessary for the plant to build up resistance. Our one-week inoculation was without any doubt too premature. By the sixth week, injections had finally become efficacious (*P*=0.01) in slowing down pathogen growth.

Experiment fourteen

Phosphite injections and bark applications of phosphite+organosilicate carrier were tested in the field on adult coast live oaks

> *Q. agrifolia*, adult trees,
> Treated in Jnauary 2003, inoculated 6 weeks later, experiment ended 4.4.03
> Marin County
>   Phosphite injection, Sidewinder system, Agrifos 400, 8% a.i.
>   Phosphite bark application, 5% organosilicate carrier, 13% a.i.
> Paired tree experiment

Both treatments were effective in reducing canker size on adult coast live oaks (*P*<0.05). Results in Experiment fourteen were clearer than in Experiment thirteen because of the better tree selection and longer waiting time between injection and inoculation. Bark application was the most successful treatment.

Figure 6. Effect of preventative phosphite treatments (AF400=injections, 90T=bark application) on canker size (NSEW) caused by *P. ramorum*

CONCLUSIONS

The evidence presented in the numerous experiments here presented, clearly indicates the potential for effective treatment of cankers caused by *P. ramorum*. Both oaks and tanoaks responded well to the treatments. Phosphites or phosphonates were the most effective compounds, when injected or applied to the bark with the addition of an organosilicate carrier. Among them, Aliette was in general the least effective one. Injectable Aliette is also currently unavailable on the market.   Phosphites appeared to be comparable in efficacy when applied in a preventive trial (Experiment eight). Only significant differences were due to application method: efficacy of these compounds appears to be broad, independent of actual commercial product used, at least for the products tested at U.C. Berkeley between 2000 and 2003.

All drenches, including those with Subdue, were not effective. Products already registered for the control of other *Phytopthora* species were not effective. Topical

application of products known to be effective either systemically such as phosphites, or by contact such as copper derivatives were not effective. The addition of an organosilicate carrier turns bark applications into effective treatments. No registered product for Phytophthora species tested by us was consistently effective. There is currently no effective treatment for Sudden Oak Death.

Phosphites can either be injected, applied to the bark if mixed with the carrier tested in our experiments, or sprayed on the leaves. Foliar sprays are the least recommended option: not very effective, variability in efficacy, and the only treatment causing significant phytotoxicity symptoms. They are also environmentally unacceptable because of the potential air drift. Some experiments have shown that the effect of foliar sprays is ephemeral or short-lived.

The most effective treatments are the preventative ones. Therapeutic ones are also effective, but they should be applied as soon as the first symptoms show. All trials suggest these treatments are durable.

Because of the scarce side effects of phosphites, they represent a viable option for treatments of SOD in California. Experiments nine showed these compounds to be effective across the spectrum of genetic variability of the pathogen. This feature is extremely important, as fungicides can exert a strong selection pressure in favor of resistant genotypes, quickly turning and effective treatment into an ineffective one. When injections and bark applications were performed, no phytotoxicity was observed. The rates used by us can certainly be adopted, but further experiments are needed to fine-tune the range of optimal application rates, and formulations to be used.

Beneficial and significant effects of phosphites were proven by experiments replicated both in space and time. Preventative treatments performed best, but therapeutic treatments were also effective. The main question regarding curative treatments is to understand at what point tree treating may become futile. Experiments are under way to answer this question. As a good empirical rule, treatments should be administered at the first onsight of confirmed *P. ramorum* symptoms.

Am analysis of percentage of control obtained in therapeutic injection trials using phosphites gave the following results when comparing hours between pathogen inoculation and treatment injection:

Hours	% control
60	95
120	56
156	44
168	30
192	35
264	5

A regression analysis (statistics shown below) shows a very strong correlation between time lapsed and percentage control obtained. These results highlight the importance of early treatment.

%control By hours

— Linear Fit

Linear Fit

%control = 112.439 − 0.4267 hours

Summary of Fit

RSquare	0.942665
RSquare Adj	0.928331
Root Mean Square Error	8.069469
Mean of Response	44.16667
Observations (or Sum Wgts)	6

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	4282.3680	4282.37	65.7649
Error	4	260.4653	65.12	**Prob>F**
C Total	5	4542.8333		0.0013

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	112.43878	9.040334	12.44	0.0002
hours	-0.426701	0.052617	-8.11	0.0013