

**Protection of lone manzanita (*Arctostaphylos myrtifolia*) stands
from *Phytophthora* root rot
E-2-P-30**

Final Report: Use of AgriFos to prevent *Phytophthora* root rot (*Phytophthora cinnamomi*) infection of lone manzanita (*Arctostaphylos myrtifolia*)

Prepared by Matteo Garbelotto, University of California, Berkeley

*ABSTRACT. The water mould *Phytophthora cinnamomi* has become an increasing threat to *Arctostaphylos myrtifolia* (lone manzanita), a rare species already federally protected. We investigated the use of AgriFos (phosphite compound) to prevent infection. AgriFos is already used to prevent *Phytophthora* infection in other species but has not been previously tested with species of manzanita. The major goals of this study were to determine 1) if AgriFos is an effective *P. cinnamomi* preventative for manzanitas, and 2) what the optimal concentration of AgriFos solution is.*

*In the initial stages of the project, work was done on commercial manzanita seedlings to determine efficacy and phytotoxicity of AgriFos treatments on live plants. Because of the lack of commercially available lone manzanita plants, we worked with two alternate species: common manzanita (*Arctostaphylos manzanita*) and white leaf manzanita (*A. viscida*).*

Results indicated a range of potential concentrations of active ingredient that did not cause excessive phytotoxicity and also indicated that white leaf manzanita tests were more reliable and applicable to the lone situation. Three genotypes of the pathogen were employed and one of them emerged as the most aggressive: this genotype was used in all subsequent tests.

Subsequent tests were performed by treating small clusters (each including about four plants) at different concentrations of active ingredient, either including or excluding the use of a surfactant. Besides these two variables (active ingredient concentration and presence of surfactant), we were very interested in testing whether timing of applications had an impact on efficacy and phytotoxicity and whether type of application (foliar and stem vs. stem only) also had an impact. To test these variables, we elected to treat plants in the late spring and in the fall. Efficacy was evaluated at different time intervals since treatment by artificial inoculations of cuttings ex situ at U.C. Berkeley.

Foliar and stem treatments were more effective than stem treatments alone. When spring treatments were compared with fall treatments, a greater efficacy, especially in terms of duration of protection, was observed for the spring treatments. In this final report, we include data from an inoculation experiment performed ex situ (as previously described) thirteen months after treatment that showed significant effects of AgriFos treatments. Although all treatments tested were somewhat effective (meaning that they significantly reduced length of necrotic lesions caused by the pathogen), the concentration of 0.01x active ingredient with surfactant consistently performed well in all tests. Based on the cumulative results, the preferred treatment is a concentration of 0.01x active ingredient with surfactant applied in the spring.

In an effort to minimize our impact on the fragile lone chaparral ecosystem, initial treatments consisted of minimal numbers of treated plants (n=16). Due to the great efficacy of the applications, this small number of treated plants was adequate to detect statistically significant differences when compared to untreated controls. The small scale of treatments, however, made evaluation of phytotoxicity less reliable. To provide stronger data on efficacy and phytotoxicity of the selected treatment, ten (10) blocks (2m x 2m) of lone manzanita were treated in early July 2009 with 0.01x active ingredient and surfactant and ten (10) equally large

blocks were simply treated with water. In December 2009, phytotoxicity appeared to be negligible on the treated blocks. Evaluation of efficacy of this treatment will be performed in January and April 2010. If both evaluations are positive (effective and without significant phytotoxicity), we will then pass to larger landscape treatments in the spring.

In conclusion, we have developed an experimental approach to test the efficacy of phosphite treatments on lone manzanita to reduce its susceptibility to the pathogen, *Phytophthora* root rot, and we have used the techniques developed to identify the best treatment. The information provided by this research will represent the basis for actual treatments at the landscape level intended as one of the few options available to arrest the ever increasing spread of this exotic pathogen in stands of the endangered lone manzanita.

SUMMARY OF RESULTS

We tested the following concentrations with and without surfactant: 1) 0.01x AgriFos without surfactant; 2) 0.05x AgriFos without surfactant; 3) 0.1x AgriFos without surfactant; 4) 0.01x AgriFos with surfactant; and 5) 0.05x AgriFos with surfactant. These treatments were applied in the spring to both leaves and branches; in the fall to branches only; and in the fall to both leaves and branches.

- One month after application, both the spring and fall treatments (both leaves and branches treated) significantly reduced lesion length in infected cuttings. All tested solutions, regardless of concentration and surfactant presence, appeared equally effective.
- Seven months after application (both leaves and branches treated), the spring treatment continued to significantly reduce lesion length in infected cuttings but results from the fall treatment were mixed. For the spring treatment, all tested solutions were still equally effective; however, only 4 of the 5 fall treatments significantly reduced lesion length and even those appeared less effective than their spring counterparts.
- Thirteen months after treatment, the spring treatment (both leaves and branches treated) continued to significantly reduce lesion length in infected cuttings. (Note: These results need to be confirmed because it was only possible to inoculated new growth, not previous season's growth as in the other inoculations, and it is unknown how this might affects lesion growth.)
- Spraying branches only (only tried for the fall treatment) is less phytotoxic, but may be less effective.

Background/Methods:

On April 10, 2008, spring treatment plots branches and leaves of lone manzanita were sprayed with the following AgriFos mixtures: 1) 0.01x AgriFos without surfactant; 2) 0.05x AgriFos without surfactant; 3) 0.1x AgriFos without surfactant; 4) 0.01x AgriFos with surfactant; and 5) 0.05x AgriFos with surfactant. We sprayed a fall treatment on November 5, 2008, repeating the previous concentrations and treatment regime (both leaves and branches) as well as adding another set of five (5) plots (all previous concentrations) with only branches treated (branches only).

At various times after treatment, cuttings from these plots were inoculated with *P. cinnamomi* strain P3232 and a control (V8). Eleven days after inoculation, lesions were measured to determine the spread of *P. cinnamomi* through the plant. For each cutting, we measured the visible lesion, marked by the darkened bark extending from the site of inoculation, and noted the foliar health of both the overall cutting and the inoculated branch using a 1-5 scale. (5=

perfectly health, 4= less than 10% foliar damage, some problems but still healthy, 3=10-90% foliar damage, significantly damaged 2=less than 10% healthy foliage, almost dead, 1= dead)

Results and Discussion:

1. Treatment efficacy 1 month after spring and fall treatments (both leaves and branches).

To determine the initial efficacy of the treatments, cuttings were inoculated from both the spring and fall treated plots one (1) month after treatment. (Note: For the spring treatment, the 0.1x w/surfactant cuttings were misinoculated; therefore, there is only data for the other four (4) treatments.) Both the spring and fall treatments appeared equally effective at reducing lesion length (Figures 1 and 2). In all cases, the treatments exhibited significantly smaller lesions than the untreated controls.

Figure 1: Spring (1 month after)

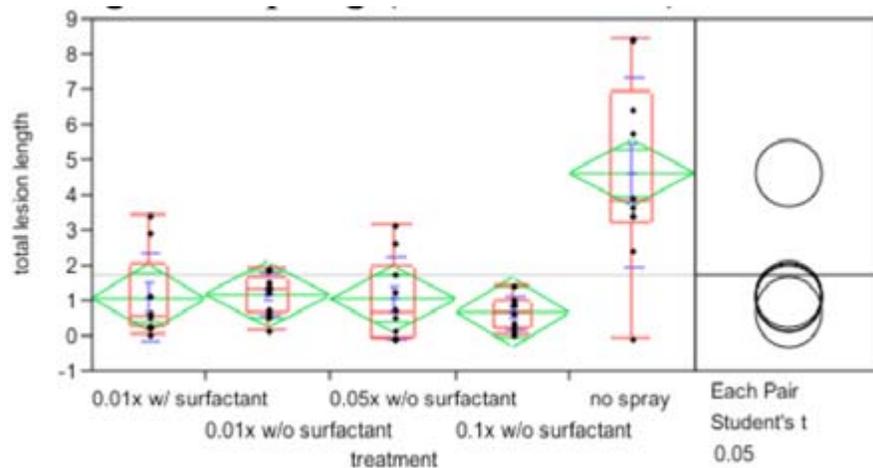
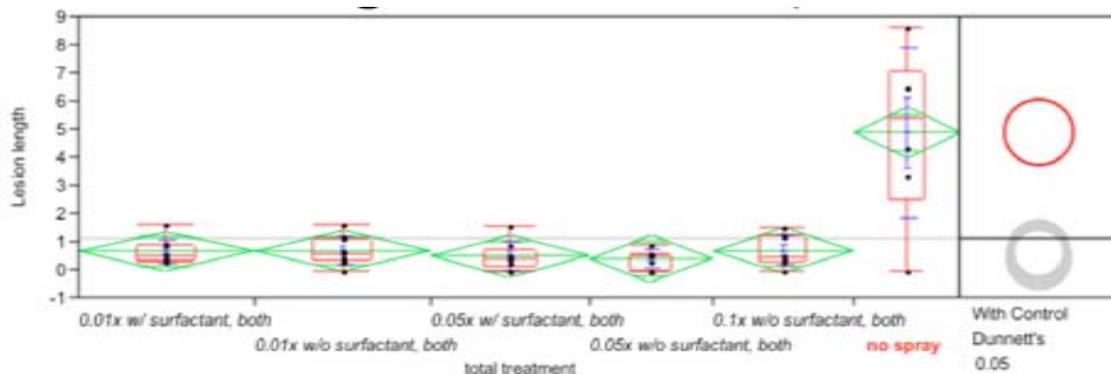


Figure 2: Fall (1 month after)



2. Treatment efficacy 7 months after spring and fall treatments (both leaves and branches).

To see how well this acquired resistance would hold up over time, late inoculations were conducted. The spring-treated plots were inoculated 7 months after their initial treatment and the fall-treated plots were inoculated 6 and 7 months after their initial treatment. (Data from the 6 and 7 month inoculations were merged for the fall plots because of technical difficulties (faulty P3232 plates) with the initial inoculation. For these results we are assuming that there is no difference in plant defense between 6 and 7 months after inoculation.)

In the spring-treated plants, all treatment exhibited significantly smaller lesions than the untreated (control) cuttings (Figure 3). There appears to be no significant difference at this point between the treatments, or at least none that can be observed with this relatively small sample size (n= 6-10).

In the fall treatment, however, 6 to 7 months after treatment the difference between treated and untreated plants are much smaller (Figure 4). In addition there is a lot more variation within a treatment, making it harder to understand what is happening, given the small and variable sample size (n=6-18).

Figure 3: Spring Treatment (7 months after)

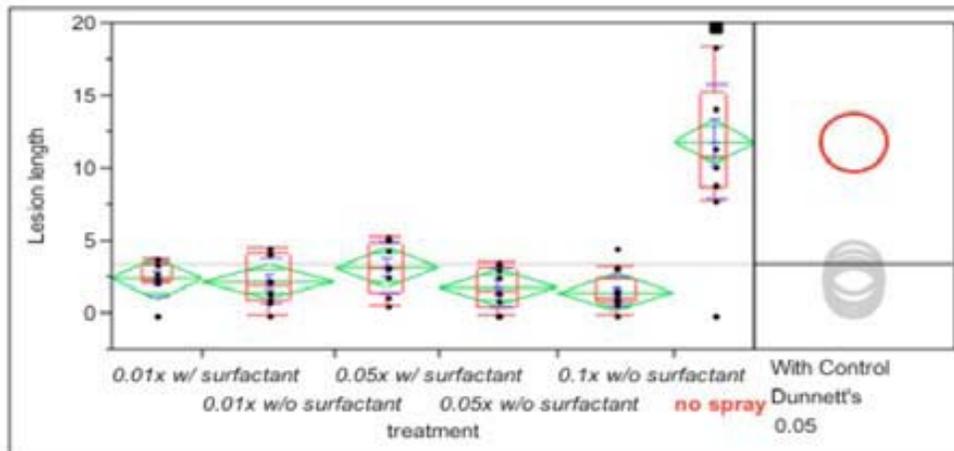
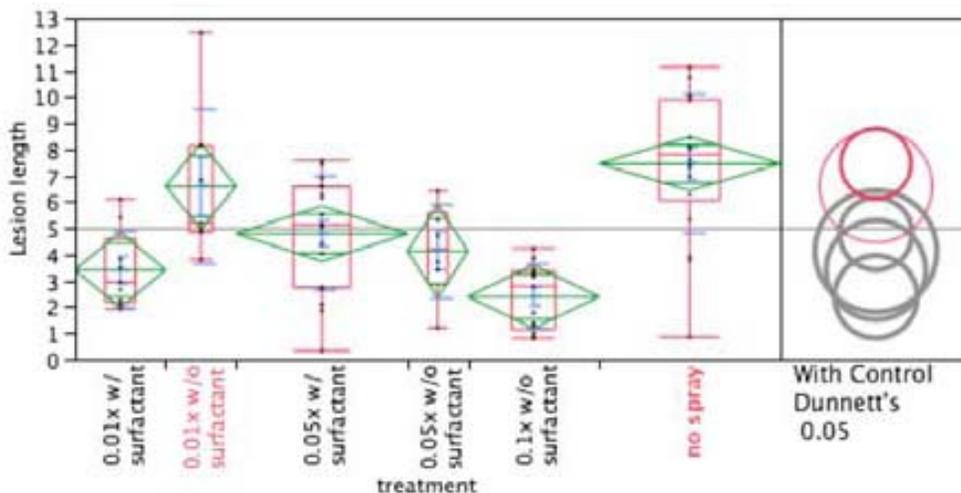


Figure 4: Fall Treatment (6/7 months after)



These results suggest that the protection provided by a spring treatment lasts longer than that of a fall treatment. This has important implications for management strategies since treating large areas of wild land would be time intensive and it would be important to minimize the frequency of spraying needed. Therefore the longer protection afforded by the spring treatment seems ideal. However, if *P. cinnamomi* is most infectious during a brief window after the fall treatment (the wet, cold winter months) the fall treatment might retain efficacy long enough to be a viable option, especially given the reduced phytotoxicity of fall treatments compared to spring.

Although these results seem conclusive it is important to note possible sources of error. The difference in treatment efficacy between fall and spring could be due to winter rains washing off treatments before they can have the desired effect, it could be an issue of growth stages, where plants inoculated in the fall are less susceptible than those inoculated in the spring. Given the physiological differences in the plant at these times, perhaps it would be helpful to see what response the AgriFos is triggering in the plant and whether the elevated levels of secondary metabolites are present at different times, instead of simply looking at lesion length.

3. Treatment efficacy both leaves and branches v branches only.

In the fall treatment half of the plots were treated by applying solution to the leaves and branches and half were treated by applying to just the branches. We compared the efficacy of these treatments to see whether the much less phytotoxic branches only treatment provided the same protection at the both leaves and branches treatment.

One month after treatment, the branches only treatment seems slightly less effective than the both leaves and branches treatment. (Figure 5) At 6/7 months after treatment there seems to be no significant difference between the two, however, both had much larger mean lesion length (Figure 6). Although the implications of this study are difficult to deduce given the overarching effect that treatment time had on efficacy, it seems as if targeting branches only could be a good way to reduce phytotoxicity. Further studies are needed to determine if this improvement is worth the possibly decreased efficacy.

Figure 5. One-way analysis of lesion length by treatment technique, 1 month after treatment.

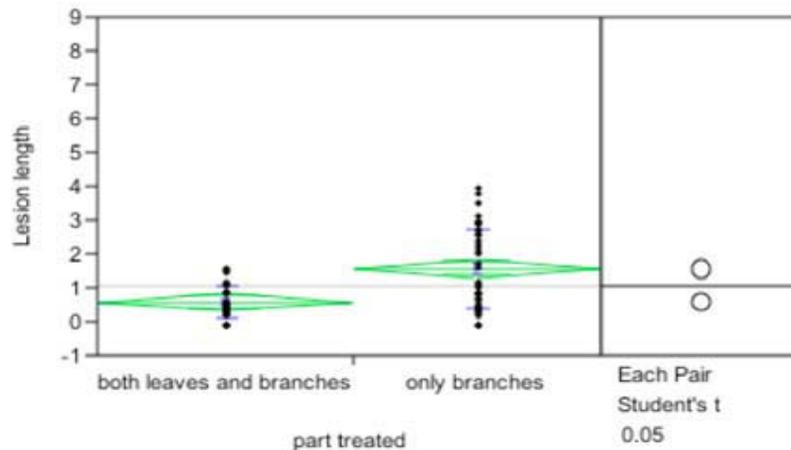
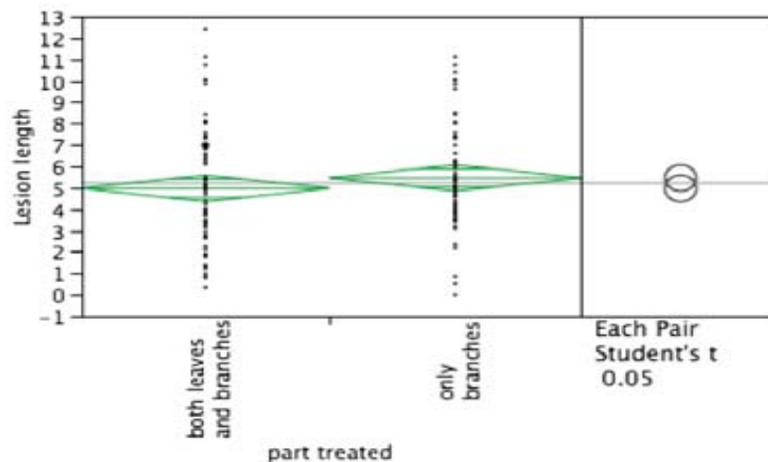


Figure 6. One-way analysis of lesion length by treatment technique, 6/7 months after treatment.

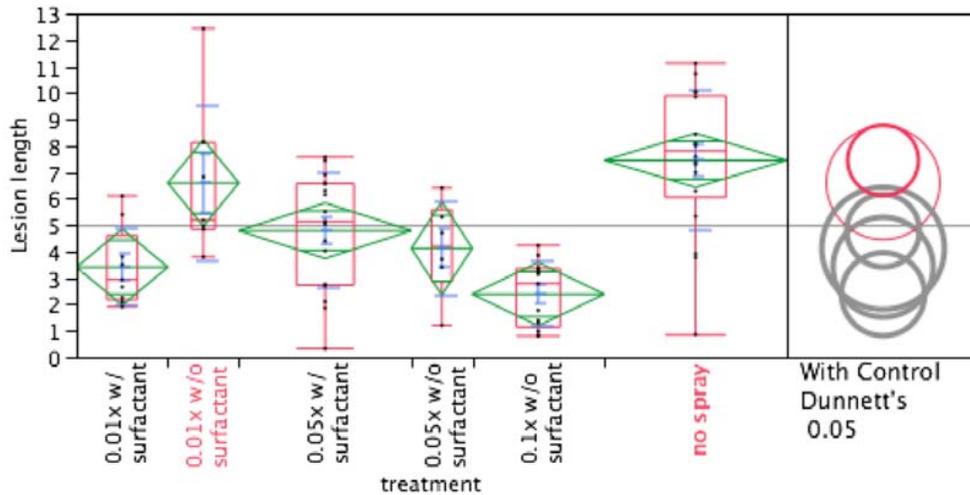


DATA SUMMARIES FROM ALL PREVIOUS INOCULATIONS

Spraying 0.1x Agri-Fos®/surfactant solution prevents *P.cinnamomi* infection in whit leaf manzanita while minimally damaging the plant.

- The total lesion length of *P.cinnamomi* in infected plants is much higher in those sprayed with water alone than those sprayed with both 0.1x and 0.5x Agri-Fos®/surfactant solution.
- White leaf manzanita plants treated with a 0.1x Agri-Fos®/surfactant solution remain healthy, while plants treated with a 0.5x solution are severely harmed and do not appear to recover .
- Although white leaf manzanita plants treated with 0.1x are harmed by the spray, the decrease in health is no greater than the decrease caused by *P.cinnamomi* infection of the same duration.
- The *P.cinnamomi* isolate MC 6, a common type found on avocados, is the most virulent on white leaf manzanita while MC9, found on manzanitas, is the least.
- Common manzanita is a poor study plant for these trials because it is unable to tolerate hot, dry conditions such as characterize the habitat of lone manzanita, but white leaf manzanita is suitable to use as proxy for the lone manzanita.

Figure 7. Lesion length by treatment (*P. cinnamomi* inoculated only). Fall treatment, branches and leaves inoculated April and May 2009 (6/7 months after treatment).

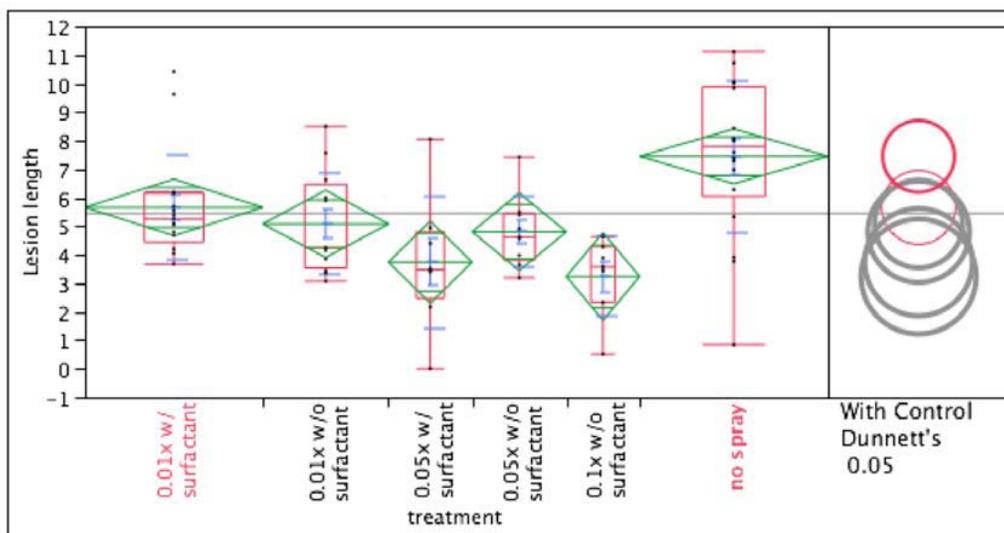


| Level | Number | Mean | Std Dev | Std Err Mean | Lower 95% | Upper 95% |
|----------------------|--------|---------|---------|--------------|-----------|-----------|
| 0.01x w/ surfactant | 9 | 3.40333 | 1.47631 | 0.4921 | 2.2685 | 4.5381 |
| 0.01x w/o surfactant | 7 | 6.59000 | 2.94617 | 1.1135 | 3.8652 | 9.3148 |
| 0.05x w/ surfactant | 17 | 4.78294 | 2.17117 | 0.5266 | 3.6666 | 5.8993 |
| 0.05x w/o surfactant | 6 | 4.11667 | 1.79713 | 0.7337 | 2.2307 | 6.0026 |
| 0.1x w/o surfactant | 13 | 2.38231 | 1.23305 | 0.3420 | 1.6372 | 3.1274 |
| no spray | 18 | 7.44722 | 2.67445 | 0.6304 | 6.1172 | 8.7772 |

Summary of the Spring 2008 trials:

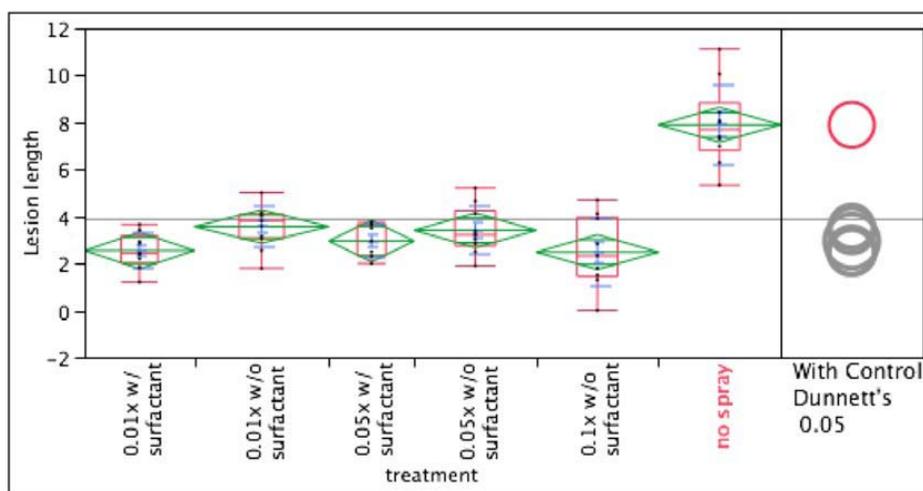
Two treatments were used for the Spring and Fall 2008 trials. Spraying of branches only was used only in the Fall 2008 trials (Figure 8). Although this approach was somewhat slower than the foliar applications, it was feasible. Because both Spring 2008 (Figure 9) and Fall 2008 trials were ongoing, the efficacy of trials including their side effects will be evaluated in Spring 2009. Based on the results, the most effective treatment (variables to be selected include timing of application, concentration of AgriFos, presence or absence of surfactant, foliar vs. branch applications) and which causes the least phytotoxicity will be evaluated in field trials in at least three different sites in the lone area.

Figure 8: Lesion length by treatment (*P.cinnamomi* inoculated only). Fall treatment, branches only inoculated April and May 2009, (6/7 months after treatment).



| Level | Number | Mean | Std Dev | Std Err Mean | Lower 95% | Upper 95% |
|----------------------|--------|---------|---------|--------------|-----------|-----------|
| 0.01x w/ surfactant | 17 | 5.65882 | 1.81244 | 0.43958 | 4.7270 | 6.5907 |
| 0.01x w/o surfactant | 12 | 5.08000 | 1.78960 | 0.51661 | 3.9429 | 6.2171 |
| 0.05x w/ surfactant | 8 | 3.74000 | 2.30483 | 0.81488 | 1.8131 | 5.6669 |
| 0.05x w/o surfactant | 9 | 4.80444 | 1.24410 | 0.41470 | 3.8481 | 5.7607 |
| 0.1x w/o surfactant | 7 | 3.23714 | 1.40522 | 0.53113 | 1.9375 | 4.5368 |
| no spray | 18 | 7.44722 | 2.67445 | 0.63037 | 6.1172 | 8.7772 |

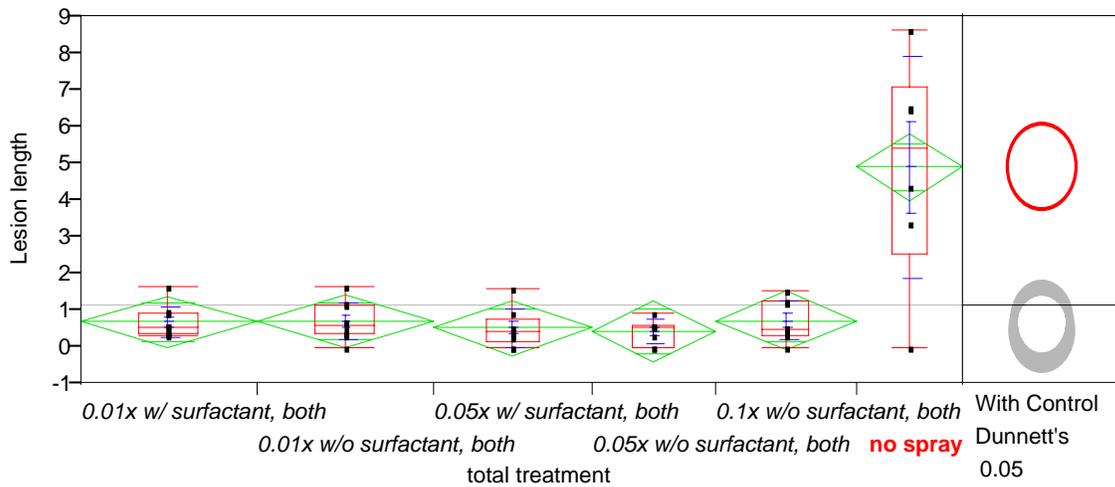
Figure 9. Lesion length by treatment (*P.cinnamomi* inoculated only). Spring treatment, branches and leaves inoculated May 2009 (13 months after treatment).



| Level | Number | Mean | Std Dev | Std Err Mean | Lower 95% | Upper 95% |
|----------------------|--------|---------|---------|--------------|-----------|-----------|
| 0.01x w/ surfactant | 9 | 2.54556 | 0.76386 | 0.25462 | 1.9584 | 3.1327 |
| 0.01x w/o surfactant | 11 | 3.56364 | 0.87899 | 0.26503 | 2.9731 | 4.1542 |
| 0.05x w/ surfactant | 7 | 2.95000 | 0.70193 | 0.26530 | 2.3008 | 3.5992 |
| 0.05x w/o surfactant | 10 | 3.40500 | 0.99645 | 0.31511 | 2.6922 | 4.1178 |
| 0.1x w/o surfactant | 10 | 2.47800 | 1.44227 | 0.45608 | 1.4463 | 3.5097 |
| no spray | 10 | 7.89300 | 1.70167 | 0.53811 | 6.6757 | 9.1103 |

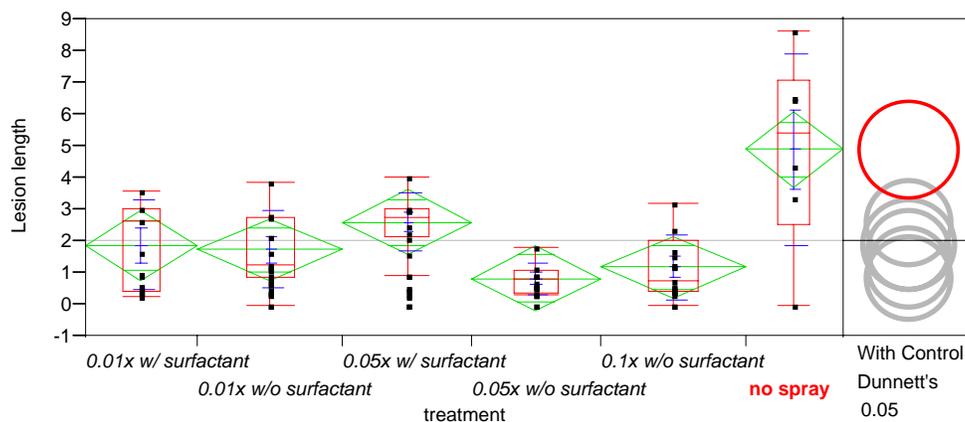
All treated plots, regardless of treatment concentration or technique, reduced lesion length in inoculated cuttings. However, this difference is more pronounced in cuttings where both branches and leaves (Figure 10) were treated. While all of the both branches-and-leaves treated cuttings had significantly smaller lesions than the non-treated cuttings, one of the branches-only plots (0.5x with surfactant) was not significantly different from the untreated control (Figures 11 and 12).

Figure 10. Lesion length of both branches-and-leaves treated plots. Fall treatment, December 8, 2009 inoculation (1 month after treatment).



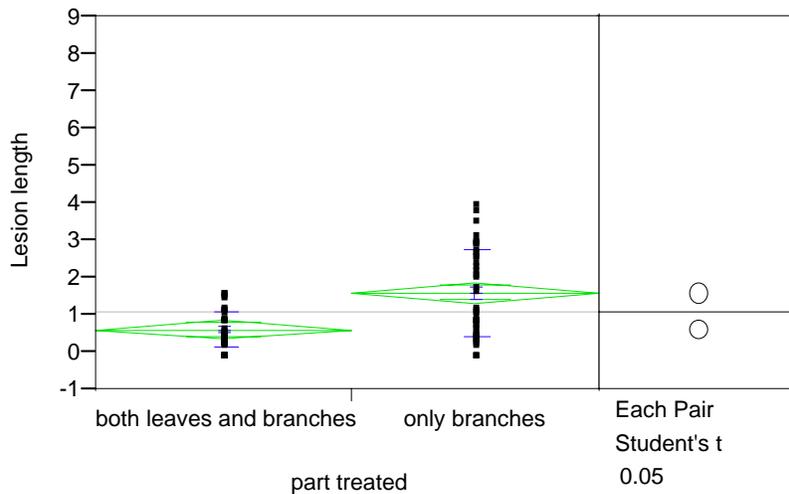
| Level | Number | Mean | Std Dev | Std Err Mean | Lower 95% | Upper 95% |
|----------------------------|--------|---------|---------|--------------|-----------|-----------|
| 0.01x w/ surfactant, both | 10 | 0.66800 | 0.41854 | 0.1324 | 0.3686 | 0.9674 |
| 0.01x w/o surfactant, both | 10 | 0.68400 | 0.50096 | 0.1584 | 0.3256 | 1.0424 |
| 0.05x w/ surfactant, both | 9 | 0.50222 | 0.50485 | 0.1683 | 0.1142 | 0.8903 |
| 0.05x w/o surfactant, both | 7 | 0.42286 | 0.33320 | 0.1259 | 0.1147 | 0.7310 |
| 0.1x w/o surfactant, both | 8 | 0.71000 | 0.54148 | 0.1914 | 0.2573 | 1.1627 |
| no spray | 6 | 4.89833 | 3.03046 | 1.2372 | 1.7181 | 8.0786 |

Figure 11. Lesion length of branches-only treated plot. Fall treatment, December 8, 2008 inoculation (1 month after treatment).



| Level | Number | Mean | Std Dev | Std Err Mean | Lower 95% | Upper 95% |
|------------------------------|--------|---------|---------|--------------|-----------|-----------|
| 0.01x w/ surfactant, only b | 7 | 1.86429 | 1.41494 | 0.5348 | 0.5557 | 3.1729 |
| 0.01x w/o surfactant, only b | 9 | 1.73000 | 1.22671 | 0.4089 | 0.7871 | 2.6729 |
| 0.05x w/ surfactant, only b | 8 | 2.60125 | 0.90885 | 0.3213 | 1.8414 | 3.3611 |
| 0.05x w/o surfactant, only b | 8 | 0.82875 | 0.49738 | 0.1758 | 0.4129 | 1.2446 |
| 0.1x w/o surfactant, only b | 9 | 1.18111 | 1.03973 | 0.3466 | 0.3819 | 1.9803 |
| no spray | 6 | 4.89833 | 3.03046 | 1.2372 | 1.7181 | 8.0786 |

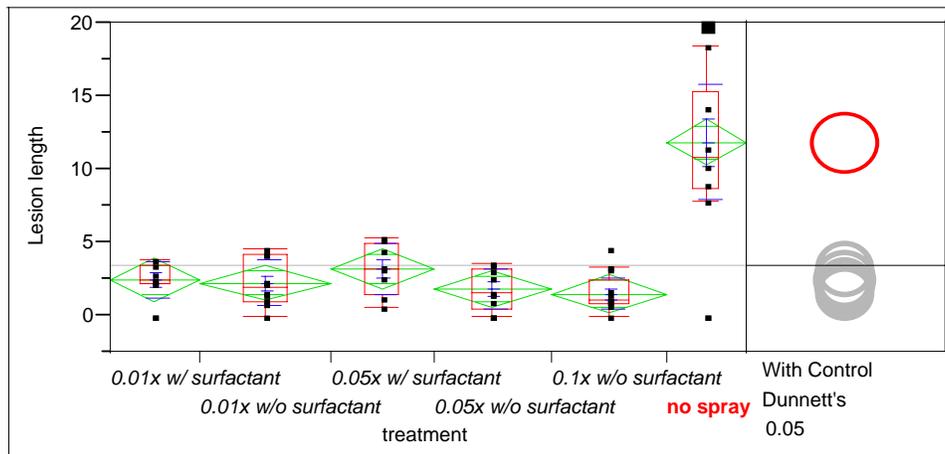
Figure 12. One-way analysis of lesion length by treatment technique. Fall treatment, December 8, 2008 inoculation (1 month after treatment).



| Level | Number | Mean | Std Dev | Std Err Mean | Lower 95% | Upper 95% |
|--------------------------|--------|---------|---------|--------------|-----------|-----------|
| both leaves and branches | 44 | 0.60636 | 0.46029 | 0.06939 | 0.4664 | 0.7463 |
| only branches | 42 | 1.58786 | 1.18698 | 0.18315 | 1.2180 | 1.9577 |

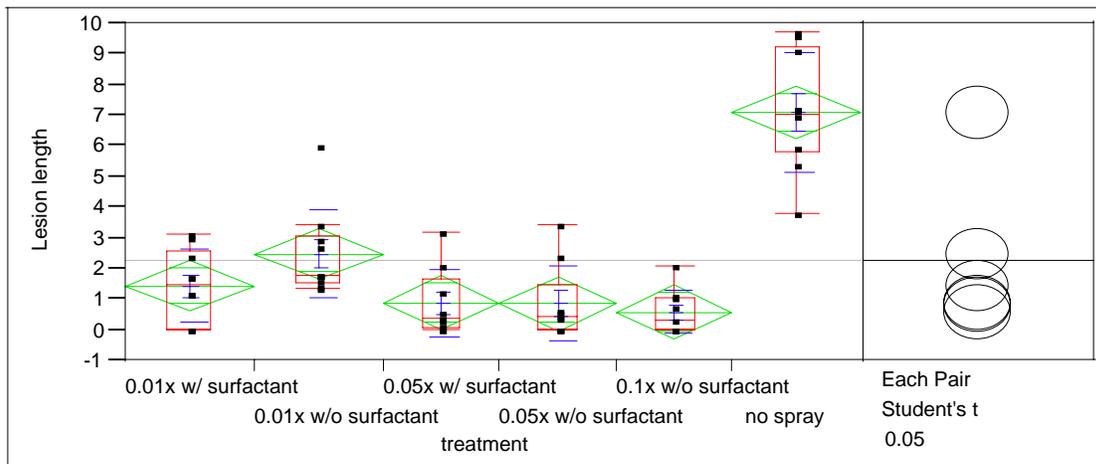
On April 10, 2008 the following treatments were applied to small plots of lone manzanita: 0.01x AgriFos without surfactant, 0.05x AgriFos without surfactant, 0.1x AgriFos without surfactant, 0.01x AgriFos with surfactant and 0.05x AgriFos with surfactant. On May 8 and July 23, 2008 cuttings from these plots were inoculated and results showed that all treatments significantly reduced lesion size (Figures 13, 14, 15).

Figure 13: One-way analysis lesion length by treatment. Spring treatment November 2008 inoculation (7 months after treatment).



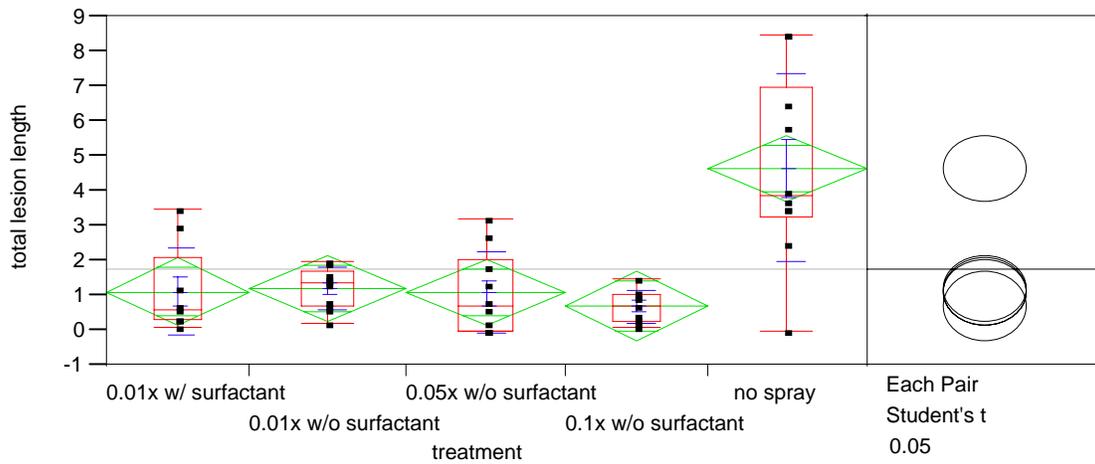
| Level | Number | Mean | Std Error | Lower 95% | Upper 95% |
|----------------------|--------|---------|-----------|-----------|-----------|
| 0.01x w/ surfactant | 7 | 2.4371 | 0.72018 | 0.985 | 3.890 |
| 0.01x w/o surfactant | 10 | 2.2350 | 0.60255 | 1.020 | 3.450 |
| 0.05x w/ surfactant | 8 | 3.2087 | 0.67367 | 1.850 | 4.567 |
| 0.05x w/o surfactant | 9 | 1.8367 | 0.63514 | 0.556 | 3.118 |
| 0.1x w/o surfactant | 9 | 1.4933 | 0.63514 | 0.212 | 2.774 |
| no spray | 6 | 11.8400 | 0.77789 | 10.271 | 13.409 |

Figure 14. One-way analysis lesion length by treatment. Spring treatment, July 2008 inoculation (3 months after treatment).



| Level | Number | Mean | Std Dev | Std Err Mean | Lower 95% | Upper 95% |
|----------------------|--------|---------|---------|--------------|-----------|-----------|
| 0.01x w/ surfactant | 10 | 1.43400 | 1.18544 | 0.37487 | 0.586 | 2.2820 |
| 0.01x w/o surfactant | 10 | 2.48200 | 1.42067 | 0.44926 | 1.466 | 3.4983 |
| 0.05x w/ surfactant | 9 | 0.88222 | 1.10226 | 0.36742 | 0.035 | 1.7295 |
| 0.05x w/o surfactant | 9 | 0.86000 | 1.20663 | 0.40221 | -0.067 | 1.7875 |
| 0.1x w/o surfactant | 9 | 0.58333 | 0.71939 | 0.23980 | 0.030 | 1.1363 |
| no spray | 10 | 7.08500 | 1.93143 | 0.61077 | 5.703 | 8.4667 |

Figure 15. One-way analysis lesion length by treatment. Spring treatment May 2008 inoculation (1 month after treatment).



| Level | Number | Mean | Std Dev | Std Err Mean | Lower 95% | Upper 95% |
|----------------------|--------|---------|---------|--------------|-----------|-----------|
| 0.01x w/ surfactant | 9 | 1.11111 | 1.26040 | 0.42013 | 0.1423 | 2.0799 |
| 0.01x w/o surfactant | 10 | 1.20000 | 0.59628 | 0.18856 | 0.7734 | 1.6266 |
| 0.05x w/o surfactant | 10 | 1.06000 | 1.16733 | 0.36914 | 0.2249 | 1.8951 |
| 0.1x w/o surfactant | 9 | 0.68889 | 0.47288 | 0.15763 | 0.3254 | 1.0524 |
| no spray | 10 | 4.65000 | 2.67924 | 0.84725 | 2.7334 | 6.5666 |