Use of phosphite to protect Ione manzanita (*Arctostaphylos myrtifolia*) stands from root rot caused by *Phytophthora cinnamomi*

Final Contract Performance report

31 August 2016

Contract: L12AC20094 - BLM / CESU Institute for Wildlife Studies, Sept 2012 - Aug 2016
Phytosphere project 2012-1001

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Cover photo: Phosphite-treated (12.4 kg/ha at 300 L/ha spray volume) *Arctostaphylos myrtifolia* plot 3B (right) and nontreated control plot 3A (left). Tape marks the plot front of the treated plot (right). When plots were established in 2011, the edge of the disease front was along the same line (extending beyond the tape to the left) in both treated and control plots. Mortality due to *Phytophthora cinnamomi* root disease has obliterated most of the plants in the control plot and the buffer separating the control and treated plots (left). The left edge of the treated plot, which receives a lower spray rate due to the sprayer design, shows some recent plant mortality. Photo date: 5 August 2016.

Acknowledgements

Funding for this research was provided by the Bureau of Land Management (BLM) under contract L12AC20094 with the Institute for Wildlife Studies. Phytosphere Research would like to thank Beth Brenneman, the project manager at BLM, for providing support and encouragement for this project. We also thank Brian Hudgens and Dick Johnson at IWS for their collaboration and support for this project. We also thank Eric Kleinfelter, our contact at California Department of Fish and Wildlife, for his assistance and cooperation. We also thank Al Franklin, retired BLM staff, for his assistance with the 2013 spray application.

Executive Summary

This report presents results on experimental phosphite treatments to protect native stands of *Arctostaphylos myrtifolia* from root rot caused by the introduced pathogen *Phytophthora cinnamomi*. Treating plants at two-year intervals with a foliar application of potassium phosphite at 12.4 kg/ha using a standard spray volume of 300 L/ha provided good control of disease. *P. cinnamomi* propagule density was lower at the disease front of treated plants compared to nontreated plants, but levels of inoculum would be sufficient to allow disease to progress if phosphite treatment was discontinued. This application rate was also effective in reducing mortality among seedlings which had started to grow in old mortality centers.

We initiated a series of experiments to find an effective phosphite dose using ultra low volume (ULV) applications. Due to the amount of liquid used for standard rate applications, a successful protocol for ULV treatment is necessary to allow for application by air or in off-road situations that commonly exist in Ione manzanita habitat. ULV treatments use approximately one tenth the spray volume of standard volume sprays, i.e., about 30 L/ha, and therefore require a large increase in the concentration of the spray solution. Microplot tests indicated that phytotoxicity could develop after ULV applications of 12.4 kg/ha. To reduce the likelihood of phytotoxicity, initial ULV treatments were applied at 8 and 10 kg/ha. However, these lower applications rates showed inadequate levels of disease suppression. We initiated a second set of studies using split ULV applications, i.e., two applications separated by a period of 4-6 weeks, which allowed us to double overall application rates (to 16 and 20 kg/ha) while minimizing phytotoxicity. Initial evaluations indicate greater disease control at 20 kg/ha compared to 16 kg/ha, but repeat applications and further evaluations are needed to determine whether ULV applications will adequately suppress disease progress if applied at two-year intervals.

Introduction

Ione manzanita, *Arctostaphylos myrtifolia*, is a rare and threatened California endemic plant that is limited to the unusual, highly acidic soils of the Ione formation in the central Sierra Nevada foothills. Significant disease problems in natural stands of *A. myrtifolia*, including death of large patches of plants, were noted as early as 1988 (Wood and Parker 1989). In the summer of 2002, we determined that *Phytophthora cinnamomi* was the cause of a root and crown rot that has killed large areas of *A. myrtifolia* and *A. viscida* in an area of *A. myrtifolia* habitat south of Ione, California (Swiecki and Bernhardt 2003; Swiecki et al 2003).

In subsequent studies (Swiecki et al 2005), we determined that mortality due to *P. cinnamomi* has affected large portions of Ione manzanita habitat north of the town of Buena Vista. Genetic analyses showed that this widespread infestation apparently arose from several distinct introductions of the pathogen to the area and subsequent spread from established disease centers. A second *Phytophthora* species, *P. cambivora* was also associated with declining Ione manzanita in one site.

Phytophthora cinnamomi can persist for many years in the soil and it is very difficult to eradicate. Hence, efforts to prevent spread from existing infestations will form the basis for protecting Ione manzanita's dwindling habitat from this pathogen for the foreseeable future. In Australia, where *P. cinnamomi* has infested and devastated several hundred thousand hectares of native forests, large scale application of phosphite has been used to help slow spread of the pathogen and protect rare plant communities (Barrett

2003, Barrett et al 2003, 2004, Hardy et al 2001b). Phosphites (also called phosphonates) are simple inorganic salts (mono- and di-potassium salts of phosphorous acid) that are classified as biopesticides by the USEPA. Phosphite functions as a systemic and selective fungicide that protects plants against *Phytophthora* species but has very low toxicity to non-target organisms (Guest and Grant 1991). Phosphite residues in the soil are converted by soil microorganisms to phosphate, a plant nutrient and naturally-occurring constituent of soils.

Plants do not metabolize phosphite. In plants, phosphite moves in both the phloem and the xylem. In the phloem, it moves towards metabolic sinks for sugars and amino acids. Thus, the phenology of the plants at the time of application affects which tissues accumulate the most phosphite from any one application. The amount of phosphite applied to a plant is proportional to the amount of phosphite absorbed, but various species absorb phosphite at different rates. Drought stressed plants do not absorb phosphite well. Absorption of high amounts of phosphite can cause phytotoxicity in some plants (Guest and Grant 1991, Hardy et al 2001a).

Phosphite has a complex mode of action that is not totally understood. Concentrations in a plant may be high enough to be directly toxic to *Phytophthora* in some cases. At lower concentrations, phosphite stimulates the plant to mount a resistant reaction in response to infection (Guest and Grant 1991, Hardy et al 2001a). Experimentation is required to optimize phosphite dose and timing to achieve disease control for each plant/pathogen interaction.

In 2011, under a previous project, we set up a field trial to test efficacy of potassium phosphite against *P. cinnamomi* root rot (Swiecki and Bernhardt 2012). This study continued that initial field trial and established additional trials to test efficacy of various application rates and treatment volumes.

Objectives

The objectives of this project were as follows.

1. Continue the study initiated in 2011 and initiate new studies to test the effectiveness of phosphite in slowing the spread of mortality caused by *Phytophthora cinnamomi* in natural stands of Ione manzanita. .

2. Make results of the study available to assist land managers and researchers in the management of this disease on Ione manzanita and other affected native plant species. Results were made available to Bureau of Land Management (BLM) via quarterly reports.

Plot phasing plan

We have followed a phased approach in our program for testing whether phosphite can be used to protect stands of Ione manzanita from mortality caused by *P. cinnamomi*. In general, it is necessary to establish efficacy for the application methods tested in one phase before proceeding to the next related phase. To accelerate progress to the degree possible, we were able to work on several phases in parallel and combine some elements from different phases. Plot phase numbers referred to in this report are the same as those in the narrative for contract Amendment 1 (12/27/2013).

Phase 1 plots: Standard volume application rates. We started a study in 2011 (prior to this contract) in which phosphite was applied at a standard spray volume (300 L/ha) at 12.4 kg/ha. This treatment has

been reapplied at a two year retreatment interval. The intent of the study was to determine whether phosphite application was effect in reducing mortality caused by *P. cinnamomi* under field conditions. Based on positive results from this study, we have progressed to subsequent phases.

Phase 2 plots: Ultra-low volume (ULV) application rates. Use of phosphite over large patches of Ione manzanita would require aerial application, which requires much lower total spray volumes per unit area than used for standard volume applications (phase 1 plots). To maintain the 12.4 kg/ha application rate with a lower spray volume, a higher phosphite concentration must be used in the spray solution, increasing the risk of phytotoxicity. Under this contract, we conducted additional microplot phytotoxicity tests using higher concentration / ultra low volume applications to determine rates that can be tolerated without significant damage.

Results from phytotoxicity tests were used to set ULV application rates near the maximum tolerated dosage. Because early results showed poor efficacy at these rates, we converted most of our plots to a split application regime. Under this regime, two sprays are separated in time by a period of at least 4-6 weeks. This allows some phosphite to be translocated from the leaves so that phytotoxicity is avoided even though the total dose if doubled.

Phase 3 plots: Treatment band width. Phase 1 plots phosphite plots extended 7 m into the healthy stand from the edge of active root disease centers. The level of efficacy seen in the phase 1 plots suggested that a narrower band of plants along a disease front could be treated to prevent expansion of *P. cinnamomi* into the noninfested stand. This has the potential to both reduce treatment cost and make application with ground equipment more feasible. In phase 2 testing of ULV applications, we concurrently tested use of narrower treatment band, mostly 2 to 3 spray swaths wide (2.4 to 3.6 m).

Phase 4 plots. Use of phosphite to protect seedling regeneration in old mortality centers. Treating individual surviving plants in mortality centers is effectively the ultimate reduction in treatment band width (phase 3). In parallel with phase 2 studies, we established a study to determine whether it was feasible to protect individual *A. myrtifolia* seedlings that had become established in older portions of mortality centers. Without treatment, these seedlings typically do not survive for more than a few years. Treating these recruits to allow them to survive to reproductive age would help maintain genetic diversity and provide options for potential rehabilitation of affected stands.

Phase 5 plots. Extended retreatment interval. Once efficacy has been established under any of the above treatment regimes, the final step is determining the longest retreatment interval that can be used to maintain efficacy. Our standard retreatment interval is 2 years. Given the limited duration of the project and the need to repeat efficacy tests over at least two cycles, it has not been possible to reach this phase of testing to date.

Table 1 presents a summary of all phosphite plots established, treated, and monitored under this contract.

Table 1. Phosphite treatment plots at Apricum Hill Preserve. Currently all plots are set up with a 2 year retreatment interval.

Plot type	Phosphite rate, kg/ha	Spray volume, L/ha	Plot depth, m (band width)	Plot numbers	Treatment dates	Evaluation dates	Monitoring method
Standard rate (phase 1)	12.4	300	7	1A, 1C, 2B, 3A, 4A, 8A	4/28/2011 4/19/2013 3/4/ 2015	6/8/2011 1/24/2013, 9/30/2013, 4/21/2014, 3/9 or 4/20/2015, 3/25/2016 8/5/2016	Measurements along disease front
ULV: initial single application, then split application (phase 2/3)	Single: 10 Split: 20 (10+10)	30	Single: 2.4 Split: 2.4 (plot 10 irregular)	9E, 10, 11W, 12W	5/6/2014 initial at 10 kg/ha 1/23/2015 & 3/9/2015	2/18 or 3/9/2015 3/25/2016 8/5 & 8/6/2016	Measurements along disease front, dieback ratings
ULV: initial single application, then split application (phase 2/3)	Single: 8 Split: 16 (8+8)	30	Single: 2.4 Split: 2.4	9W, 11E, 12E	5/6/2014 initial at 8 kg/ha 1/23/2015 & 3/9/2015	3/25/2016 8/5 & 8/6/2016	Measurements along disease front, dieback ratings
ULV split application (phase 2/3)	20 (10+10)	30	2.4 – 5	13, 14, 15, 16, 17, 18	11/26/2014 & 2/20/2015	4/1/2016 8/4/2016	Photopoints
ULV split application (phase 2/3)	20 (10+10)	30	2.4 - 6	19, 20, 21, 22-23, 24-25	1/2/2015 2/20/2015	4/1/2016 8/4/2016	Photopoints, Measurements along disease front (19)
ULV single application (phase 2/3)	10	30	3.6 (average)	26/28, 27	1/2/2015	4/1/2016 8/4/2016	Photopoints
Standard rate[1], individual seedlings (phase 4)	12.4	300	0.5 or less	Individually tagged seedlings	2014 cohort: 3/24/2014 or 4/9/2014 2014 and 2016 cohorts: 3/18/2016	7/17/2014 4/20/2015 3/18/2016 8/5/2016	Individual plant ratings and measurements

[1] Ten seedlings initially treated using 10 kg/ha ULV application, all converted to standard rate at second application date.

1. Phase 1 plots: standard application volume (300 L/ha)

Plots were first established at the California Department of Fish and Wildlife (CDFW) Apricum Hill Preserve in 2011, prior to the start of this contract (Swiecki and Bernhardt 2011). We subsequently retreated these plots at 2 year intervals. The disease suppression achieved with standard volume (300 L/ha) treatment is used as a basis for comparison for disease suppression achieved with the ultra low volume (ULV) phase 2 and 3 plots.

1.1. Methods

1.1.1. Spray materials

Reliant® Systemic Fungicide (Quest Products LLC, Linwood KS EPA reg. No. 83416-1) was used as the source of phosphite. Undiluted Reliant® contains 619.5 g/L of active ingredient (a.i.), consisting of a mixture of mono- and dipotassium salts of phosphorus acid. It is a 45.8% a.i. liquid concentrate that is fully miscible with water. It is chemically identical to Agri-Fos®, which was used for the 2011 and 2013 applications. To enhance spray deposition and uptake, a non-ionic organomodified trisiloxane surfactant (Break-thru®, Plant Health Technologies, Lathrop, CA; CA Reg. No. 65343-50003) was added at 0.03% by volume in all years.

1.1.2. Plot layout

In 2011, we established five sets of plots containing six treated plots and five nontreated control plots in healthy stands of *A. myrtifolia*, most with some intermixed *A. viscida* (Figure 1). These and all other plots described in this report are located at the CDFW Apricum Hill Preserve. The preserve, which is not open to the public, is located on the west side of Jackson Valley Road about 4 km southeast of the Town of Ione in Amador County.

One plot set (plot 1) was made up of three matched plots. Two of these were treated and the third was left as a nontreated control. Each of the other plot sets (2, 3, 4, and 8) consisted of matched pairs of plots. We applied phosphite to one plot in each of these plot pairs. Each plot is 6 m wide and 7 m long. For plot sets 1, 2, 3 and 4, one of the 6 m plot faces was located along an active *P. cinnamomi* root disease front; the plot extended 7 m from the disease front into the healthy part of the stand. In plot set 2, one of the 7 m plot sides of each plot was also parallel to another disease front, so disease spread into these plots could also occur along these long sides. Plot set 8 was set up in a patch of Ione manzanita that was not directly adjacent to an active root disease center. The front (west) edges of plots 8A and 8B (6 m wide side) were located along on old unpaved road that leads to a large root disease center about 7 m away. The road was considered to be the most likely route by which *P. cinnamomi* would be introduced into the plot area. Mortality due to *P. cinnamomi* has also been advancing toward the old road from the west.

All plot corners were marked with carriage bolts (6 mm diameter, 100 mm long) driven into the ground through a 40 mm-diameter fender washer atop a square piece (about 10 cm across) of white vinyl flashing. GPS coordinates were recorded for all plot corners. These coordinates were used in conjunction with aerial imagery to develop a GIS layer of plot polygons (Figure 1).

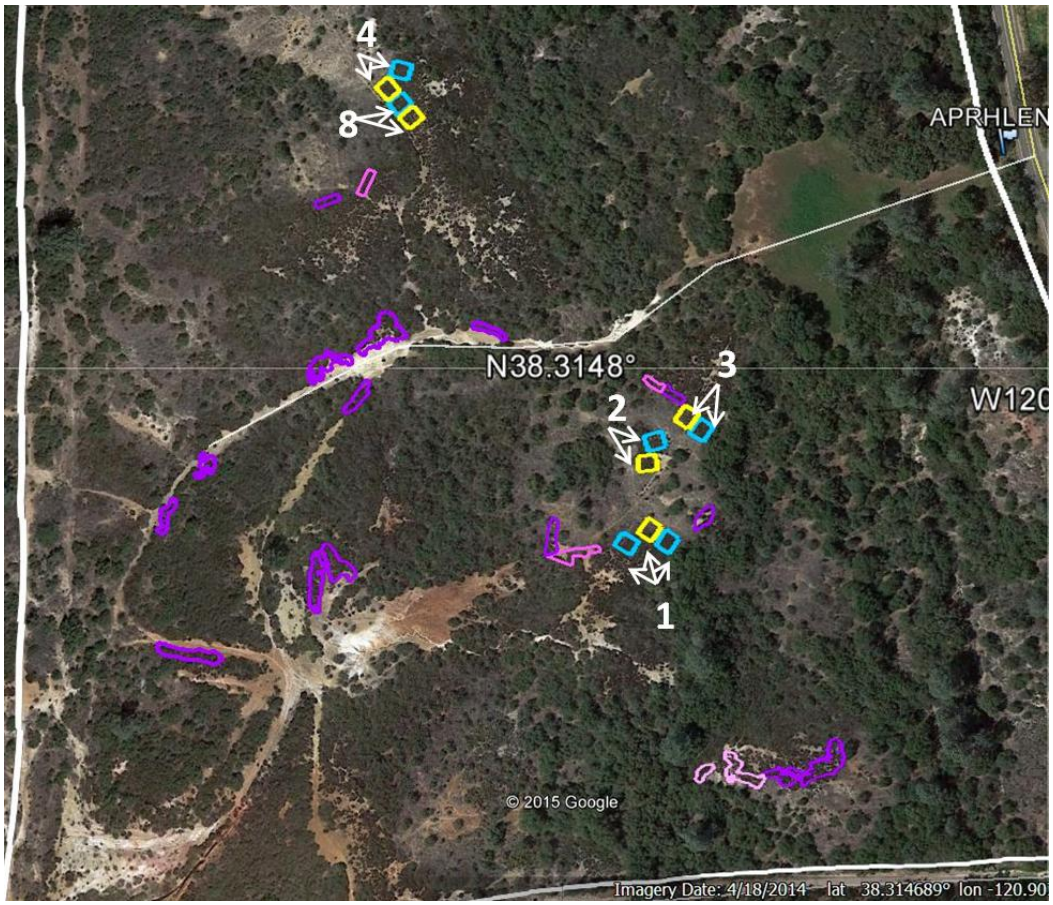

Figure 1. Locations of phase 1 phosphite-treated plots (cyan rectangles) and matched control plots (yellow rectangles). Plot set numbers are shown in white. Purple shaded plots are phase 2/3 ULV plots described below. White lines at left and right of image are approximate borders of the California Department of Fish and Wildlife Apricum Hill Preserve. Jackson Valley Road is visible at the upper right corner of the image (Google Maps image).

1.1.3. Phosphite application

We used the custom spray rig we designed and built in 2011 to reapply phosphite to the plots. This spray apparatus:

- allows us to spray the plots at the desired application volume (300 L/ha) with a high degree of accuracy and repeatability;
- minimizes the amount of travel between infested and noninfested ends of the plots;
- avoids mechanical damage to Ione manzanita that would be associated with straight line walking or vehicle traffic through the plot area;
- is light and portable enough to transport into the plot areas, which are accessed with some difficulty by foot;
- requires no more than two people to operate.

The spray rig consists of a short spray boom that travels along a tubular monorail that is suspended over the plot area (Figure 2). The monorail is supported by a pair of reinforced PVC pipe legs on each end that can be adjusted to obtain the desired height of the boom above the canopy. Poles are inserted into the monorail tubing and extend past the legs. During the application, these end poles are pulled downward,

which flexes the monorail into a slight upward arch that overcomes the sag that otherwise develops in the unsupported center of the monorail. The spray boom is shuttled across the monorail by winding a line attached to the boom onto a large-diameter spool. Rotating the spool at a regulated speed causes the boom to travel over the plot at a constant velocity. The entire spray apparatus is easily assembled and dissembled into sections for transport (Figure 3).

The spray boom that travels along the monorail is about 1 m wide and has three Teejet XR 11001VS nozzles mounted on 0.5 m centers. At a spray height of 0.5 m above the canopy, the boom produces a 1.5 m-wide spray swath after accounting for necessary nozzle pattern overlap. Four swaths centered 1.5 m apart were used to spray the 6 m width of each plot. The length of each swath was 7 m, the distance that the boom travels along the monorail. The outer 7 m edges of each plot (to right and left when facing the plot) receive a lower rate since there is no overlap in the spray pattern at the outer edges.

The spray solution is delivered from a polyethylene tank to the spray boom via a battery-operated diaphragm pump (SHURflo Model 8009-541-236) which has a rated output of 3.79 L/min (1 gal/min). The speed of the pump is varied using a DC motor speed controller that allows us to reduce pump output below this rating. We calibrated the sprayer by adjusting the pump speed to register a given line pressure (96.5 KPa=14 psi) on a gauge that was mounted at the end of the monorail closest to the pump (Figure 4). The tank, pump, controller, and battery were all mounted on a modified bicycle. We used an electric switch on a wire that extended to the end of the monorail to start and stop the sprayer (Figure 4).

For the April 2015 application, sprayer pressure was 96.5 KPa, and sprayer output was 9.7 ml/sec through the three nozzles of the spray boom. To produce the desired spray volume of about 300 L/ha (1.27 L for each 42 m^2 plot), the uptake spool was rotated so that the boom traversed the 7 m plot length in about 33 seconds. This required 8.5 revolutions of the spool. To pace the spool rotation rate, we used a metronome (Ludwig metronome application on iPod Touch) that provided audio signals for each quarter and full revolution at the desired rate of travel. For this configuration, a metronome speed of 62 beats per minute (0.97 sec/beat) provided the proper pace for controlling the speed of the boom to approximately 0.21 m/sec. All spray swaths were sprayed in one direction, starting at the far (healthy) end of the plot and ending at the near (disease front) end.

Phosphite applications 2011 and 2013

We originally sprayed the six phosphite-treated plots on 28 April 2011 at 12.4 kg/ha with 0.03% Break-thru® surfactant. Winds were variable during the applications, which were conducted between 11:25 and 18:55 PDT. Due to plot orientations, spray drift beyond the plot borders was minimal and in most cases was directed away from control plots. Plots were treated at a spray volume of about 300 L/ha (1.27 L for each 42 m^2 plot). Applications were made using the monorail spray apparatus described above (Figures 1-5).

We retreated the six phosphite-treated plots using the same rates and methods on 19 April 2013. This was about a week earlier than the date of our 2011 application. However, because winter/spring rainfall had been low in 2013, plant phenology and water stress levels were similar to conditions in the original 2011 applications. Skies were clear and sunny and the air temperature ranged from about 26 to 30 C. Winds were light and from the west during the applications, which were conducted between 12:05 and 18:45

PDT. As before, spray drift beyond the plot borders was minimal and did not affect control plots. Al Franklin, retired BLM staff serving as a BLM volunteer, assisted us with the spraying operation.

Phosphite applications 2015

We retreated the six phosphite-treated plots using the same rates and methods as described above on 3 April 2015. This was earlier in the spring than the two previous applications. However, because winter/spring rainfall had been low in 2015 and phosphite uptake is poor in drought stressed plants, we scheduled the application earlier in the season. Plant phenology was similar to conditions in the previous two applications. Skies were clear and sunny and the air temperature was about 22 C. Winds were light and from the west during the applications, which were conducted between 11:38 and 18:20 PDT. As before, spray drift beyond the plot borders was minimal and did not affect control plots. Beth Brenneman (BLM) assisted us with the spraying operation (Figure 5).

Plots were treated with 12.4 kg/ha and 0.03% surfactant spray solution that was made a day in advance of the application. Due to the amount of spray held in the lines and tank and the losses associated with calibration, line purging and other processes, we made up 15 L of spray, about twice the volume actually needed for the plots, which provided an adequate buffer of spare material. The spray volume target was 300 L/ha (1.27 L for each 42 m^2 plot, 7.6 L total). From measurements of leftover materials and additional estimates of small losses, we calculated that the applied amount was about 99% of the target volume.

Figure 2. Spraying apparatus used to apply phosphite to phase 1 (300 L/ha) plots. Spray apparatus set up over plot 8A in 2011 (top) and in use in the same plot in 2013 (center; photo by Al Franklin). Bottom: apparatus in use in plot 1A in 2011. Note lack of disease front for plot 8A. Turning the spool at a regulated rate pulls the three-nozzle spray boom along the rail at a constant speed.

Figure 3. Spraying apparatus dissembled and packed for transport between plot areas. The entire apparatus is assembled and disassembled by hand by sliding sections together. Most joints are held together through the use of spring-action buttons. Four Phillips-head screws are used to lock two stiffeners in place.

Figure 4. Pump end of monorail sprayer showing pump power switch (center left) and pressure gauge (center right). Photo by Al Franklin.

Figure 5. Beth Brenneman and Ted Swiecki confer during phosphite application 3 April 2015.

1.1.4. Disease evaluation

For each plot, we laid a measuring tape along the 6 m wide front edge of the plot (adjacent to diseased area for plots 1, 2, 3, and 4) using the two plot corners markers as reference points. Starting at this reference line, we measured the distance to the first healthy foliage (either *A. myrtifolia* or *A. viscida*) in 20 cm-wide belt transects placed at 0.5 m intervals along the reference line. The belt transects were centered at the sample distance and oriented perpendicular to the tape. Distances toward the plot center were recorded as positive numbers and distances in the opposite direction were recorded as negative numbers. The species of the first live foliage encountered was recorded (either *A. viscida* or *A. myrtifolia*). Initial baseline data were collected 6/8/2011. Subsequent evaluation dates were 1/24/2013, 9/30/2013, 4/21/2014, 3/9 and 4/20/2015, 3/25/2016, and 8/5/2016 (Table 1). We measured distance to the first live *A. myrtifolia* as a separate variable beginning with the 9/30/2013 evaluation.

1.2. Results

Repeated measures multivariate analysis of variance (MANOVA) of the distance to the first live *A. myrtifolia* from the plot front showed that plot set, treatment, plot set by treatment, and date of evaluation, were all significant at $p<0.0001$. Plot set 8, which is not yet at an active front, was not included in this analysis. Disease advance from the front edge of the phosphite-treated plots continued to be suppressed through August 2016 (Figures 6-8, cover photo). Plants have also died at the edges of some of the treated plots where these edges are now adjacent to *P. cinnamomi*-related mortality. As noted above, the

phosphite application rate is one-half of the target rate at these outer plot edges due to lack of overlapping spray from an adjacent nozzle.

The average advance of the disease front in the phosphite-treated plots (excluding plot set 8, which is not yet at an active front) from 2011 through August 2016 was 26 cm, compared to an average advance of 396 cm in the control plots (significantly different at $p<0.0001$, one-sided t test). A few plants have died near the leading edge of the disease front in some of the treated plots. This scattered mortality suggests that we are operating near the dosage limit for efficacy and that it may not be possible to reduce the phosphite rate (12.4 kg/ha) or extend the retreatment interval beyond 2 years without a reduction in efficacy.

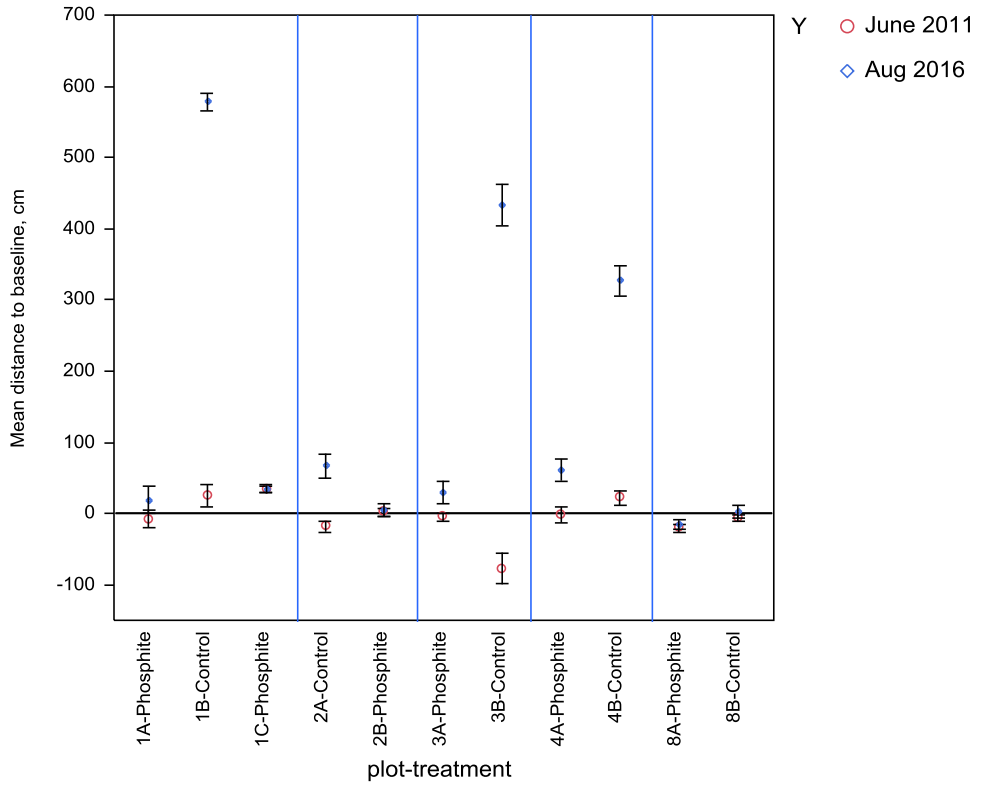

Figure 6. Change in average position of disease front from baseline between June 2011 to August 2016 in phosphite-treated and adjacent control plots at five locations at the Apricum Hill preserve. Potassium phosphite was applied as an overhead spray at 300 L/ha at a rate of 12.4 kg/ha in spring 2011, 2013, and 2015. Error bars represent one standard error of the mean. Plot set 8 is not yet along an active disease front.

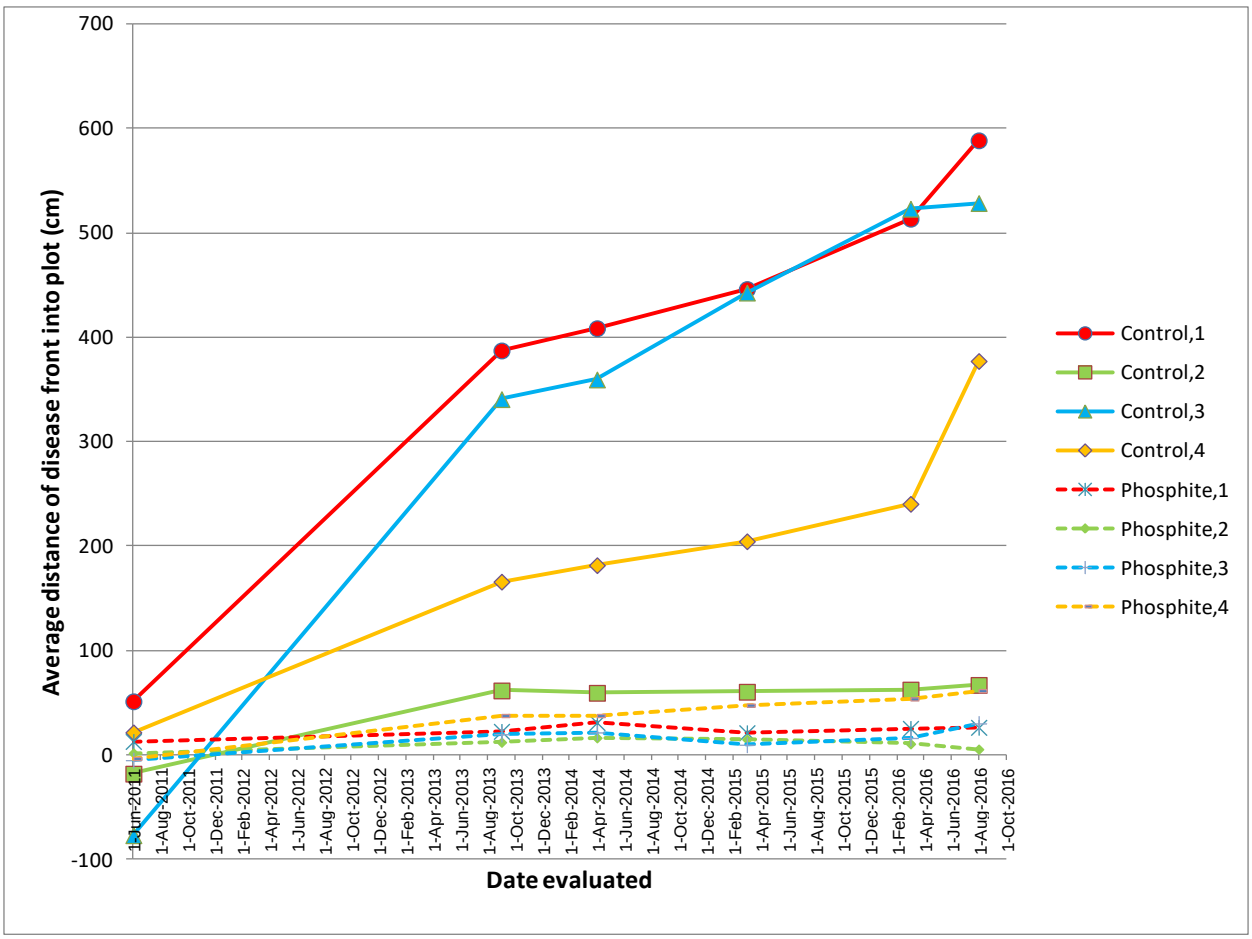

Figure 7. Average distance (cm) to the first live *A. myrtifolia* foliage along each plot front edge between June 2011 and July 2016 by treatment and plot. Negative distances seen in the baseline 2011 data indicate that some plant edges were to the outside of the reference line used to mark the plot front.

As shown by the significant MANOVA model term for plot set and illustrated in Figures 6 and 7, different rates of disease advance have been observed in the various control plots. In particular, disease advance into the control plot in plot set 2 has been particularly slow. This plot is on a slight mound, and the drought conditions that have prevailed over most of the period since the plots were established may account for the slow movement of disease into the plot. The difference in disease advance between plots also accounts for the significant plot set by treatment interaction term in the MANOVA model. The effect of phosphite treatment on disease advance is greatest in those plot sets where the most disease has developed in the corresponding control plot.

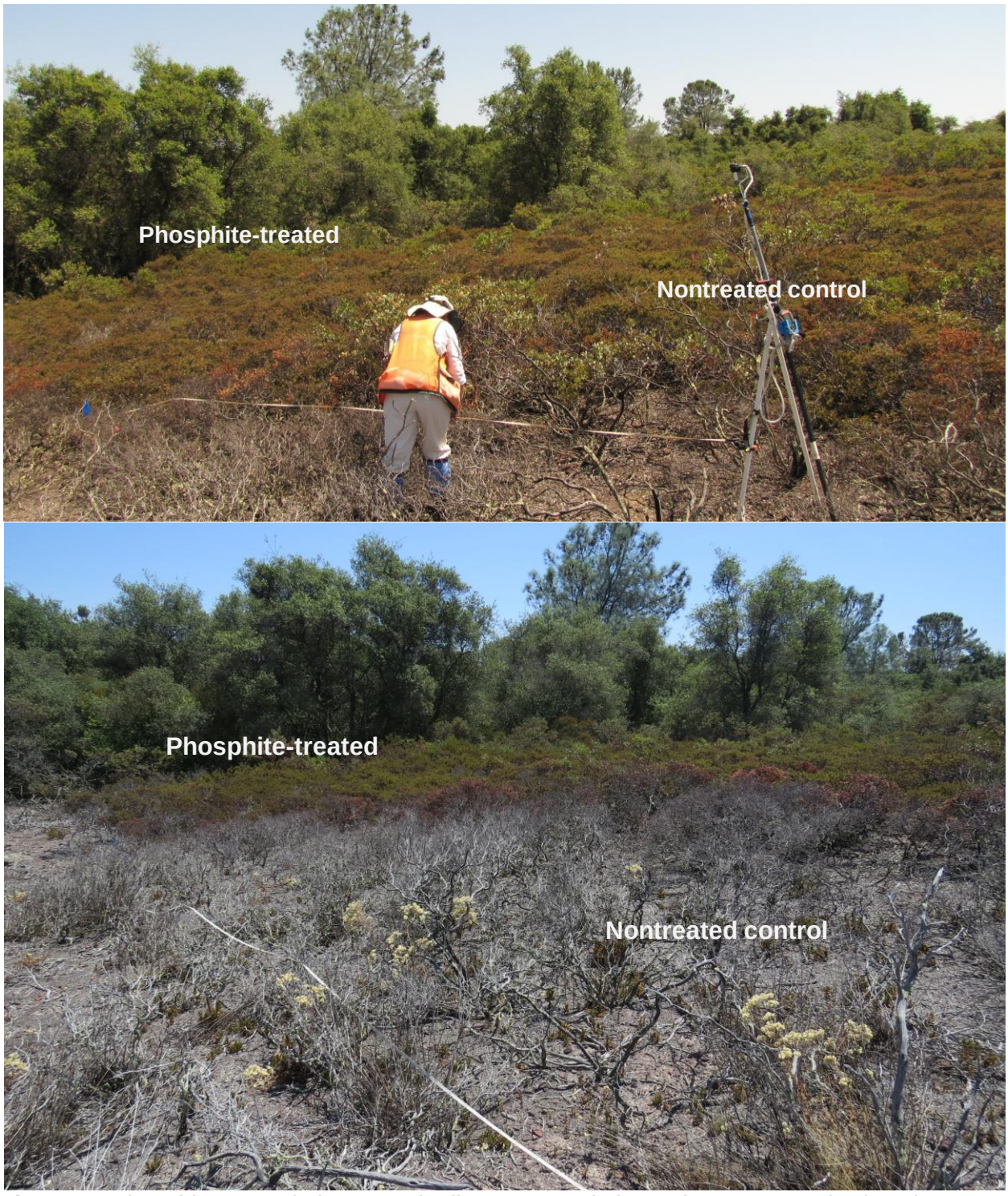

Figure 8. Phosphite-treated plot 1A and adjacent control plot 1B in June 2011 (top) and August 2016 (bottom). All manzanitas in the control plot to an average distance of 6 m from the baseline (measuring tape in both images), and in the untreated buffer between the two plots, died. Only a small amount of mortality along the front edge (to left in image) has been seen in the phosphite-treated plot. Some recent dead plants are visible that extend into the edge of the treated plot along the nontreated side, where application rate is reduced.

2. Phase 2 plots: Ultra-low volume (30 L/ha) application phytotoxicity trial

Results reported above showed that alternate year phosphite applications in standard volume sprays (300 L/ha) at 12.4 kg/ha significantly reduced the rate at which *P. cinnamomi* mortality centers expand. It is practical to apply phosphite at this rate in areas accessible by vehicle or small areas with only foot access (using backpack or bicycle-mounted sprayers). However, it would be difficult to treat larger patches in relatively inaccessible terrain at this application volume due to the amount of liquid that needs to be transported. Alternatives for such areas include aerial application or the use of much lower application volumes applied by hand. These situations require a shift to ultra low volume (ULV) applications.

As application volume is reduced, the phosphite solution concentration needs to be increased to maintain a constant rate of phosphite per unit area. However, increasing phosphite and/or surfactant concentration may increase the risk of foliar phytotoxicity. We therefore conducted a series of spray mixture compatibility tests and additional microplot phytotoxicity tests using higher concentration / ULV applications to determine rates that would not cause significant damage.

2.1. Methods

2.1.1. Ultra low volume sprayer

We researched several different sprayer types which would allow us to treat plots at about a 30 L/ha application volume. This volume is typical for a helicopter application. Note that this is a 10-fold reduction in application volume per hectare compared to the standard ground application volume (300 L/ha) used in phase 1 plots. To minimize the production of fine droplets that are prone to drifting and evaporating, we selected controlled droplet application (CDA) equipment for use in this system.

We purchased a Dramm MH-1 Microfit Herbi® Standard Lance CDA sprayer for the study applications. These sprayers are widely used for ULV herbicide applications and have been used by Australian researchers for phosphite applications in *P. cinnamomi* management studies. This sprayer uses gravity to drip spray solution though a tube with a plastic tip onto a spinning disk. The disk, which is powered by a 6 VDC motor, breaks the solution into fine droplets within a relatively narrow diameter range and spreads them out in a 1.2 m diameter ring (=spray swath width).

To eliminate the need to keep the spray solution reservoir elevated and to provide a more constant flow rate, we removed the sprayer reservoir and connected the spray line to a 12 VDC variable flow peristaltic pump (SP300, APT instruments). The pump was fitted with tubing to provide a flow rate of 0.86 ml/sec with the pump motor running at its maximum speed. Power to both the 12 VDC pump and 6 VDC spinning disk motors are supplied by a sealed compact 12 VDC battery. An adjustable 1.5-37V DC/DC buck converter board (Marlin P. Jones & Assoc. Inc.) was used to step down the voltage from the battery to the sprayer disk motor. The battery, pump, solution reservoir, and DC/DC converter were consolidated in a modified tackle box. Switches to separately control the motor and pump were mounted on a modified extendable pole to which the spinning disk applicator was attached. The extendable pole allowed us to extend the spray swath to a distance of more than 2.5 m from the operator. The final spraying apparatus is shown in Figure 9. The extendable pole and dual switch box were not incorporated in the initial version of the sprayer used for the phytotoxicity tests.

2.1.2. Phosphite concentration

The phase 1 plots were treated with 12.4 kg/ha, applied using a solution of 0.0413 kg/L at a volume of 300 L/ha. To maintain the same rate when spraying only 30 L/ha requires a 10 fold increase in phosphite concentration (to 0.413 kg/L, Table 2). However, we were concerned that absorption of the phosphite might be less efficient with the ULV spray, so we also tested a higher rate, 15 kg/ha, which represents a 20% increase in rate per unit area. At this rate, the solution concentration is 0.5 kg/L, which is 81% of the strength of the undiluted product. Another reason for using this higher rate was to see if some light phytotoxicity would develop with the ULV spray. To ensure that we were applying phosphite close to the maximum tolerated dose, we needed to see some evidence of phytotoxicity at one of these concentrations to verify that the plants were taking up the applied phosphite.

2.1.3. Surfactant compatibility tests

In phase 1 tests, we used the organosilicate surfactant Break-thru® at 0.03% by volume in the spray solution. This was the surfactant used by the Garbelotto lab (UC Berkeley) for their initial phytotoxicity and efficacy assays. However, this surfactant is not on the BLM-approved adjuvant list, so we looked for alternatives from the approved list that might perform similarly. We obtained samples of the non-ionic surfactant Activator90® and Freeway®, a surfactant blend of alcohol ethoxylates, silicone-polyether copolymer, propylene glycol and dimethylpolysiloxane, from the manufacturer, Loveland Products Inc. (Greely, CO) for use in our tests.

Rates of surfactant used for ULV herbicide applications vary widely, and we could not find much published information on the use of surfactants with ULV phosphite applications. For example, experiments in Australia have used 0.2% BS1000 (Cropcare Australasia, Queensland) surfactant (Shearer and Crane 2009) and 2% Synertrol Oil (Barret 2003). Some of the herbicide literature we examined suggested that efficacy was superior if the concentration of surfactant was maintained at the same application rate per hectare. This would require a 10-fold increase in the concentration of surfactant (to 0.5% v/v for Break-thru and Freeway, 1.25% for Activator90) beyond the level used in standard spray volume applications.

We mixed small batches of phosphite and each of the three surfactants prior to our initial application and observed that spray solution precipitates developed. Consequently, we performed a series of mix tests to find compatible mixtures that would not form precipitates. Distilled water was used in all solutions to avoid potential interactions related to water quality. Activator90 was incompatible with the 0.413 kg/L phosphite solution used for the 12.4 kg/ha ULV applications, even when added at both the standard high (0.5% v/v) and low (0.125% v/v) label rates. The higher rate flocculated immediately and formed a surface scum. The lower rate had similar problems that developed some minutes after mixing. The flocculates formed in the solution would clog nozzles and result in an uneven spray pattern. Freeway formed a fine precipitate with the 0.413 kg/L phosphite solution at concentrations of 0.5% and 0.25%, as well as the high label rate of 0.125% v/v. However, the low label rate of Freeway (0.05%) was compatible with 0.413 kg/L phosphite, as was 0.05% Break-thru.

Figure 9. Ultra low volume sprayer used for study applications. Top: sprayer in use. Pump and spinning disk motor are turned on with a single switch. A second switch deactivates the disk motor, allowing only the pump to run. This setting is used for filling the tubing to the spray head. Bottom: box holding spray reservoir (bottle in left image) as well as the pump, battery, and voltage converter (right image) plus electrical connections and tubing connections to the pump.

Table 2. Rates and concentrations of potassium phosphite and surfactants used in standard volume (300 L/ha) and ULV (approximately 30 L/ha) applications.

Plot type:	Phase 1 and 4 plots disease control	Phase 2 plots ULV phytotoxicity microplots low rate	Phase 2 plots ULV phytotoxicity microplots high rate	Phase 2 and 3 plots disease control low rate	Phase 2, 3, and 4 plots disease control high rate
Potassium phosphite rate, kg/ha	12.4	12.4	15	8	10
Volume, L/ha	300	30	30	28.3[1]	28.3[1]
Potassium phosphite concentration, kg/L	0.04133	0.4133	0.5	0.2824	0.3529
Surfactant concentration v/v	0.03%	0.05%	0.05%	0.05%	0.05%

[1]Final configuration of the sprayer for 2015 applications had a calibrated flow rate of 0.85 ml/s; phosphite concentrations were adjusted to reflect this actual application volume, spray head velocity (1 m/s), and number of passes (2 passes).

2.1.4. Phytotoxicity test using microplots

We set up 14 1-meter square microplots in three separate areas (Figure 10). The corners of each microplot were marked with flagging tape and the microplot number was marked on a wire stake flag. GPS coordinates were also recorded for each plot.

Based on our surfactant compatibility tests, we discontinued consideration of Activator90. The phytotoxicity microplots were sprayed at two phosphite rates (12.4 and 15 kg/ha; 0.413 and 0.5 kg/L, respectively). The two rates were duplicated in mixtures using one of two surfactants (Break-thru and Freeway), both at 0.05% v/v. This provided four different surfactant/phosphite treatments. In each of the three plot areas, one plot was randomly assigned to be sprayed with each of the phosphite concentration × surfactant combinations. In addition, one area had two additional microplots. One was sprayed with Break-thru and the other with Freeway mixed only with distilled water (0.05% v/v).

Plots were sprayed 9 April 2014, under mild weather conditions with minimal wind (Figure 11). A PVC pipe frame was laid over the plot to clearly mark its boundaries. A PVC pipe marked in 24 cm increments was laid along one edge of the frame and was used as an index to help regulate sprayer travel rate. A metronome running at 60 beats per min (Ludwig metronome application on an iPod Touch) was used to set a cadence for moving the spray head at a rate of 0.24 m/s across the plot.

Unlike the flat fan-shaped pattern produced by the spray nozzles used in phase 1 plots, the CDA sprayer pattern is a 1.2 m ring with an open center. To obtain uniform coverage at the target volume, both edges of the ring need to pass over the sprayed area. For phytotoxicity microplots, it was necessary to start with the spray head at least 60 cm from the microplot edge and finish with the head 60 cm beyond the plot edge to make sure that both sides of the spray pattern passed over the whole plot.

As a result of this spray pattern, plants outside of the plot on either side actually receive a reduced phosphite dose, about 50% of the dose applied within the 1 m^2 plot. Observations of these areas allowed us to estimate the phytotoxicity of doses that were about half of the applied doses, i.e., about 7.5 and 6.2 kg/ha.

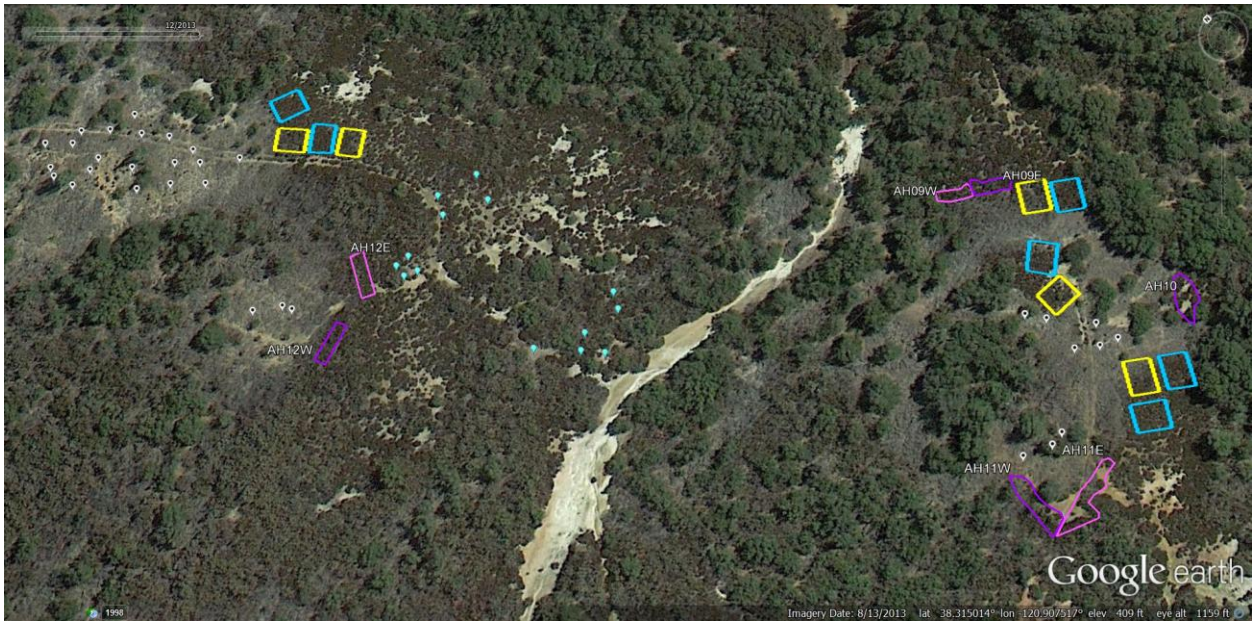

Figure 10. Locations of phase 2 phytotoxicity microplots for ULV applications (cyan dots) at Apricum Hill. Phase 1, 2, 2/3 (plot sets 9-12), and 4 phosphite study plots are also shown. Phase 1 plots (300 L/ha at 12.4 kg/ha - blue rectangles, control - yellow rectangles) are the same as shown in Figure 1. Phase 2/3 ULV plots sprayed at approximately 30 L/ha are outlined in pink (8 kg/ha) and purple (10 kg/ha). White and black symbols mark individual seedlings that were either treated with phosphite or left as nontreated controls. Only a portion of the individually-treated study seedlings had individual GPS points; others (not shown) were located near these symbols. Imagery: Google Earth, August 2013.

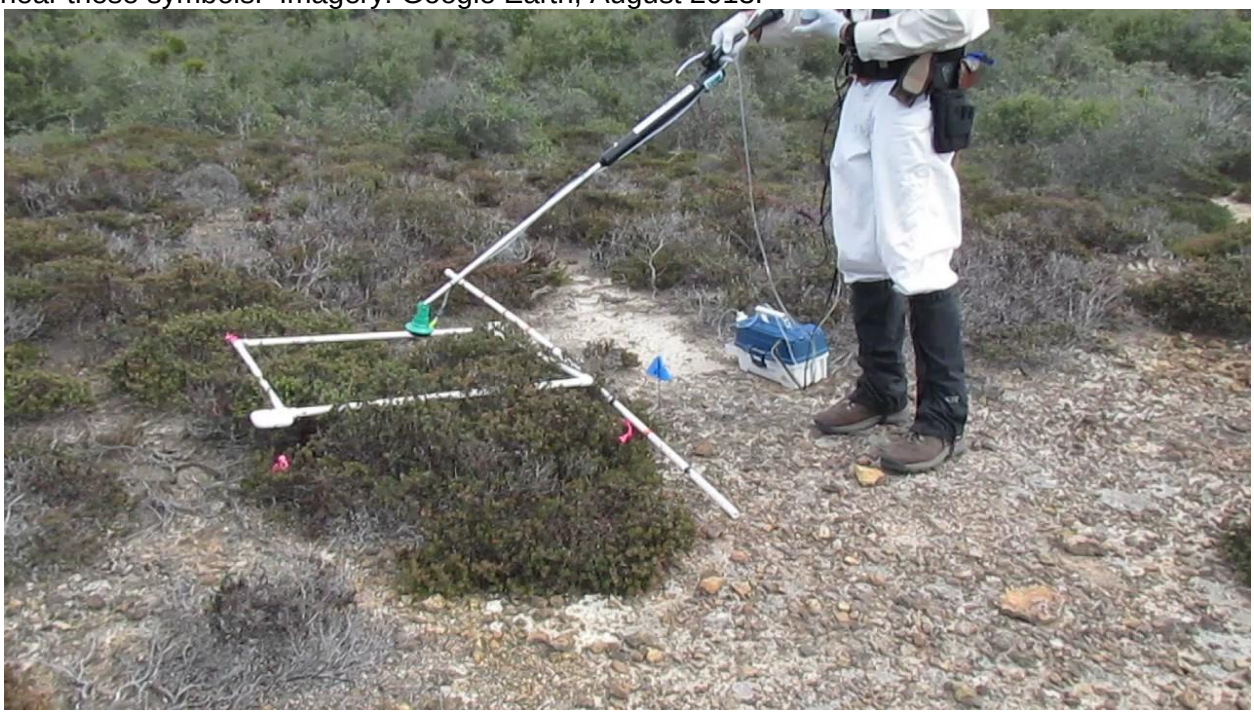

Figure 11. Application of potassium phosphite to microplot with the CDA sprayer. The PVC pole along one edge of the frame is marked in 24 cm increments. IPOD metronome was set so the audible signal prompted the applicator to move the spray head at the appropriate rate. Pink flags mark the corners of the microplot.

Each microplot was photographed prior to the application. The microplots were rephotographed and visually evaluated for phytotoxicity symptoms on 21 April and 6 May 2014.

2.3. Results and discussion

The surfactants alone caused no visible symptoms on the treated plants. However, microplots treated with phosphite showed varying amounts of phytotoxicity (Table 3). The primary symptom associated with treatment was leaf tip necrosis, and the proportion of leaves within each microplot with phytotoxicity symptoms varied between and within the plots. Phytotoxicity was generally seen in a higher proportion of leaves treated at 15 kg/ha than at 12.4 kg/ha. Buffer areas on each side of the plots that received approximately half rates (7.5 and 6.2 kg/ha) did not show phytotoxicity symptoms.

The 12.4 kg/ha rate used in this trial was previously tested at the 300 L/ha rate on 31 March 2011, 3 weeks earlier that the application date in the current experiment (Swiecki and Bernhardt 2012). No significant phytotoxicity was observed in that 300 L/ha application, in which the phosphite concentration was 0.041 kg/L. In the current test, even though the dose rate per unit area was the same (12.4 kg/ha), some phytotoxicity developed. This suggests that the higher phosphite concentration (0.41 kg/L) is more likely to cause phytotoxicity. This may be related to slight variations in the amount of solution delivered to small areas, which cannot be avoided.

Because the 0.41 kg/L concentration was somewhat phytotoxic and provided no margin of safety, we decided to reduce the phosphite concentration and rate per unit area for larger scale tests. For our initial ULV disease control plots, we decided to test two lower rates, which would provide a margin of safety. However, it was possible that at these lower dosages efficacy might be reduced and/or a shorter retreatment interval would be required. The rates we selected for initial phase 2/3 plots were 10 kg/ha (high rate) and 8 kg/ha (low rate), which have corresponding phosphite concentrations of 0.33 and 0.27 kg/L. The lower rate (8 kg/ha) was close to the 7.5 kg/ha half rate at the edges of the phytotoxicity plots which did not result in phytotoxicity. The 10 kg/ha represents nearly the midpoint between the 8 kg/ha rate and the 12.4 kg/ha rate.

Table 3. Results of microplot phytotoxicity test comparing two phosphite rates and two surfactants, 3 plots per dilution/surfactant combination.

Phosphite rate (kg/ha)	Phosphite concentration (kg/L)	Surfactant	Phytotoxicity rating (number of microplots)		
			Acceptable	Intermediate	Not acceptable
15	0. 5	Break-thru	0	3	0
15	0. 5	Freeway	1	2	0
12.4	0. 41	Break-thru	0	3	0
12.4	0. 41	Freeway	1	2	0

Spray application patterns ULV versus standard rate

We used white vinyl tags to visualize the spray droplet pattern produced by the CDA sprayer versus a pump-up sprayer calibrated to deliver a standard rate application using a Teejet XR 11001VS nozzle. As shown in Figure 12, the 300 L/ha application provides more complete wetting of surfaces with a wide range of droplet sizes. The spray deposit from the 30 L/ha application with the CDA sprayer leaves much

more space between droplets, but the distribution uniformity is quite good and the droplets show less variation in size. Nonetheless, the small sized droplets may dry more quickly and reduce the amount of material absorbed into the plant to below the target amount.

3. Phase 2/3 plots: Efficacy tests using ultra-low volume (30 L/ha) application and reduced treatment band width

Because potential plot areas are limited at Apricum Hill, we incorporated elements of both phase 2 (ULV application efficacy) and phase 3 (application band width) tests into the new plots we established in spring 2014. Based on the appearance of phytotoxicity in plants treated with phosphite via ULV application, it was clear that phosphite was being taken up by the sprayed plants. Hence, we could reasonably expect to see some level of efficacy in plants treated via ULV application.

Furthermore, our monitoring of previously treated plots (12.4 kg/ha at 300 L/ha) indicated that the *P. cinnamomi* disease front has not moved more than about a meter into any of the treated plots (Figures 6 and 7) and that few *P. cinnamomi* propagules were detected 0.75 m from the disease front (see Section 5 below). Previous work (Swiecki and Bernhardt 2005) showed the *P. cinnamomi* was rarely detected 2.5 m from a disease front. Based on these observations, it seemed likely that the narrowest sprayed area that is likely to offer reasonable protection from disease spread would be about 2.5 meters.

We combined these two factors and set up disease control plots that were sprayed using an ULV application in a band that was 2.4 m wide (2 spray swaths using the ULV sprayer). We used two different application rates (8 and 10 kg/ha) that were low enough to avoid phytotoxicity. These rates are lower than the rate used in phase 1 plots (300 L/ha), so the new plots were expected to demonstrate the minimum spray application parameters (phosphite rate and treated area width) needed to obtain acceptable levels of efficacy.

3.1. Methods

3.1.1. ULV sprayer: calibrating and regulating the speed of the spray head

The main technical issue associated with the ULV sprayer setup had been finding a way to precisely regulate the speed of the spray head as it passes over the vegetation. This is challenging because the spray head is typically moved using a combination of motions, both walking and sweeping the pole over the vegetation. There are various techniques that can be used to help maintain a calibrated speed along straight-line paths, but the incorporation of curves or arcs in the spray pattern complicates matters significantly. Initial spraying of ULV plots set up 6 May 2014 used a calibrated rope as a guide to keep spray head velocity at the desired speed. The rope was marked at 0.25 m increments. The applicator moved the spray head across the plot using an audible beat from a metronome to time movement (1 beat per increment) (Figure 13).

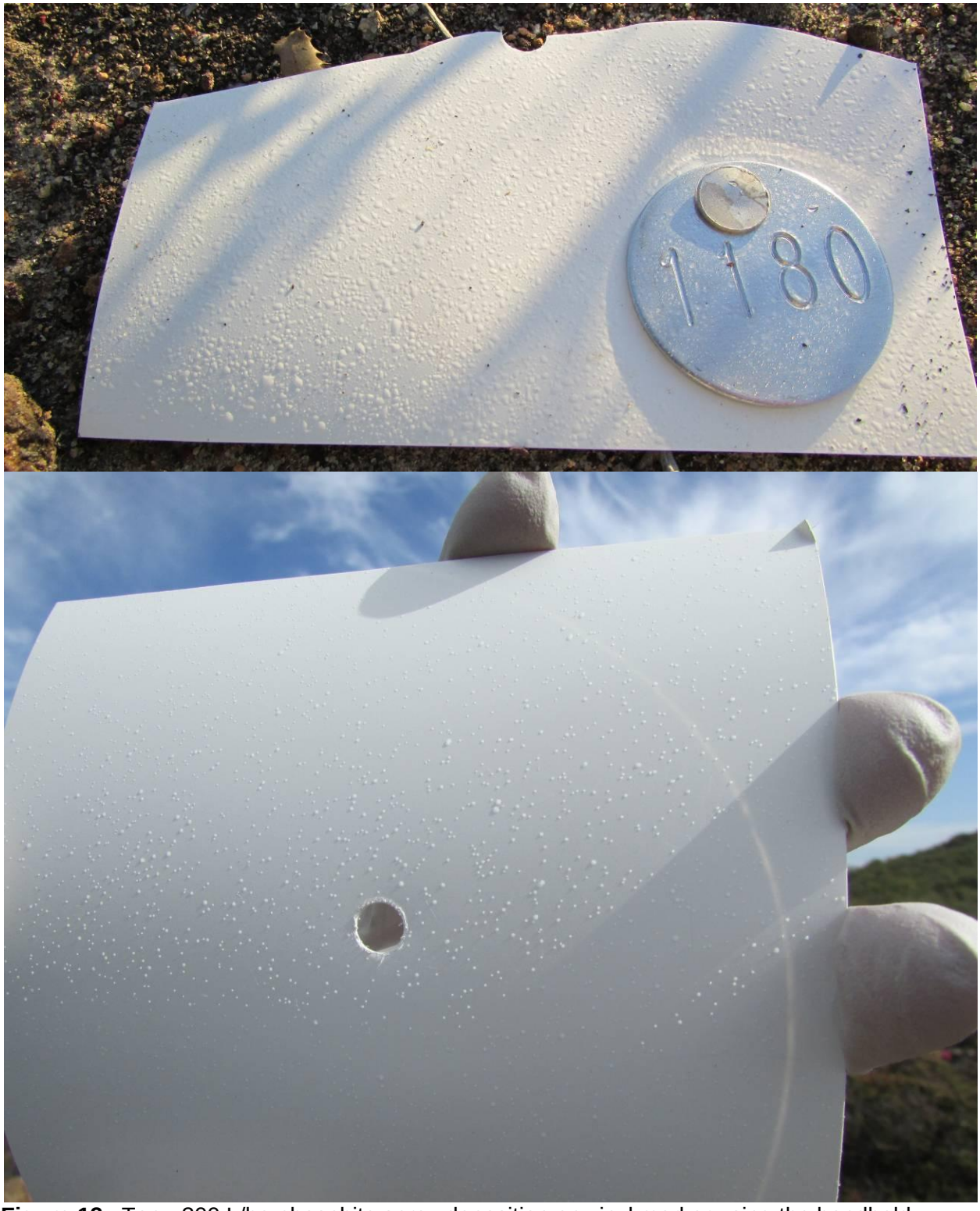

Figure 12. Top - 300 L/ha phosphite spray deposition on vinyl marker using the handheld sprayer shown in Figure 26. Bottom - 30 L/ha (ULV) spray droplet distribution on vinyl placed in a phytotoxicity trial microplot sprayed with the CDA sprayer.

Figure 13. Spray being applied with CDA sprayer to plot 9W. The marked yellow rope was used as a guide to keep spray head velocity at the desired speed. The rope was marked at 0.25 m increments. The applicator moved the spray head across the plot using an audible beat from a metronome to time movement (1 beat per increment).

To use the ULV sprayer effectively, we needed a more efficient way to allow the spray operator to modulate the speed of the spray head to keep it within the target speed. We thought that a sensor mounted on the spray head that tracks true ground speed and provides an auditory output would be the best option for hand-operated equipment. We identified a compact unit, the FlySight GPS (http://www.flysight.ca/index.htm), which calculates Doppler speeds and is programmed to provide auditory feedback. This instrument was designed to provide feedback on glide ratio to skydivers via audible tones. The stock software in the unit was not ideally suited to our use, but the product's developer, Michael Cooper, indicated that he could work with us to modify the software. We specified that we would like the unit to produce a solid, mid-frequency tone (middle C, about 262 Hz) when operating at the target speed. As the speed deviates from the target, the tone rises (if speed is too high) or drops (if speed is too low) while at the same time being broken up into pulses that increase in frequency as the deviation increases. This provides fairly intuitive auditory feedback that allows the operator the speed up or slow down the motion of the spray head as needed to maintain it in the target range. Michael Cooper programmed this behavior into the firmware and software of a FlySight unit that we purchased.

Given the limitations of the Doppler GPS resolution, we selected a target speed of 0.5 m/s, which is twice as fast as we had used in previous ULV applications. To apply the target dose of 10 kg/ha, we maintained our solution concentration at 0.35 kg/L and made 2 passes over each treated swath from opposite directions. This application pattern is likely to provide a more uniform and complete application than a single swath, given that there are some irregularities in any given spray pass. Because the FlySight GPS is light (about 60 g with a mounting bracket we added) and compact (about 1.5 cm tall, 4.8 cm length and width), we were able to mount it to the top of the CDA sprayer head (Figure 14). Sound output is routed

via an audio cable to a portable battery-powered speaker (we used a HMDX model HX-P140) that is attached to the spray boom handle near the control switches.

We tested and practiced with the FlySight to check the tone output against ground speed measured on a tape measure. The unit appeared to work well, although we had intermittent problems getting the sound to start, which required repeated powering on and off of the GPS unit. Despite this issue, we used this unit in the field on 26 November 2014 to treat plots AH13-18. The intermittent starting problem reappeared and eventually got to the point that we could not get the sound to activate after the first swath of the last plot treated (plot AH18). After this, we contacted Michael Cooper with a detailed description of the problem we were having with the sound after our return. He was eventually able to replicate the error and correct the firmware to solve this intermittent problem, which was the result of a failure of the sound to restart after a memory buffer dump.

Figure 14. Head of the CDA with attached FlySight GPS unit. The FlySight is mounted on a metal plate that is magnetically attached to bracket on the spray head. The audio cable (black cord) runs back to portable mini speaker that is attached to the spray handle. The unit is place in a plastic bag to protect it from blown spray.

3.1.2. ULV plot set up and spray application initial plots: 9E, 9W,10,11E, 11W, 12E and 12W

We set up seven plots 6 May 2014 along active disease fronts to test the efficacy of ULV treatment (Table 4, Figure 10). ULV plots were applied as banded treatments, generally 2.4 to 3.6 m wide, along disease fronts rather than the strictly rectangular plots as used for the standard volume treatments. These treatments were done in a manner that would be suitable for ground application on an operational basis. Plot layout varied slightly from plot to plot, depending on the nature of the disease front. In plots 9E and 9W (Figure 15), 10, and 11E and 11W, phosphite was applied to plants that were at the edge of an active disease front, although in plots 10 and 11, there were some unvegetated gaps between the recently killed plants and live plants. Furthermore, the active disease front for plot 11W is at the back of the plot, which is not easily accessed. The edge of the disease front adjacent to plots 12E and 12W was very irregular, so

the treated areas were variable distances (0.27 to 3.1 m) from the disease front. Where enough area was available to establish two adjacent plots, we treated one plot at 8 kg/ha and the second plot at 10 kg/ha. Plot 10 was too small to subdivide, so we used only the 10 kg/ha rate. We used the Freeway® surfactant blend at 0.05% (v/v) for all plots. Phosphite concentrations of the spray solution for each plot are shown in Table 4. Spray applications were made on 6 May 2014 under mild temperatures with variable overcast (Figure 13).

Figure 15. Plot 9 at time of initial plot setup, viewed from within the adjoining *P. cinnamomi* mortality center. Pink flags mark the edge of healthy foliage. The left side of the plot (9W) was treated at 8 kg/ha), and the right side (9E) was treated at 10 kg/ha).

We checked these plots 2 Jan 2015. Based on advance of the disease fronts into the treated areas, particularly for plots 9W and 9E, we decided to convert the plots to a split application regime to double the applied dose. We planned for 4-6 weeks between the two applications. The first dose of the split application was applied 23 Jan 2015 and the second dose was applied 9 March 2015, for a total dose of either 16 or 20 kg/ha.

Table 4. Initial application rate and monitoring system used for plots treated by ULV application 6 May 2014.

Plot number	Phosphite rate (kg/ha)	Monitoring system		Monitoring line length (m)
		Individual plant ratings	Distance to first healthy *A. myrtifolia*,	
9W	8	yes	yes	5.5
9E	10	yes	yes	6
10	10	yes	yes	6
11E	8	no	yes	6
11W	10	yes	no	n.a.
12E	8	no	yes	8
12W	10	no	yes	8

3.1.3. ULV plots AH13- AH18 - 26 November 2014 / 20 February 2015

The first ULV plots were set up in May 2014, after spring rains finished. We intended to set up another set of plots after the first rains of fall. It is known that drought-stressed plants do not absorb phosphite. We had hoped to set up several ULV plots on County of Amador property west of the transfer station Buena Vista Road. However, as discussed in previous progress reports, we encountered several issues when scoping this property. For several reasons, including access and security difficulties, we did not attempt to establish study plots at this location.

At Apricum Hill, we investigated several different parts of the preserve we had not previously explored and set up six new plots (plots AH13- AH18) along disease fronts in several areas (Figure 15). All plots were placed in areas where they could prevent or slow movement of *P. cinnamomi* into remaining live stands or patches of *A. myrtifolia*. Many of these new plots were located along a main path/drainage that runs through the center of the preserve. Mortality due to *P. cinnamomi* affected many stretches along this drainage. Depending on the plot geometry, we treated bands of vegetation that were two to three spray swaths wide, i.e., 2.4 to 3.6 m from either the disease front or from unvegetated areas along the main path. We used the FlySight to time spray applications for five of the six new plots. For the last plot (plot AH18), which was both linear and adjacent to a strip of open ground, we reverted to timing the last swaths using a metronome as had been done with the spring ULV applications due to technical difficulties with the FlySight.

Linear fronts of this set of plots total approximately 75 meters. We treated these plots at 10 kg/ha on 26 November 2014. After noting the lack of control associated with the ULV treatments on 2 January 2015, we decided to convert these plots to split application plots by reapplying phosphite at 10 kg/ha (20 kg/ha total application) on 20 February 2015.

3.1.4. ULV plots AH19-AH25 - 2 January 2015 / 20 February 2015

We set up an additional set of ULV plots (AH19-AH25) on 2 Jan 2015. These plots were treated at 20 kg/ha in two split applications of 10 kg/ha each. The second application occurred 20 February 2015. In treated stands with fairly linear edges, plots were generally 3.6 m wide (3 swaths). In areas with more irregular stands, patterns were varied, resulting in effective widths from 2.4 m to about 6 m wide.

3.1.5. ULV plots AH26-AH28 - 2 January 2015

Plots 26/28 and 27, which were treated with a 10 kg/ha ULV application on 2 Jan 2015 showed moderate levels of phytotoxicity on 20 Feb 2015, when the second application was scheduled. Phytotoxicity generally indicates high levels of phosphite in the plant, so we did not reapply phosphite to these plots to avoid excessive phytotoxicity. We have previously noted variation in sensitivity to applied phosphite in Ione manzanita, which may be due to localized soil chemistry or plant phenotype. The edges of these plots were somewhat irregular, and the width of the treated band was mostly around 3.6 m. The current set of phosphite-treated plots and application dates are summarized in Table 1 and shown in Figure 16.

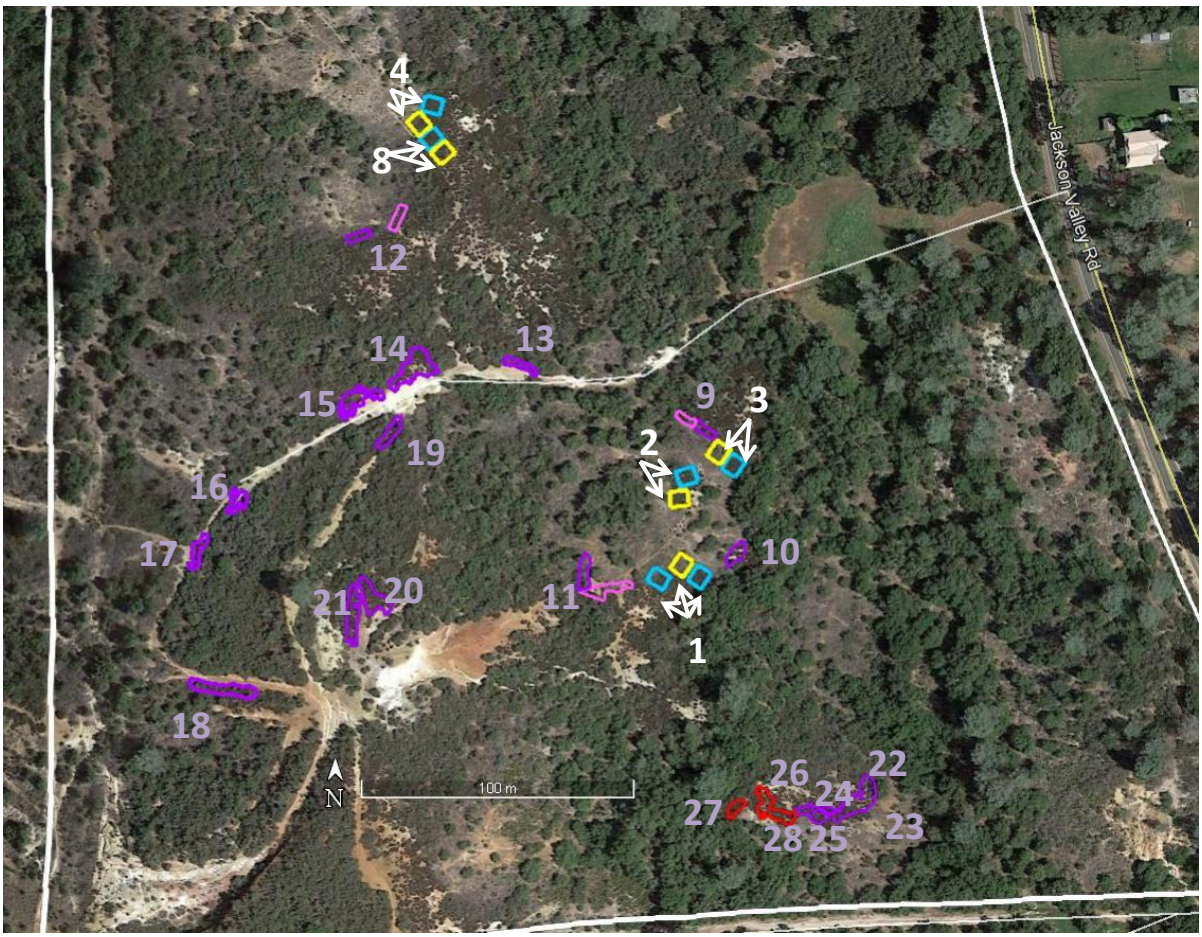

Figure 16. Location of phosphite-treated plots listed in Table 1 at Apricum Hill Preserve (seedling plots not shown). Yellow (control) and turquoise (treated) squares are standard volume (300 L/ha) plots treated at 12.4 kg/ha. ULV plots (30 L/ha) are color coded to show final application rates: purple plots=split application, 20 kg/ha; pink plots= split application, 16 kg/ha; red plots=single application, 10 kg/ha. *Arctostaphylos myrtifolia* appears low growing and olive green in the aerial image, areas killed by *Phytophthora cinnamomi* appear gray. Imagery date 4/18/2014, Google Earth.

3.1.6. Plot baseline data

For ULV plots 9E, 9W, 10, 11E, 12E, 12W, and 19, we have monitored progress the *P. cinnamomi* disease toward and into the treated areas following the same methods used in the phase 1 plots. We used two markers (carriage bolt driven into the soil through a fender washer and a square of vinyl flashing) to mark the ends of a straight monitoring transect line along the advancing disease front. At 0.5 m intervals along the monitoring transect line (1 m intervals for plot 19 which has a 12 m long monitoring line), we measured the distance to the first live *A. myrtifolia* along a perpendicular line projected from the monitoring transect line. We did not use this methods in plot 11W because it was subject to disease ingress from multiple directions.

In addition, for ULV plots 9E, 9W, 10, 11E, and 11W, we tagged individual healthy plants along the disease front and visually rated the amount of canopy dieback. We estimated canopy dieback using a

pretransformed (arcsine transformation) 7 point scale, where: 0 = no dieback seen, 1= less than 2.5%, 2 = 2.5% to 19%, 3 = 20% to 49%, 4 = 50% to 79%, 5 = 80% to 97.4%, 6 = 97.5% or more.

3.1.7. Photopoint monitoring for ULV plots 13-28

The monitoring method we have used with the original standard volume plots is not well suited to the more irregular geometry of the plants in plots set up in Nov 2014-Jan 2015. Therefore, we developed a method for taking overhead photopoints that can be rephotographed periodically to evaluate disease progress in the plots, irrespective of the pattern of disease progress into the plots.

Overhead photopoints were set up along disease fronts or the outer edge of the treated area if it was not adjacent to a defined disease front. At each photopoint, an aluminum nail (7.6 cm long) was used to pin a numbered aluminum tag and underlying square of vinyl flashing (to improve visibility) to the ground. GPS coordinates were also recorded for each photopoint.

Digital images were recorded at the photopoints using a remotely activated Canon SX50HS digital camera mounted on an extendable pole (Figure 17), the base of which was set onto the tag. The pole was extended to bring the lens surface 3.4 m above ground when the pole was vertical. The camera was mounted on a bracket that was inclined away from the pole (toward the plot) by 18 degrees. The mounting bracket was on an extendable arm that placed the center of the lens 53 cm horizontally from the center of the pole axis toward the plot. The camera aspect ratio was set at 3:4, and the lens at 28 mm equivalent zoom (wide angle).

An angle gauge attached to the pole was used to adjust the pole to a vertical orientation. A 1 m length of PVC pipe (marked in 25 cm increments) was set in the plot facing in the same direction as the camera bracket arm and served as a linear dimension reference for each photo. The azimuth of the reference stick (and camera bracket arm) was recorded for each photopoint. Initial overhead photos were taken 18 and 20 February and 9 March 2015. Photos were retaken 1 April 2016, and 5 August 2016. Photopoint images taken on different dates were compared visually and increased areas of canopy dieback were identified. The area of new canopy dieback was computed for each pair using the length of reference stick to estimate the area of new canopy dieback.

Figure 17. Overhead photo monitoring using camera mounted on an extendable pole. An angle gauge (partly visible below left forearm) was used to adjust the vertical angle of the pole. A 1 m reference stick (laying on top of the plants) is visible to left of camera pole.

3.2. Results and discussion

3.2.1. Application precision

The FlySight GPS has the advantage of providing GPS tracks showing the movement of the spray head. After downloading the tracks, we converted them to KLM files that we imported into ArcGIS. Points beyond the sprayed areas were deleted and we merged 0.6 m buffers (radius of the spray pattern from the head) around the remaining points to develop the polygons shown in Figures 9 and 15 and calculate plot areas (Table 5).

Table 5. Areas of ULV plots 13-28, calculated from polygons generated by buffering around recorded tracks from the FlySight GPS mounted on the spray head.

Plot	Area, m^2
13	29.01
14	116.87
15	77.09
16	34.79
17	38.02
18	89.29
19	43.65
20	76.90
21	78.70
22-23	125.05
24-25	56.03
27	25.84
26+28	84.42
Total	875.64

About 2.5 L of spray was nominally required to treat the plots shown in Table 1 at the actual delivered volume of 28.3 L/ha. The actual amount needed is actually somewhat less, due to gaps in the vegetation and other factors that reduce the treated area somewhat. Based on our best estimates, it appears that we applied 90 to 100% of the target volume in our applications. This indicates that the audio signals provided by the FlySight GPS allowed us to move the spray head at a rate very close to the target velocity, despite the wide variations in plot geometry. It also appears that actual applied rates were probably slightly under rather than over the target, though within any plot, some variation in dosage can be expected due to small areas of gaps or overlap. However, the use of two passes per swath (necessitated by the GPS limitation for minimum velocity) should have helped to even out the applied rate within plots.

3.2.2. Assessment of plot evaluation methods

As a group, the ULV plots have irregular disease fronts compared to the standard rate plots, so various monitoring methods were tested to monitor disease progress in the ULV plots. Where the disease front is fairly linear, measurements of change from a permanent, monumented baseline was generally the simplest and was adequate for showing progressive disease spread into a healthy stand. This method, used in the phase 1 plots discussed above, was also used in plots 9, 12, and 19, and portions of plots 10 and 11W. This method is subject to some noise in year to year measurements associated with slight changes in the alignment of the distance tape along the baseline. While distances are normally quite repeatable in dense stands, gaps between plants can lead to widely different readings if the alignment of the sample point is off. Comparisons with previous data are helpful for avoiding these errors. A related issue is that if portions of a plant near the baseline survive for an extended period while plants behind it are killed, no change in the disease front will be reflected in the data. When the front plant does die, the data will show a sudden large advance in the disease front at that point. The use of many points along the disease front generally decreases the influence of these two issues.

Rating canopy dieback of individual plants was used in plots 9, 10, and 11, and was the method used for phase 4 plots described in the next section. This methods is the best way to monitor health of scattered individual plants, as in the phase 4 plots. This method also provided a fairly sensitive way to document mortality along a disease front, especially an irregular front. However, it was more time consuming to set up and rate than other assessment methods for monitoring large numbers of grouped plants. This method also does not provide data on further advancement of disease once the individual tagged plants are dead.

Photomonitoring using low-altitude aerial images from fixed locations provided a fairly sensitive way for monitoring irregularly-scattered dieback and mortality in a group of plants (Figure 18). This method can account for mortality that may move in from different directions more readily than measurements from a baseline. Evaluation of the images can be geared to data needs. Initial visual comparisons between images were used identify whether any changes had occurred. For the purposes of our work, estimating the areas of new mortality, using the reference stick as a size scale, was sufficient for identifying the magnitude of change in the plots. Issues with photomonitoring include getting good exposures without strong shadows, which make photointerpretation more difficult. We also had some reliability problems with our remote shutter release, particularly at the last evaluation. An adequate workaround was to take the photos using a custom self-timer setting. With our equipment, we were not able to preview images before shooting. The ability to preview images remotely would make alignment simpler and would minimize the number of images with suboptimal exposures.

3.2.3 Single ULV applications at 8 or 10 kg/ha

Plots 9 - 11

As noted above, our initial ULV plots were established using rates that were below the threshold of phytotoxicity. Plots 9E and 9W are adjacent to standard rate plot 3 and its control and are along the same extended disease front (Figure 15). Plots 9W and 9E were initially treated 6 May 2014 at rates of 8 and 10 kg/ha, respectively, at 30 L/ha spray volume (ULV). Initial evaluations of the advancing disease front in February 2015, showed that disease suppression at these ULV rates was inadequate to slow disease progress, particularly when compared to plot 3B, which was treated at the standard volume and rate (Figure 19).

As seen in Figure 20, plots 9W and 9E also showed large increases in dieback among tagged plants from the 2014 to 2015 ratings. The tagged plants in plots 9W and 9E were directly adjacent to an active disease front, but this was not the case for plots 10 and 11, which were also initially treated 6 May 2014. Mortality was somewhat scattered around these plots, resulting in ragged disease fronts that could not be effectively monitored with the distance from a linear baseline. Because of the uneven disease pressure along plots 10 and 11, the average change in dieback ratings was much lower than seen in plot 9. Nonetheless, canopy dieback increased significantly in plot 10 (10 kg/ha ULV) between 2014 and 2015, again indicating that this ULV rate was not providing adequate disease control (Figure 20). There was little change in plot 11.

Figure 18. Overhead photo monitoring images (tag 1370) taken with the camera pole in February 2015 (top) and August 2016 (bottom). Orange marks on the 1 m reference stick are 25 cm apart. New plant mortality (brown foliage) is visible in the later (bottom) image compared to the initial image.

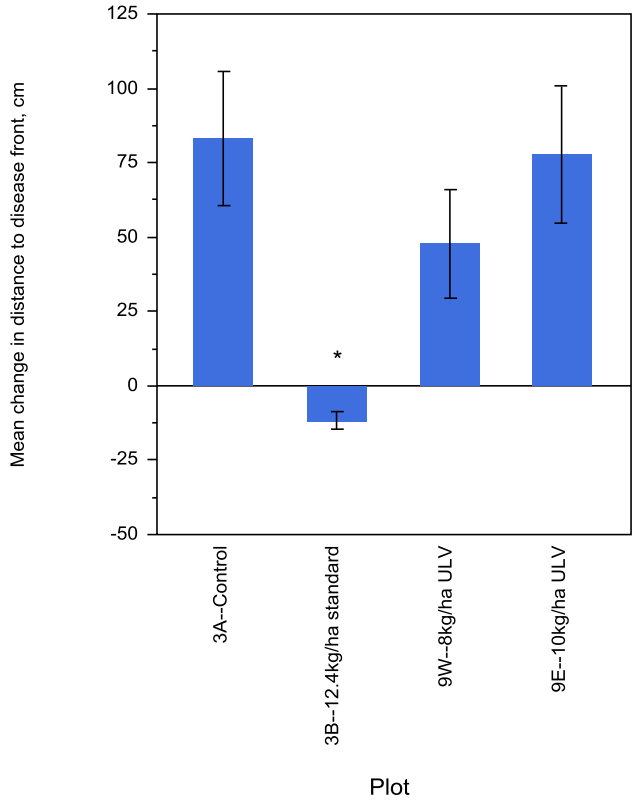

Figure 19. Changes in average distance to first live *A. myrtifolia* from April/May 2014 and March/April 2015 for plots 3 and 9. Bars marked with an asterisk differ significantly from the control at $p<0.05$ (Dunnett's test). All plots are along the same extended disease front. Changes were measured relative to a fixed baseline. Negative value in plot 3B (phosphite-treated, 12.4 kg/ha at 300 L/ha, last treated spring 2015) represents growth of live plant canopy toward the baseline. Plot 9 phosphite treatments were a single ULV (30 L/ha) application at 8 or 10 kg/ha at (ULV) in May 2014. Error bars are one standard error of the mean.

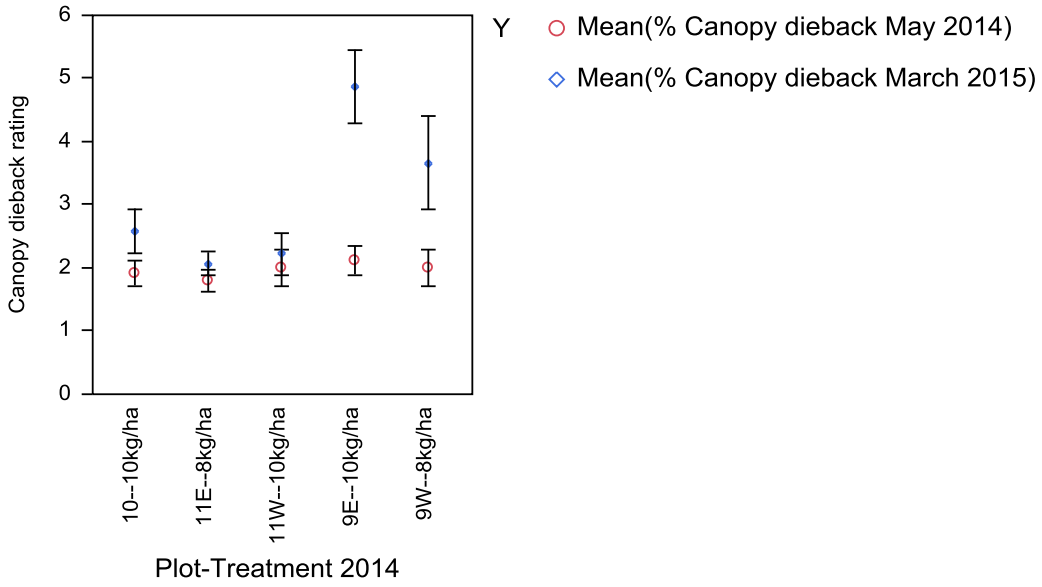

Figure 20. Percent canopy dieback measured for individual tagged *A. myrtifolia* along plot fronts for ULV plots 9, 10, and 11 in May 2014 and Feb/Mar 2015. Application rate was either 8 or 10 kg/ha in 2014. Each error bar is constructed using one standard error from the mean. Canopy dieback was rated using a 0-6 scale based on the following arcsine-transformed percentage scale: 0: Symptom not seen, 1:< 2.5%, 2: 2.5% to <20%, 3: 20% to < 50%, 4: 50% to < 80%, 5: 80% to < 97.5%, 6: 97.5% to 100%

Several factors could have contributed to the poor efficacy seen in these initial ULV phosphite treatments. These include:

1. The lower ULV application rates (reduced to 8 and 10 kg/ha to avoid phytotoxicity) were inadequate. The standard volume plots received 12.4 kg/ha.
2. Uptake of the phosphite applied via ULV was less efficient than when applied at standard spray volumes.
3. Due to the dry spring, plants were already too water-stressed by 8 May 2014 to absorb adequate amounts of phosphite.

The end result of any or all of these factors is that the level of phosphite in the plants was too low to halt disease progress. Increasing the applied dose was the most likely way to increase efficacy in these plots. Increasing the phosphite concentration in a single ULV application was not a viable option, based on our earlier phytotoxicity tests. However, the applied concentration can be effectively increased by using a split application, two successive treatments within about a two month span. Split ULV applications have been used in Australia as a way to increase applied dose while minimizing potential for phytotoxicity.

3.2.4. Split ULV applications at 16 or 20 kg/ha

Based on results from the single application ULV treatments described above, we decided to convert treatments in all phosphite ULV plots to split applications with total applied rates of 16 kg/ha (two successive 8 kg/ha applications) and 20 kg/ha (two successive 10 kg/ha applications). The only exception, discussed below, were plots 26/28 and 27. All of the split application treatments were

completed in spring 2015, and were last evaluated in early August 2016. Results to date for the split application plots are described below.

Plots 9 - 11

Initial results a little more than one year after the split applications were applied indicate that suppression of disease advance is most effective at the 20 kg/ha split application (Figures 21, 22). This shows up most clearly in the disease front evaluation (Figure 21) where the disease progress in the 16 kg/ha split application did not differ significantly from the control plot. Individual plant dieback ratings did not change significantly between 2015 and 2016 (Figure 22) suggesting that the split applications were having an effect in those plots where disease was present. In the case of plot 9E, the rating did not change largely because most of the rated plants were already dead. As noted above, a drawback of this evaluation method is that it is limited to the plants that were originally tagged and provides no further data once those plants have died.

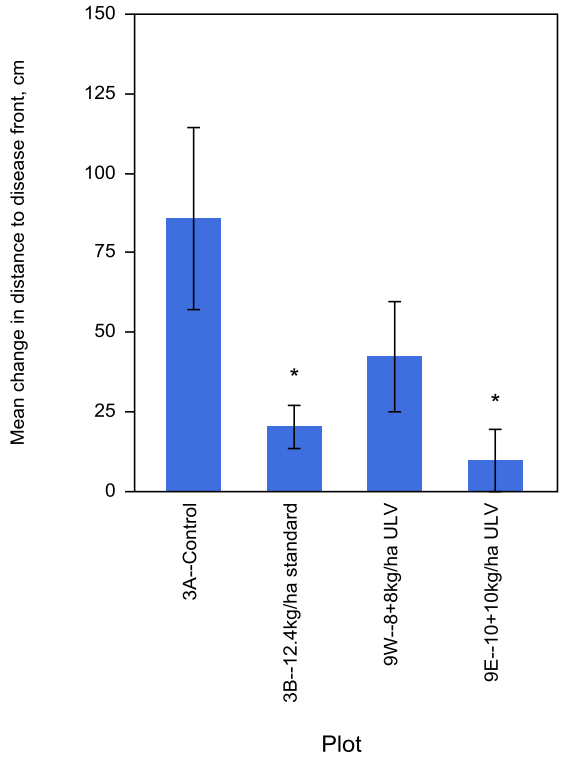

Figure 21. Changes in average distance to first live *A. myrtifolia* from March/April 2015 to August 2016 for plots 3 and 9. Bars marked with an asterisk differ significantly from the control at $p<0.05$ (Dunnett's test). All plots are along the same extended disease front. Changes were measured relative to a fixed baseline. Phosphite was applied to plot 3 at 12.4 kg/ha at standard volume of 300 L/ha (last treated spring 2015). Plot 9 treatments were split ULV (~30 L/ha) applications applied in spring 2015, at overall rates of 16 or 20 kg/ha. Error bars are one standard error of the mean.

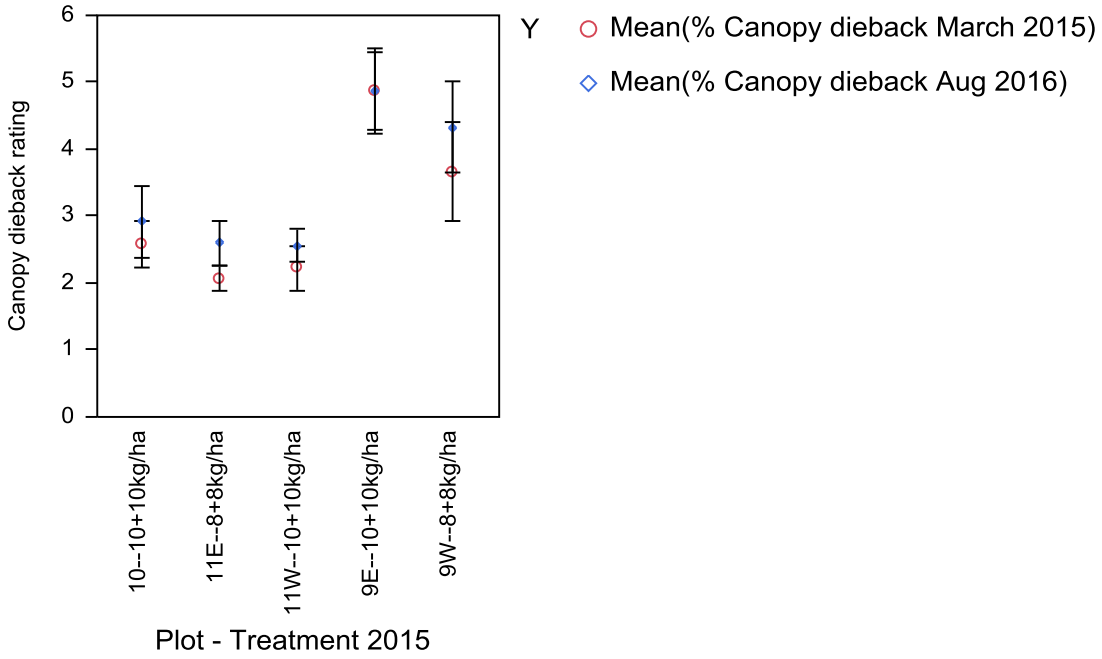

Figure 22. Percent canopy dieback measured for individual tagged *A. myrtifolia* along phosphite ULV plot fronts for plots 9, 10, and 11 in Feb/Mar 2015, and Mar/Apr 2016. Phosphite treatments were split ULV (~30 L/ha) applications applied in spring 2015, at overall rates of 16 or 20 kg/ha. Each error bar is constructed using one standard error from the mean. Canopy dieback was rated using a 0-6 scale based on the following arcsine-transformed percentage scale: 0: Symptom not seen, 1:< 2.5%, 2: 2.5% to <20%, 3: 20% to < 50%, 4: 50% to < 80%, 5: 80% to < 97.5%, 6: 97.5% to 100%

Plot 12

The design of ULV plot 12 differed from other plots in that the phosphite was applied in a band starting some distance from the active disease front. This was necessary in large part because the initial disease fronts were very uneven (Figure 23). This plot configuration provides a different way to gauge treatment efficacy. The rate of disease progress can be followed for several years in the nontreated portion of the plot. As the disease front reaches the phosphite-treated plants, disease progress should slow substantially or stop if the treatment is effective.

Disease progress in the plots, as measured from a fixed baseline, is shown in Figure 23 and summarized in Figure 24. Plot 12E and 12W were originally set up as a single application ULV plots, with rates of 8 kg/ha and 10 kg/ha, respectively, applied on 6 May 2014. The edge of the treated area was 250 cm from the 2014 baseline for plot 12E, about 50 to over 300 cm from the disease front. By the time that the 16 kg/ha ULV split application was applied in 2015, the disease front had advanced to the edge of the treated area in several spots (Figure 23, top).

Plot 12W initially received a single 10 kg/ha ULV application (May 2014) and was subsequently treated with a split ULV application (20 kg/ha total) in January-March 2015. The 2014 plot baseline was only 140 cm from the treated area, with the disease front nearly at the treatment line at several points and 200

cm away at other points (Figure 23). At least one and preferably two or more years of observations are still needed to determine the efficacy of treatments in these plots. When evaluated in 2016, recent mortality was observed within the treated area in both plots, but some of these plants may have been infected by the time the split application was made. Larger areas of mortality were seen in the treated area of plot 12E, which had the lower phosphite rate (16 kg/ha split application). However, this plot showed a higher rate of disease progress overall than 12W between March 2015 and August 2016.

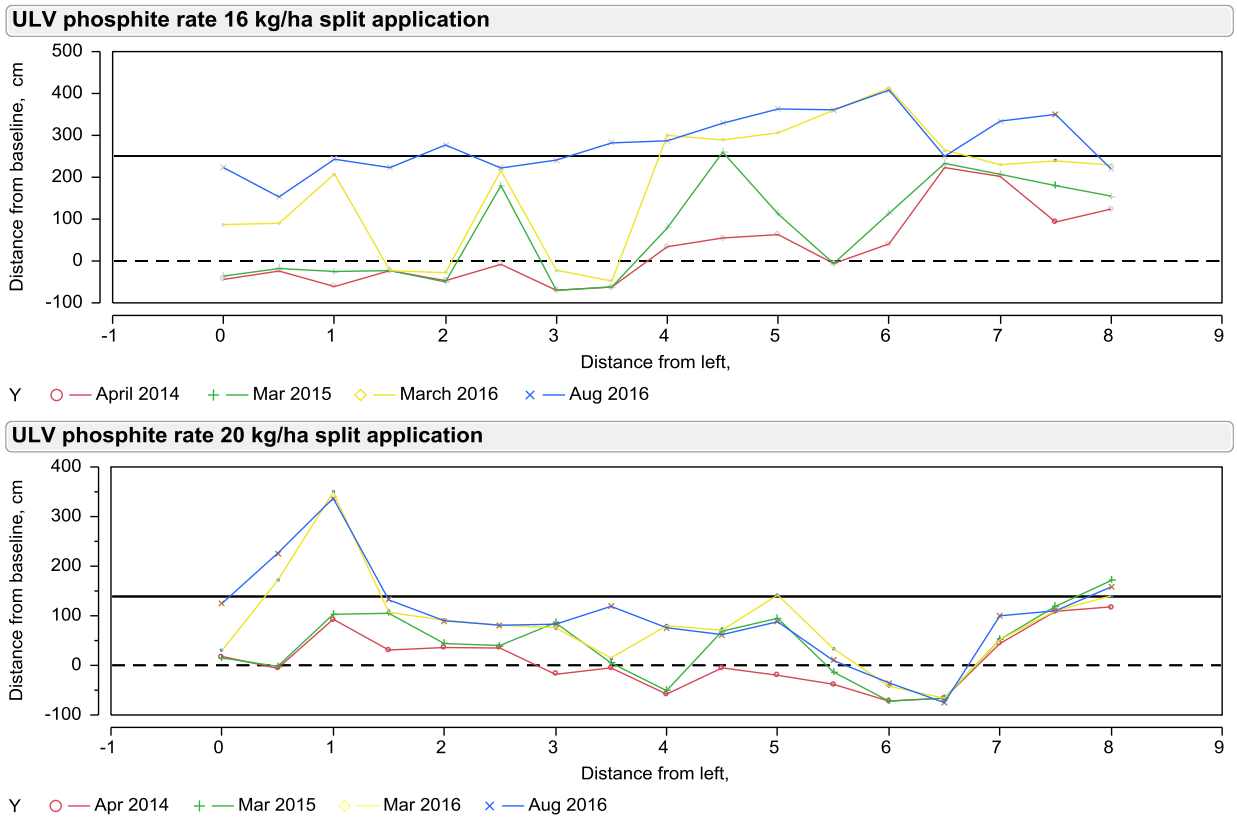

Figure 23. Distances from a baseline (dashed line) to the first live *A. myrtifolia* foliage between April 2014 and August 2016 in plots 12E (top) and 12W (bottom). Plots were treated with phosphite beyond the distance shown with the solid line in May 2014 (single ULV application) and early 2015 (split ULV application). Rates used were 8 (single) and 16 kg/ha (split) for 12E and 10 (single) and 20 kg/ha (split) for 12W.

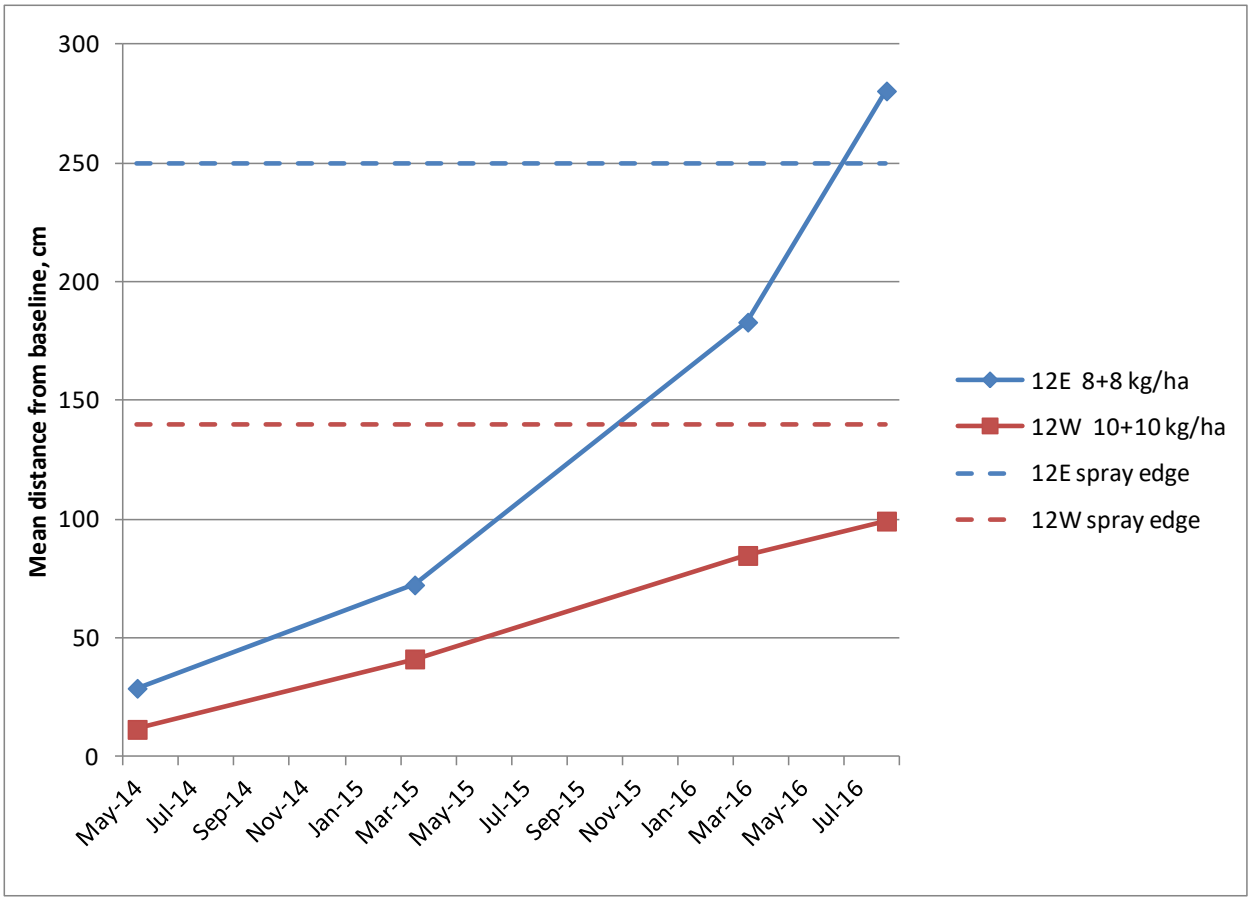

Figure 24. Average distance from 2014 baseline to first live *A. myrtifolia* foliage between April 2014 and August 2016 in plots 12E (top) and 12W (bottom). Plots were treated with phosphite beyond the distance shown with the dashed lines in May 2014 (single ULV application) and early 2015 (split ULV application).

Plots 13-25

Plots 13 though 25 were all treated via ULV applications at the 20 kg/ha total rate, with final applications occurring in February 2015 (Table 1). One objective in setting up these plots was to protect as many as possible of the at-risk *A. myrtifolia* stand edges at Apricum Hill, to help conserve these stands and keep options open for future studies. Hence, these plots varied greatly with respect to their proximity and orientation relative to active disease fronts, overall plot geometry, and the maximum treated band widths. Due to the complex monitoring issues posed by these plots, we used fixed aerial photopoints to monitor disease progress in these plots. Initial photos were taken in February 2015 and all photopoints were rephotographed in April and August 2016. Nontreated control photopoints were established in close proximity to treated areas to provide comparative data on disease progress.

April 2016 photographs showed few changes from those taken February 2015, but many August 2016 photographs showed substantial changes (e.g., Figure 18). The increase in the area of dead manzanita canopy between February 2015 and August 2016 for treated and control plots is shown in Figure 25. Trends in this data should be considered preliminary for several reasons. As noted above, plots vary greatly with respect to proximity to disease fronts. Furthermore, data for treated plots have not been analyzed for the effect of band width. For example, an outlying single plant in a site with high disease

pressure was treated by split ULV application at 20 kg/ha and was dead in August 2016. The effective treated band width for this plant would have been only about 1 m. Observations of the treated sites and photopoints suggest that the 20 kg/ha split applications are suppressing disease so far, but the two-year efficacy cannot be assessed at this point. As with other plots noted above, additional data collection, preferably through at least one more full treatment cycle, will be needed to evaluate these treatments.

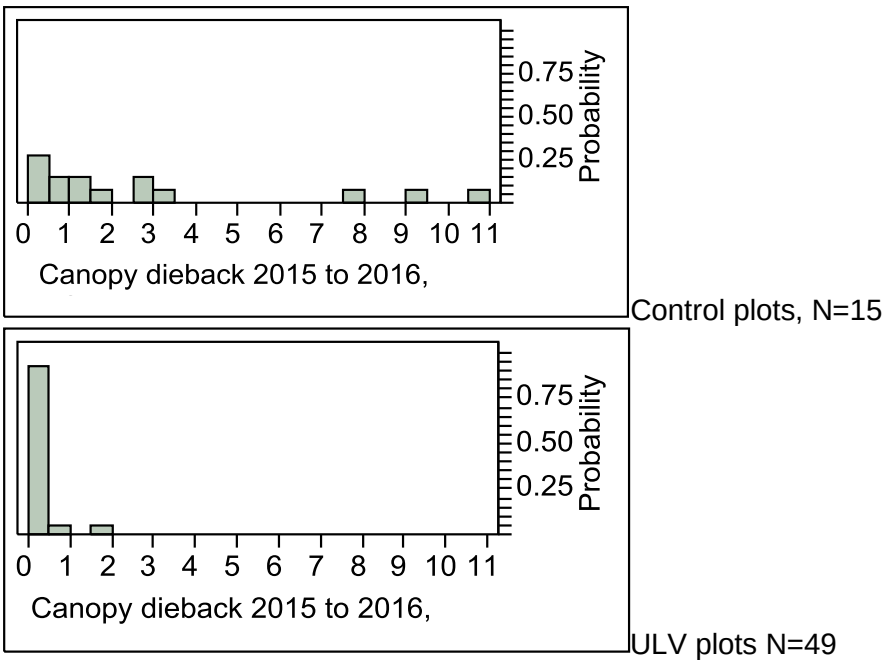

Figure 25. Increase in mortality (m^2) February 2015 to August 2016 assessed from photomonitoring images in nontreated (top) and phosphite-treated (bottom) ULV (20 kg/ha) split application plots 13-25.

Plots 26/28 and 27

These plots were scheduled to be treated as split application plots along with nearby plots 22-25. However, substantial amounts of foliar phytotoxicity were observed on 20 Feb 2015, when the second application was scheduled. Rather than risk further phytotoxicity, we skipped the second application for these plots. The plots in question were higher on the slope and may have been on a different soil type than plants in nearby plots. This might have affected their phosphorus levels and sensitivity to phytotoxicity. Alternatively, absorption of the spray may have been greater among these plants due to their physiological condition or leaf morphology (e.g., thinner cuticle).

Phytotoxicity symptoms were not evident in these plants in the 2016 evaluations. Photomonitoring of these plots has not yet shown any new mortality in the plots. As with the other ULV plot discussed above, continued monitoring will be needed to obtain definitive results. It would also be instructive to retreat these plots to determine whether the observed hypersensitivity to phosphite is repeatable.

4. Phase 4 plots: Use of phosphite to protect seedling regeneration in old *P. cinnamomi* mortality centers

We have previously observed that *A. myrtifolia* seedlings commonly become established in areas where the previous *A. myrtifolia* stand has been killed by *P. cinnamomi*. Based on our recent observations at the Apricum Hill Preserve, it appears that these seedlings are most likely to establish where all the mature plants have been dead for many years. By that point, the dead manzanitas have generally broken down to a few large diameter stems. Manzanita seedlings are generally absent from areas where the stand has been killed more recently.

These observations suggest that in the absence of hosts, *P. cinnamomi* propagule populations in the soil decrease over time to the point that susceptible seedlings can become established. However, we also commonly observe that these seedlings are killed at varying ages well before they attain the size of mature plants. Presumably, as their root systems expand, they eventually encounter scattered pockets of *P. cinnamomi* inoculum, which infect and reproduce on the roots, leading to severe root rot and plant death.

In our phase 4 plots, we are investigating whether *A. myrtifolia* seedlings that establish in old mortality centers can be protected for an extended period, perhaps indefinitely, if they are treated with phosphite. If the plants can at least survive to reproductive age, this strategy could help maintain genetic diversity and provide options for potential rehabilitation of affected stands.

4.1. Methods

4.1.1. Initial treatment and assessments — 2014 cohort

In spring 2014, we located live *A. myrtifolia* seedlings in several old *P. cinnamomi* mortality centers. Suitable seedlings were tagged with round, numbered aluminum tree tags, which were placed over small rectangles of vinyl flashing and fastened to the ground with aluminum nails (7.6 cm long). Tags were placed a short distance from individual plants or small groups (generally 2 or 3) of plants. For groups of plants, each seedling was identified by its distance and azimuth from the tag. To facilitate relocation, we placed a wire stake near each tagged plant or plant group (white for controls, blue for treated) and collected GPS waypoints within the areas where tagged seedlings were located.

For each seedling, we measured the longest canopy axis (length) from leaf tip to leaf tip and measured the perpendicular axis (width) in the same way. Plant height was measured from ground to highest leaf tip. To estimate the live cross-sectional canopy area of the seedlings, we used the formula for an ellipse:

$$(\text{Plant length}/2) \times (\text{Plant width}/2) \times \pi$$

Individual seedlings (or small groups of seedlings with a single tag) were assigned to be treated with phosphite or left as controls. Assignment was not completely random because we needed to allow sufficient separation to avoid overspray or drift of phosphite onto controls, but treated and control plants were intermixed throughout the study areas. We collected data on 113 seedlings in total.

Fifty-three seedlings were left as nontreated controls. Most of the remaining seedlings were sprayed with phosphite at a rate of 12.4 kg/ha at the standard volume of 300 L/ha. We used a modified garden sprayer

pressurized to 138 kPa (20 psig) attached to a hand wand with a TeeJet XR 11001VS nozzle (Figure 26). The nozzle delivered 5 ml/s at 138 kPa, and was moved over the ground surface at 0.33 m/s to apply the desired 300 L/ha volume. We used a PVC frame marked at 33 cm intervals and the Ludwig iPod metronome application set at 60 beats/min to provide the appropriate timing for each marked increment. The spray nozzle height above the canopy top was maintained at approximately 50 cm to provide the proper pattern width. Seedlings were initially treated on either 24 March or 9 April 2014.

Eleven individual seedlings near the edge of plot 11W were treated with phosphite at 10 kg/ha with the CDA (controlled droplet applicator) sprayer at an ultra low volume (ULV) of 30 L/ha. Phosphite was applied to these plants on 6 May 2014. In total, 60 seedlings were treated with phosphite in 2014.

We made an initial evaluation of the seedlings on 17 July 2014 because a number of the tagged seedlings were already dead or dying at that time. Seedlings were also re-evaluated for symptoms and remeasured on 20 April 2015, 18 March 2016, and 5 August 2016.

4.1.2. Phosphite treatment of 2016 cohort and retreatment of 2014 cohort seedlings

To maintain a 2-year retreatment interval, we retreated the surviving plants from the 2014 seedling cohort with phosphite on 18 March 2016. To help maintain the total sample size, we added a cohort of 44 additional seedlings to the study. Seedlings were measured, rated, and tagged as described above. Twenty two of these seedlings were left nontreated to act as controls. The remaining 22 seedlings in this new 2016 cohort were sprayed with potassium phosphite. We used the 12.4 kg/ha rate applied at 300 L/ha for all treated seedlings, including the seedlings that had been treated at 10 kg/ha using the ULV application (30 L/ha) in 2014. Our results in 2015 for treated ULV plots suggested that the lower 10 kg/ha rate was suboptimal, so we standardized all of the applications to the higher rate /higher volume spray. Furthermore, given the small target size of the treated seedlings and the wide spray pattern of the ULV sprayer, there was no practical advantage of using the ULV sprayer for individual seedlings over the standard sprayer.

4.2. Results

4.2.1. Effect of phosphite application on seedling survival

Seedling survival was significantly higher among phosphite-treated seedlings than controls in both cohorts. Survival of the 2014 cohort of treated seedlings is shown in Figure 27. Control seedlings consistently showed higher rates of mortality than phosphite-treated seedlings; these differences were significant ($p<0.05$) by 100 weeks after the start of the study treatment. By 5 August 2016, survival of the 2014 treated seedling cohort was 65%, compared to 19% for the nontreated seedlings. Results were equally dramatic for the 2016 cohort. Twenty weeks after the March 2016 treatment, 100% of the phosphite-treated seedlings were alive compared to 55% of the nontreated seedlings (significant at $p=0.0005$, 2-tail Fisher's exact test).

Figure 26. Phosphite application to marked seedlings at the 12.4kg/ha - 300 L/ha volume using a modified garden sprayer. The frame was placed over the seedling (visible near flag) and was marked to allow the applicator to time movement of the sprayer nozzle to an audio pulse generated by a metronome. White vinyl square with numbered tree tag is visible near the base of the seedling.

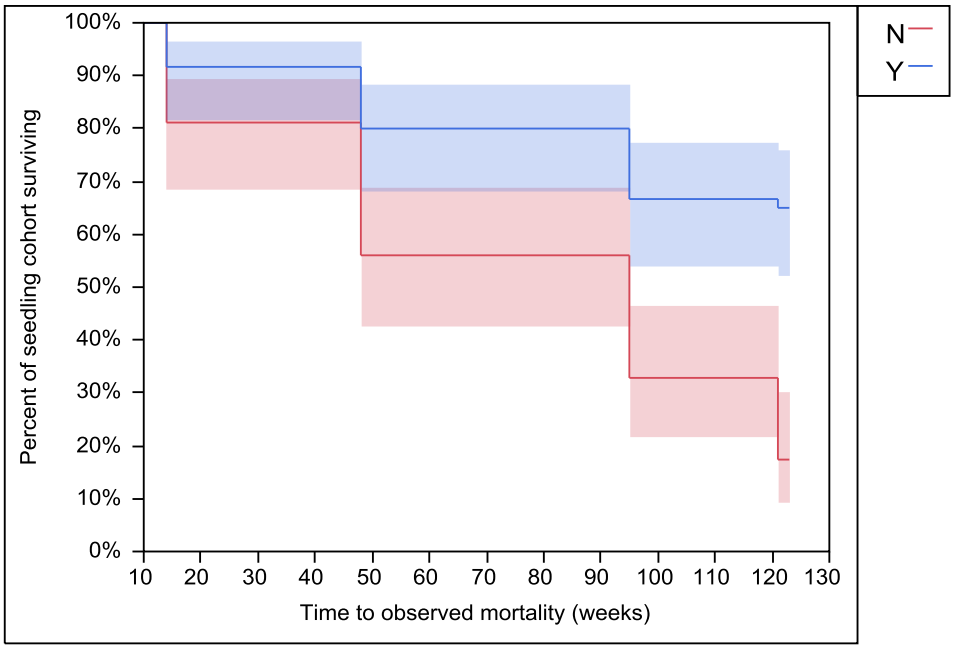

Figure 27. Survival of phosphite-treated (12.4 kg/ha, 300 L/ha, blue line) and nontreated (red line) *A. myrtifolia* 2014 seedling cohort over time. Initial treatment occurred at time 0, retreatment occurred at 106 weeks. Shading represents the 95% confidence interval of the survival percentages. Initial seedling populations: 53 nontreated (N) and 60 treated (Y) seedlings.

4.2.2. Growth of seedlings in the phase 4 seedling treatment experiment

One of the more surprising aspects of the seedling study was the fast growth rate exhibited by some of the seedlings (Figure 28). The average size of the 2014 seedling cohort did not differ significantly between treatments at either the beginning or the end of the study (August 2016), indicating the phosphite treatment did not have a negative effect on growth rate of treated seedlings. Treated seedlings exhibited a steady increase in total canopy cover over time, even though some plants in the treated cohort died. Among controls, mortality rates were so high that growth of surviving seedlings could not compensate for the loss of canopy area from killed seedlings, so the total canopy area decreased over the observation period (Figure 29).

A similar trend was seen with the 2016 seedling cohort. Average seedling size did not differ significantly between phosphite-treated and non-treated seedlings. No seedlings died among treated seedlings over the five months after treatment, and total canopy area increased by 26% as seedlings grew (Figure 30). In contrast, almost half of the non-treated seedlings died in the 5 months between March and August 2016, reducing the canopy cover of this cohort by 62% (Figure 30).

Initial seedling size also had a significant effect on mortality. A logistic regression model, using August 2016 mortality as the outcome and treatment and initial size in 2014 as predictors, was significant at $P \leq 0.0001$. Effect likelihood ratio tests showed that both treatment and initial size were significant at $P < 0.01$, but the interaction between the two predictors was nonsignificant. Phosphite-treated plants were more likely to survive than nontreated plants (odds ratio 8.96, 95% CI 3.7-23.9). In addition, larger plants were more likely to survive than smaller ones. A likely explanation of this effect is that seedlings growing in sites with more *P. cinnamomi* inoculum tend to be infected sooner and die while still small. Conversely, seedlings tend to survive longer, and therefore grow larger, if they are located where levels of *P. cinnamomi* inoculum are low. Based on our field observations, even these larger plants will eventually encounter *P. cinnamomi* inoculum in the soil and be killed. However, the time between infection to plant mortality is also likely to be longer for larger plants with more extensive root systems, than for smaller plants. This would also tend to increase the survival time of larger seedlings overall.

These results indicate that potassium phosphite treatment has potential as a means of keeping seedlings alive in areas infested with *P. cinnamomi*. However, substantial levels of mortality may still occur among phosphite-treated plants (Figure 27). This strategy seems to have the greatest potential when used on larger seedlings in areas where inoculum levels are likely to be low.

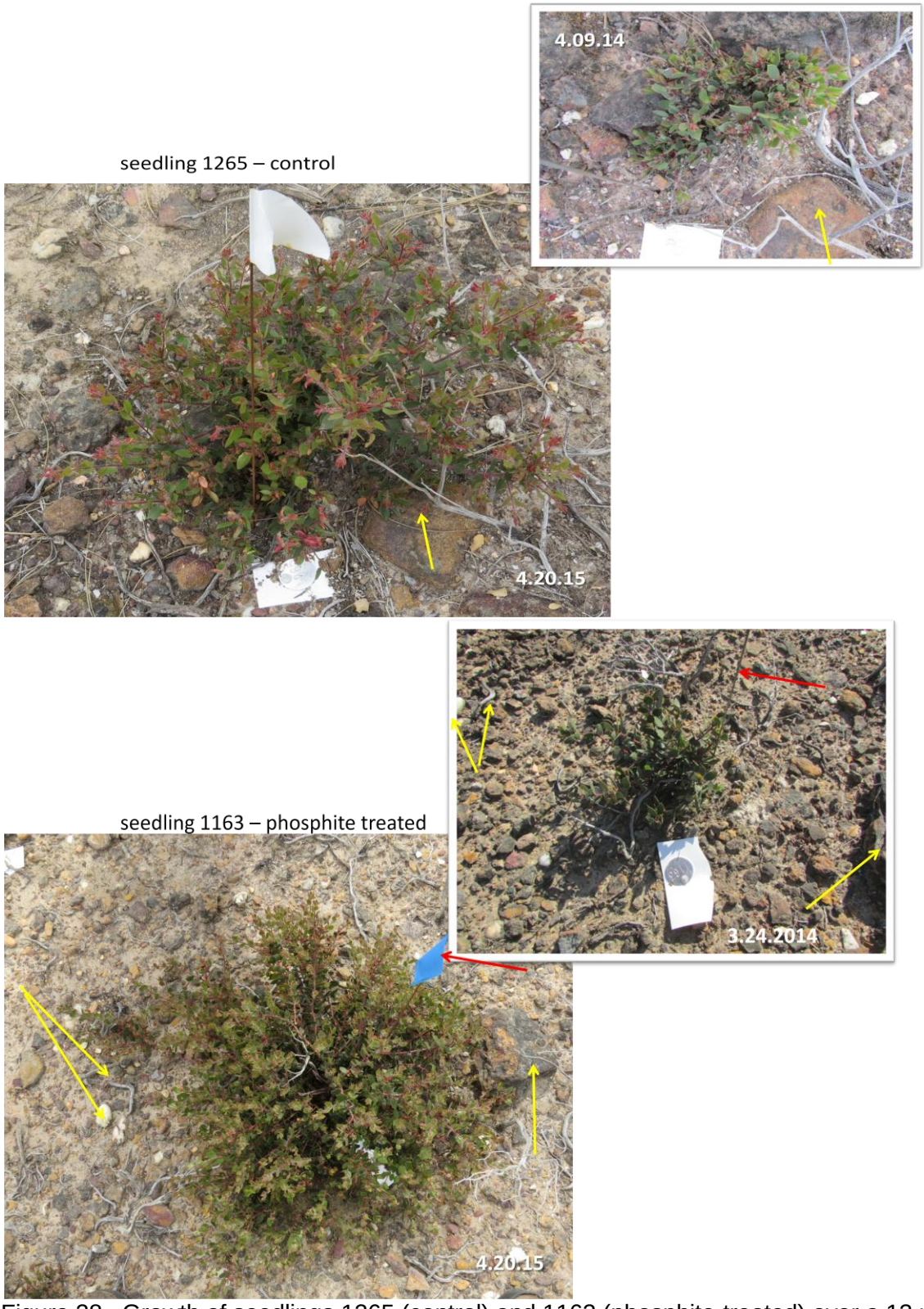

Figure 28. Growth of seedlings 1265 (control) and 1163 (phosphite-treated) over a 13 month period. Note positions of rocks, tags, and flags (arrows) for reference.

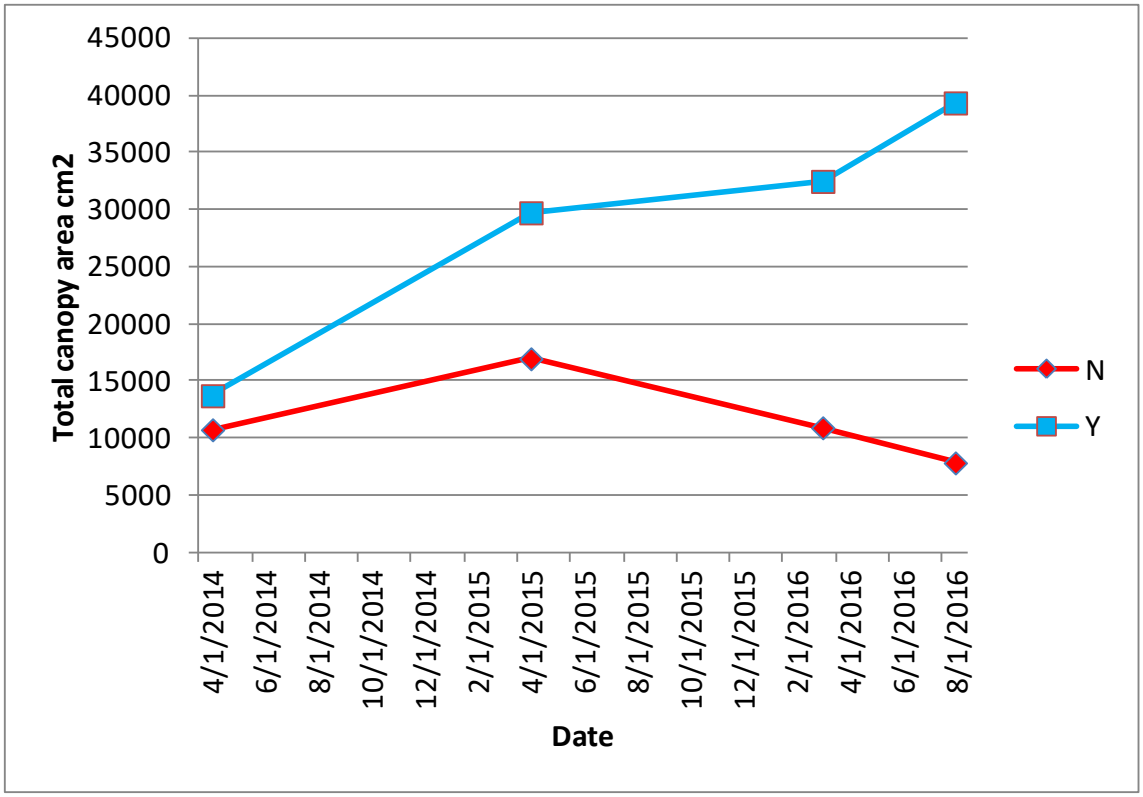

Figure 29. Change in total canopy area of phosphite-treated (12.4 kg/ha, 300 L/ha; blue line) and nontreated (red line) *A. myrtifolia* seedlings from the 2014 cohort. Phosphite treatment applied 24 March or 9 April 2014 and 18 March 2016. Initial seedling populations: 53 nontreated (N) and 60 treated (Y).

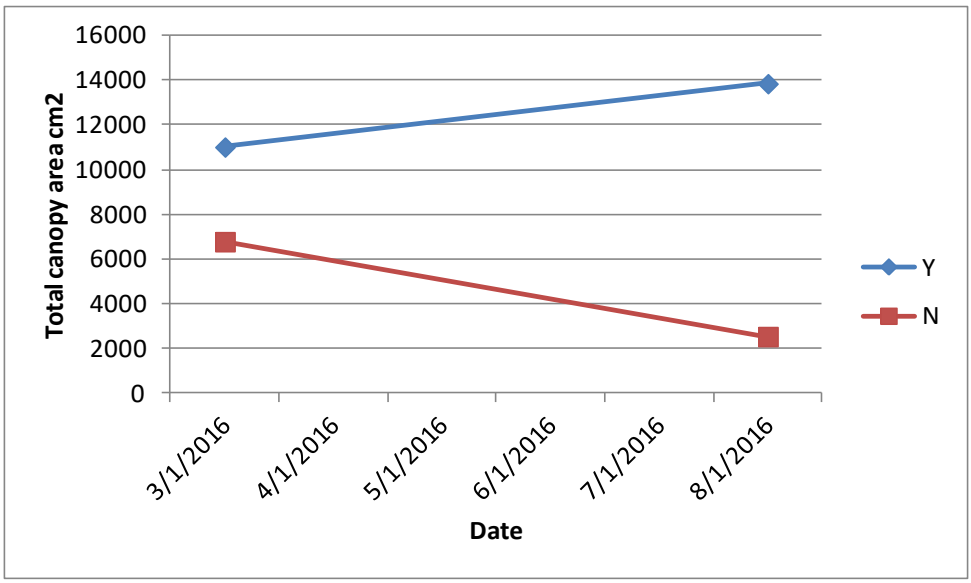

Figure 30. Change in total canopy area of phosphite-treated (12.4 kg/ha, 300 L/ha; blue line) and nontreated (red line) *A. myrtifolia* seedlings from the 2016 cohort. Phosphite treatment applied 18 March 2016. Initial seedling populations: 22 nontreated (N) and 22 treated (Y).

5. Baiting for *P. cinnamomi* in treated plots

We conducted soil sampling in 2014 to determine whether phosphite treatments in the phase 1 plots were having a detectable effect on *P. cinnamomi* populations levels in the soil.

5.1. Methods

We collected soil samples from the edge of the apparent disease front of phase 1 phosphite-treated and control plot sets 1, 2, 3, and 4 on 17 July 2014. We had previously collected a similar series of soil samples in April 2014. In the July 2014 round of sampling, we increased replication in each plot and changed the soil collection protocol slightly, to ensure that samples collected at the 0.75 m distance from the disease front were not inadvertently closer than this distance to a dead or symptomatic manzanitas.

In each plot, we collected samples at four positions relative to the visible edge of the disease front. Two samples (distance = 0 m) were taken at the edge of the first live plant along the disease front. Two other samples were collected at a distance of 0.75 m from the disease front and any other symptomatic or dead plant, but were not necessarily in line with the 0 m samples. We avoided areas where we had collected soil samples in April 2014. For each sample, we recorded the distance in meters from the left side of the plot (looking into the plot from the disease center) and the distance into the plot from the baseline edge.

Soil samples were collected by first scraping away organic debris and loose soil surface soil (generally less than 1 cm depth) with a trowel. We then used the blade end of a mason's hammer to break up the soil to a depth of about 10 cm and collected soil and associated roots from this loosened soil. This portion of the soil profile typically contains the highest density of *A. myrtifolia* roots and *P. cinnamomi* can typically be recovered from this zone if it is present. We emphasized the collection of live and dead root pieces in all samples, which are more likely than is the bulk soil to be associated with *Phytophthora* inoculum.

We placed about 0.75 to 1 liter of soil and root pieces from each excavated hole in 1 gallon zip closure plastic bags. Each sample was collected from a single sampling hole. Soil samples were placed in an insulated container for transport back to the laboratory. Sampling tools and shoes were thoroughly cleaned and disinfested with 70% isopropanol between all samples and before traveling out of known or suspected infested areas.

One day after sample collection, we added carbon-filtered tap water to the sample bags to adjust the soil moisture to about field capacity (about -30 kPa soil matric potential). Samples were maintained at field capacity for 3 days to promote sporangium production. Temperatures during this period fluctuated diurnally between 20 and 27 C.

After the 3-day adjustment period, we placed one unwounded, rinsed, green Bartlett pear fruit into each pre-moistened soil sample and added enough carbon-filtered tap water to submerge the soil and root sample to a depth of about 1 to 2 cm. We also added *Rhus integrifolia* leaf pieces as baits at this time. Baits of *Rhus integrifolia* were useful in the previous round of soil sampling in April, as diagnostic structures of *P. cinnamomi* formed on the leaves when they were subsequently incubated in water. Pear and leaf baits were incubated in the flooded soil samples for 3 days. Temperatures during the incubation period fluctuated diurnally between 20 and 27 C. After three days, symptoms had become evident on at least some of the pears and leaf baits. At that point, pear baits were removed from the flooded soil, rinsed

with water, and incubated on racks at 20 C and observed daily for further symptom development. Disease reactions on the pears were classified according to a 5 point scale (Figure 31).

Leaf baits were removed, rinsed with water, and floated in petri dishes containing nonsterile charcoal-filtered tap water and incubated at 20 to 27 C, and periodically examined using a microscope. Identification of *P. cinnamomi* was confirmed by observations of diagnostic morphological characteristics (sporangia, chlamydospores, and hyphal swellings) on leaf baits. In addition, we cut tissue pieces from the edges of brown spots on the pears and placed them in agar media in petri plates. Plates were examined periodically with a microscope for the presence of diagnostic morphological characteristics.

We used ordinal logistic regression using JMP 9.0.3 (SAS Institute Inc.) to analyze pear symptom severity, which was treated as an ordinal variable. Treatment (control or phosphite application) and distance from disease front (0 or 0.75 m) were used as predictor variables. Data from the April sample were pooled with the July data to give an overall sample size of 48 (4 plots×2 treatments×2 distances×3 samples).

Figure 31. Pears from soil baiting, illustrating 0-5 scale symptom severity. Dark brown discoloration is the normal symptom of *P. cinnamomi* infection in green pears. A black permanent marker was used to draw dotted lines around existing blemishes on the pear surfaces before baiting. These lines are visible in the two "1" rating pears and the center and left "0" rating pears.

5.2. Results and discussion

Figure 32 summarizes the results obtained through pear baiting of soil samples. At the disease front (distance = 0 m), pear symptom intensity was significantly lower in the phosphite-treated plots than in the control plots (Table 6). There was no statistical difference in detection 0.75 m from the disease front between treated and nontreated plots. The overall trends in the data were the same if the analysis was restricted to the July 2014 data only, but with the smaller sample size (N=32), the effect of treatment was not significant.

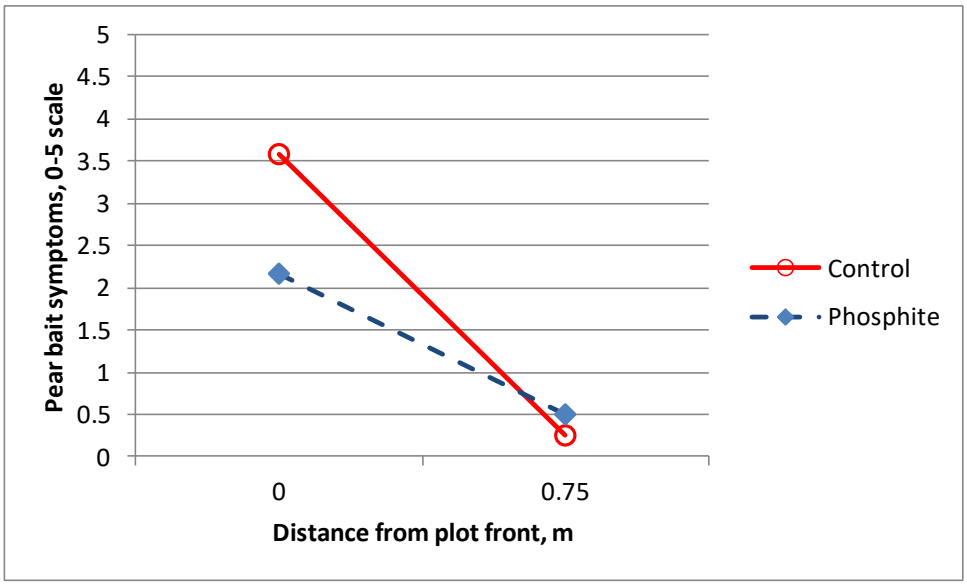

Figure 32. Average pear bait symptom severity for soil samples collected from control and phosphite-treated (12.4 kg/ha, 300 L/ha) plots, combined April and July 2014 data.

Table 6. Effect likelihood ratio tests for ordinal logistic model of pear symptom severity combined July and April 2014 data (N=48, overall model P <0.0001).

Source	DF	Likelihood ratio χ^2	P level
Treatment	1	6.1034095	0.0135
Distance from disease front, m	1	33.8988302	<.0001
Treatment × Distance from disease front, m	1	6.74129017	0.0094

These results are consistent with our previous studies, which showed that inoculum levels drop off substantially within a meter from the apparent disease front (Swiecki et al 2005). As seen in Figure 33, high levels of inoculum (ratings of 3 or more) were more common in the control plots than in the phosphite-treated plots at the 0 m distance.

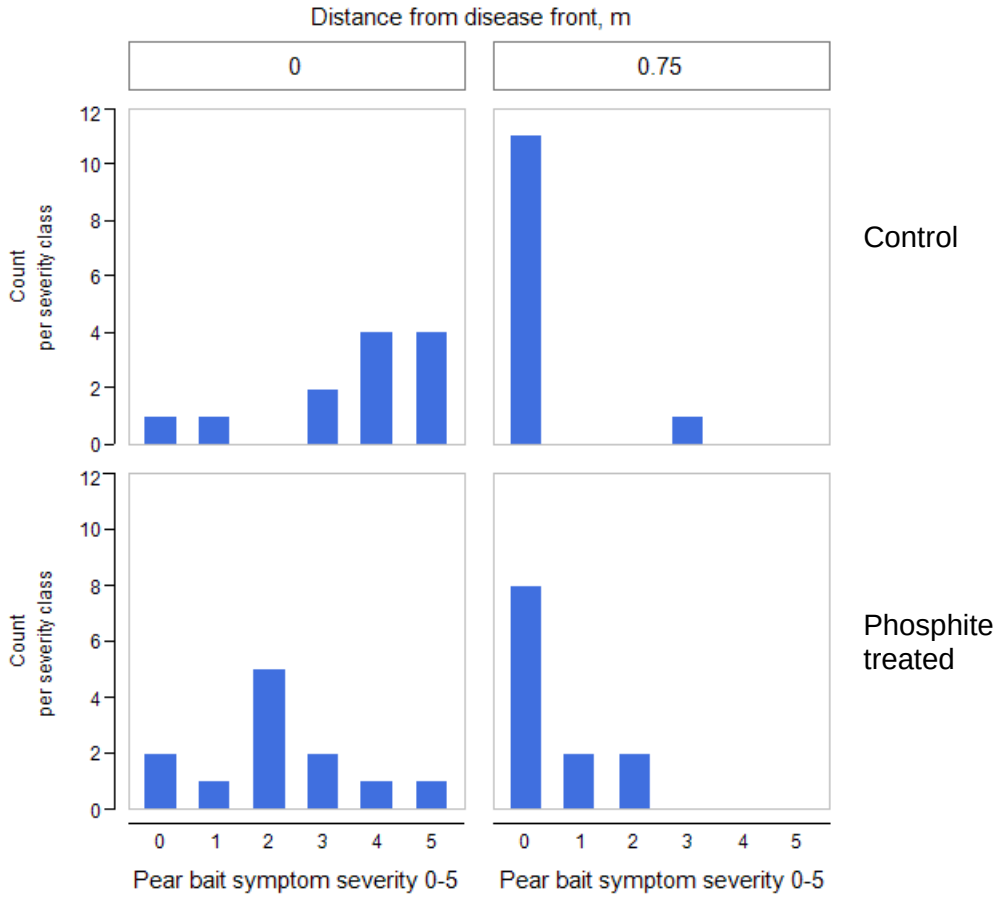

Figure 33. Histograms of pear bait symptom severity ratings (0-5 scale) for two distances from the disease front (columns 0 and 0.75 m) and treatment (top row - control; bottom row - 12.4 kg/ha phosphite spray at 300 L/ha). Each histogram contains values from 12 pears (3 pears for each treatment × distance combination × 4 plots).

It is clear from Figure 33 that *P. cinnamomi* continues to persist near the disease front in phosphite-treated plots. Wilkinson et al (2001) showed that sporangia and zoospores of *P. cinnamomi* were produced from phosphite-treated *Banksia grandis* and *Eucalyptus marginata* seedlings, albeit in lower numbers than from nontreated plants. These data suggest that the width of a phosphite-treated band should be well over a meter from the disease front edge to provide an adequate barrier to the spread of *P. cinnamomi*. These data also indicate that if phosphite treatment is terminated after a few years, disease is likely to advance into the treated area as soon as phosphite levels drop below effective levels in the plants. The fact that disease fronts have not advanced substantially into the treated plots to date indicates that effective phosphite levels are being maintained in plants under the current two-year treatment schedule with the 12.4 kg/L - 300 L/ha spray volume application.

6. Conclusions, recommendations, and further research

The research reported here conclusively demonstrates that foliar applications of potassium phosphite can be used to protect stands of *A. myrtifolia* from root rot caused by *P. cinnamomi*. Data from the phase 1 plots (Figures 6, 7) clearly show that spraying 12.4 kg phosphite/ha at a 300 L/ha spray volume on a 2-

year retreatment schedule can prevent the advance of disease along an active disease front in the presence of *P. cinnamomi* inoculum (Figures 32, 33). Although it may be possible to extend this retreatment interval to 3 years, we have not tested this possibility (phase 5). Given that there were small amounts of mortality seen along the disease front under the 2-year retreatment schedule, it seems likely that additional mortality will occur if the retreatment interval is extended. In areas where the main objective is to protect as much of the remaining occupied habitat as possible, maintaining a higher level of efficacy is preferable.

Applying 12.4 kg phosphite/ha at 300 L/ha spray volume to seedlings also shows clear potential to extend the life of individual plants that recruit in old mortality centers. The best potential appears associated with treating larger plants that have already survived for a year or more, presumably due to low local inoculum levels. The main utility of this application strategy would be to help maintain the soil seed bank and the genetic diversity associated with these plants. The 300 L/ha spray volume used to treat these plants is not a strong liability because of the very limited canopy areas being treated.

In contrast, treating the extensive borders between diseased and healthy areas in affected *A. myrtifolia* habitat would be difficult to manage with the 300 L/ha spray volume because of the difficult access to the areas needing treatment and the high volume of spray required. That is why much of our research efforts in this project have been directed at determining whether treatment at ultra low volume (ULV) is feasible and how to maximize efficacy with ULV applications.

We developed a reliable and accurate method for making ULV spray applications with hand-carried ground equipment that is suitable for use in *A. myrtifolia* habitat. We also have determined what spray concentrations can be used without causing substantial phytotoxicity. We also determined that single applications at a non-phytotoxic rate (10 kg/ha at 30 L/ha spray volume) do not appear to have acceptable levels of efficacy. We have determined that split applications can be used to apply rates up to at least 20 kg/ha in a single season. However, definitive efficacy assessments of these split applications are still pending. Initial data suggest that split applications of 16 kg/ha may not be sufficiently effective. This result alone indicates that phosphite uptake must be less efficient in ULV applications compared to standard volume applications, given that rates of 12.4 kg/ha have been effective when applied at 300 L/ha. It remains to be seen whether the 20 kg/ha ULV split application treatment will provide the efficacy seen in the phase 1 standard volume plots. Further observations of existing treated plots, including a second round of applications, would the most cost- and time-efficient way to resolve these outstanding questions.

The partial results from ULV studies to date suggest that further research is needed to optimize phosphite uptake in ULV applications. These may include changes in surfactant rate or use of different surfactants; inclusion of other adjuvants such as humectants; different timing of applications relative to plant phenology and weather conditions (rainfall, dew); or small changes in sprayer output (e.g., increase from current 28.3 L/ha to 32 L/ha). All such changes have to be checked for their potential to cause phytotoxicity, but the field screening methods we have developed allow for fairly rapid and reliable phytotoxicity testing.

One issue for future testing may be finding additional suitable test locations. Due to the expansion of *P. cinnamomi*-related mortality at the Apricum Hill Preserve, there are very few areas left to establish

additional efficacy tests. Because of the long-term nature of these tests, limited-access areas such as the Apricum Hill Preserve, where habitat is being protected over the long term, are the best locations for these studies. In addition, these tests provide a positive conservation benefit by helping to protect stand edges from further encroachment by *P. cinnamomi*.

Phosphite treatments have potential to help hold the line against *P. cinnamomi*, but in the end, these treatments mainly help to buy time. In association with other work we have been doing with *Phytophthora* root diseases introduced into restoration sites with nursery stock, we have been testing methods to eradicate *Phytophthora* from soil. This is technically challenging and relatively costly, but has the potential to allow infested habitat areas to be converted back to a state that would support *A. myrtifolia* populations. Of the methods that can eradicate *P. cinnamomi* from soil, long-term solarization of old mortality centers may be the most feasible. Its application would be limited to specific sites and would require adequate site preparation. Research would be needed to not only determine whether *P. cinnamomi* could be eliminated but also whether the heat treatment had other either adverse or beneficial effects on the soil that would affect reestablishment of *A. myrtifolia*

7. Efforts to publicize results

We met with CDFW staff members including Eric Kleinfelter, Cherilyn Burton, Jeb Bjerke, Laura Hayes and Tim Nosal at the CDFW Apricum Hill Preserve on 17 June 2014. With the exception of Eric, these staff members had not been to the Preserve before. They were interested in observing the effects of *P. cinnamomi* on the *A. myrtifolia* population first-hand. In addition, they wanted to locate the population of the endangered Ione buckwheat (*Eriogonum apricum* var. *apricum*) on the Preserve. Cherilyn, Jeb, and Laura were planning to set up a monitoring plot to follow populations of Ione buckwheat. We showed them the populations of Ione buckwheat at the preserve, discussed and demonstrated sanitation measures they should take to avoid spreading *P. cinnamomi*, and discussed the dangers associated with *Phytophthora*-infested nursery stock being used in habitat restoration projects. As a followup, we provided editorial input on an email that Cherilyn subsequently sent to all members of the CDFW botany list on 1 July 2014.

Presentations

Ted Swiecki gave the following presentations that included information from the research funded by this contract.

Testing and Implementing Methods for Managing *Phytophthora* Root Diseases in California Native Habitats and Restoration SitesSixth Sudden Oak Death Science Symposium, San Francisco, CA. 6/23/2016.

***Phytophthora* introductions into California native habitats and restoration sites.** CDFW Conservation Lecture Series: Concerns Over Plant Pathogen Introductions in Native Plant Nurseries and Restoration Sites. Sacramento, CA. 4/19/16

Can phosphite treatments limit spread of root rot caused by *Phytophthora cinnamomi* in habitat of Ione manzanita? 62nd Annual Conference on Soilborne Plant Pathogens, Parlier, CA. 3/23/16

Effects of *Phytophthora* introductions into California native habitats and restoration sites. An Expanding Threat: Exotic *Phytophthora* species entering native landscapes. 2016 Northern California Botanists Symposium, Chico, CA. 1/13/16

***Phytophthora* rising: Effects of introductions into California native habitats and restoration sites.** Do No Harm: Considerations of pathogens, pests, and plant disease in restoration activities, UC Palm Desert Campus, Palm Desert, CA. 11/5/2015

***Phytophthora* species in native plant nurseries, restoration sites, and native habitats in California .** Public meeting, Santa Clara Valley Water District, San Jose, CA. 2/12/15

Limiting the destruction of Ione manzanita habitat caused by the exotic pathogen *Phytophthora cinnamomi* . California Native Plant Society 2015 Conservation Conference, San Jose, CA. 1/15/15

***Phytophthora* species: life cycle, distribution, dispersal, impacts in California.** Responding to an Expanding Threat: Exotic *Phytophthora* Species in Native Plant Nurseries, Restoration Plantings, and Wildlands. San Francisco, CA. 12/2/14.

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