Final Report
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2014 ODA Nursery Research Program

Surfactants and Anti-Transpirants to Prevent *Phytophthora* Foliar Blight

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Project background and justification:

Phytophthora ramorum, causal agent of sudden oak death (SOD) and ramorum leaf blight, continues to be a problem for the Oregon nursery industry. In 2014, nine nurseries and one residential location were found infested with *P. ramorum* and were required to participate in the USDA Certified Nursery Protocol.

Pathogen spread within nurseries occurs aerially via plant to plant contact, splash dispersal between adjacent plants, or through inoculum splashing from ground water films. Preliminary assays with detached leaves have shown Moisturin, a commercially available anti-transpirant polymer, in combination with Tergitol, a surfactant, prevents infection of rhododendron leaves by *P. ramorum* zoospores (Peterson and Kelsey, *unpublished*). When applied post-infection, Moisturin also reduces sporulation while allowing for symptom development on infected leaves, a characteristic that could be useful during nursery evaluations of quarantined plants. Similar research in Parke's lab has shown that a biosurfactant produced by AGAE, Inc. in Corvallis lyses zoospores of *P. ramorum* and prevents infection of leaf disks in lab assays (Larson, E. et al., 2013). A new commercial surfactant product, Zonix, has recently been labeled for use as a biofungicide. The purpose of the proposed research is to test the effectiveness of these surfactants and a few anti-transpirants in preventing infection and reducing spore production by *P. ramorum,* and to determine application rates and duration of effectiveness following irrigation or rainfall.

Project objectives:

Objective 1. Determine the concentration and formulation of leaf surface treatments needed to prevent infection of rhododendron leaves by zoospores of *P. ramorum.*

Objective 2. Determine the duration of effectiveness following exposure of treated leaves to rainfall or irrigation.

Objective 3. Assess symptom development and inoculum production following post-infection application of leaf surface treatments to rhododendron.

Methods:

Objective 1. Several lab trials were conducted with detached rhododendron leaves coated with surfactants or anti-transpirants, then inoculated with zoospores of *P. ramorum.* Surfactants included the AGAE biosurfactant, Tergitol, and Zonix at 100, 400, and 1000 ppm. Anti-transpirants included Moisturin, Anti-Stress, Nu-film, Nature Shield, and Vapor Gard, each tested at 0.5% and 10% (v/v). Four of the five anti-transpirants were also tested at the 5% concentration. Disease severity (lesion area) was determined 10 days after inoculation after scanning the leaves and using image analysis software (Assess, APS, St. Paul, MN).

Objective 2. Potted (1-gal.) rhododendrons cv. Roseum Elegans (100 plants) were obtained for this experiment. In September, plants were moved to the outdoor container yard near the OSU west greenhouses and watered with overhead irrigation (30 min, twice daily, or natural rainfall) to simulate nursery conditions. For Trial 1, started on Sept. 19, plants were sprayed with Zonix at two rates (500 ppm or 1000 ppm), Anti-Stress (5%), Nu-Film (5%), or water (untreated controls). Two hours after plants were treated, three leaves from each plant were sampled (Time 0); additional sets of leaves were removed at weekly intervals (Week 1-4). Leaves were then challenged in the lab by inoculating them with zoospores of *Phytophthora ramorum*. Trial 2 was conducted beginning Oct. 10 with the remaining 50 plants. In addition to the previous treatments, a combination treatment of Zonix (500 ppm) with Nu-film (2.5%) was included.

Objective 3. In lab assays, leaves were wounded and inoculated with *P. ramorum* zoospores or agar plugs. Four days later, leaves were sprayed with Anti-Stress (5%), Nu-film (5%), Zonix (500 and 1000 ppm), a mixture of Nu-film (5%) and Zonix (1000 ppm), or not treated. Leaves were incubated for an additional four days and misted daily. Sporangia production was assessed by placing the leaves in 50-mL Falcon tubes containing distilled water. After mixing and filtering the sporangia suspensions through 5 μm polycarb filters, the number of sporangia per leaf was counted. Lesion area was also determined.

Statistical Methods. A generalized least squares model was used to test for treatment effects upon percent lesioned area, excluding treatments from analysis if no leaves were infected by *P. ramorum* in both trials. For the duration of effectiveness trials on whole plants, lesion area was normalized as a percentage of the controls for each time period and were analyzed separately by treatment. A negative binomial model was used to test if treatment impacted sporulation. For experiments in which treatment by trial interactions were non-significant all data were pooled. We performed post-hoc analysis to determine differences between treatments with a Tukey HSD. All analyses were performed in R (ver. 2.13).

Results

Objective 1: Effect of surfactants on disease severity:

In lab tests, detached leaves treated with the surfactant Tergitol at the 400 and 1000 ppm concentration, or Zonix at the 1000 ppm concentration before inoculation with *Phytophthora ramorum* zoospores had significantly smaller lesions relative to the untreated controls. AGAE was the least effective surfactant tested and did not significantly differ from the control even at 1000 ppm (Figure 1).

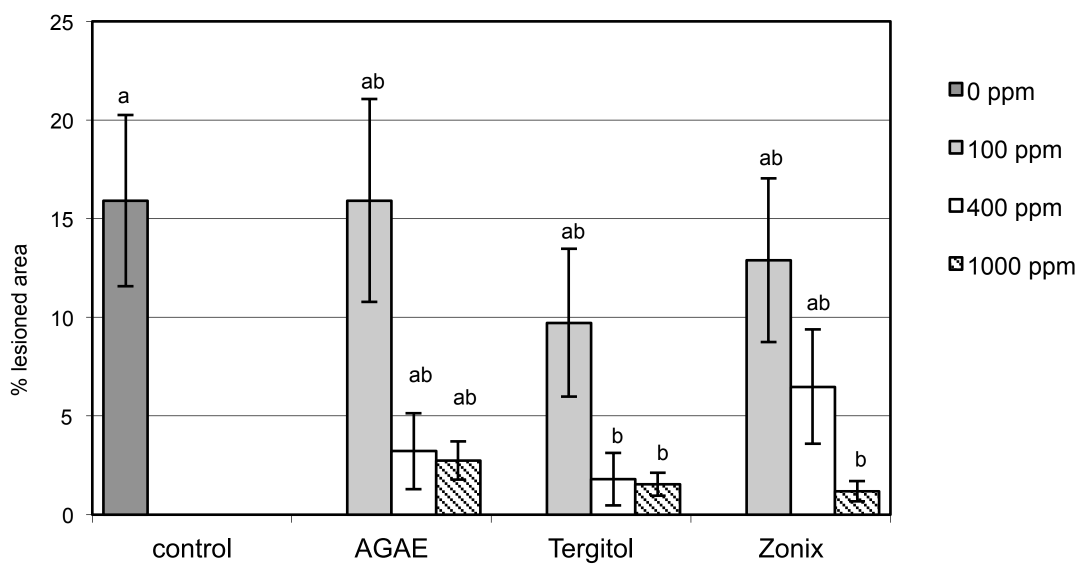


Figure 1. Average percent lesioned area of rhododendron leaves infected with *P. ramorum* zoospores post treatment with various surfactants at three concentrations, 100ppm, 400ppm, and 1000ppm, and an untreated control. Data is averaged over 2 trials; n=20. Bars represent standard error.

Objective 1: Effect of anti-transpirants on disease severity: In lab trials testing the effects of all 5 anti-transpirants (Anti-Stress, Moisturin, Nu-Film, Nature Shield, and Vapor Gard) at 0.5 and 10% concentration, there was a marginally significant difference between one or more treatments relative to controls ($p = 0.0586$). The application of either Anti-Stress or Moisturin at the 10% rate completely prevented infection by *P. ramorum* (Fig. 2). No significant differences were detected among the remaining treatments in which lesions developed.

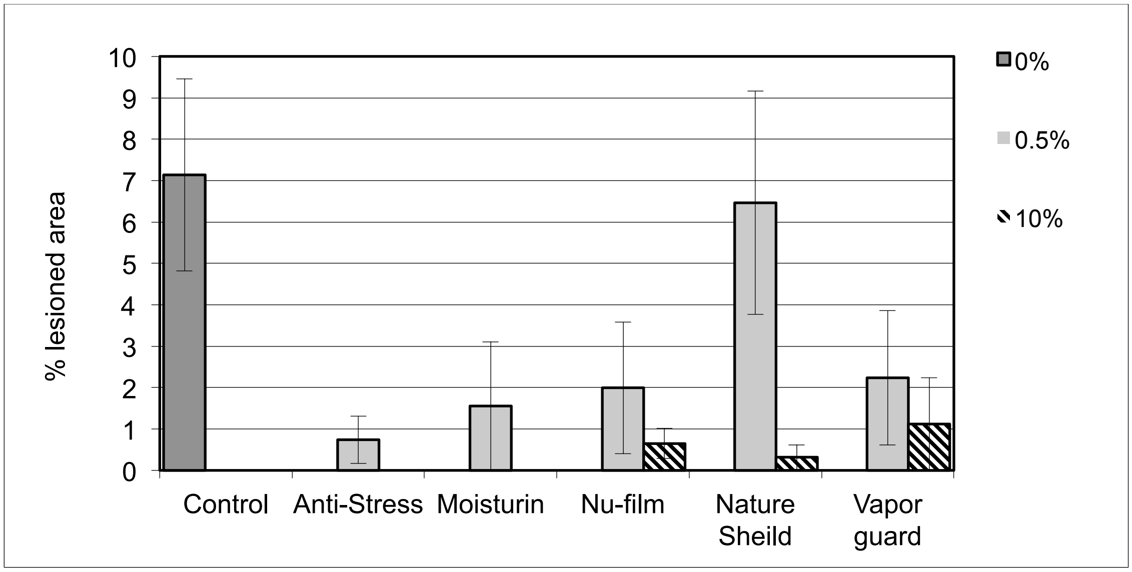


Figure 2. Average percent lesioned area of rhododendron leaves iterated with 5 different anti-transpirants at either a 0.5% or 10% concentration, and an untreated control. Anti-Stress and Moisturin at the 10% concentration were 100% effective at preventing infection by *Phytophthora ramorum* in both trials, and were not included in the analysis. No significant differences were detected among the remaining treatments and the untreated control. Data are combined for 2 trials; n=20. Bars represent standard error.

Moisturin, Anti-Stress, Nu-film and Vapor Gard were also tested at a 5% rate to better differentiate efficacy of each product and to enable selection of the most effective anti-transpirants for further testing in Objectives 2 and 3. In this trial, AntiStress, Nu-film, and Vapor Gard significantly reduced lesion size relative to the untreated control (Figure 3).

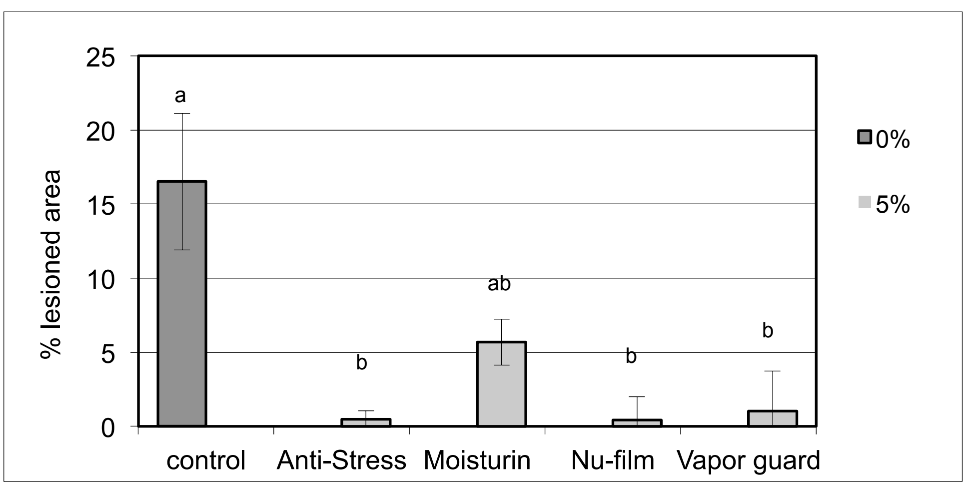
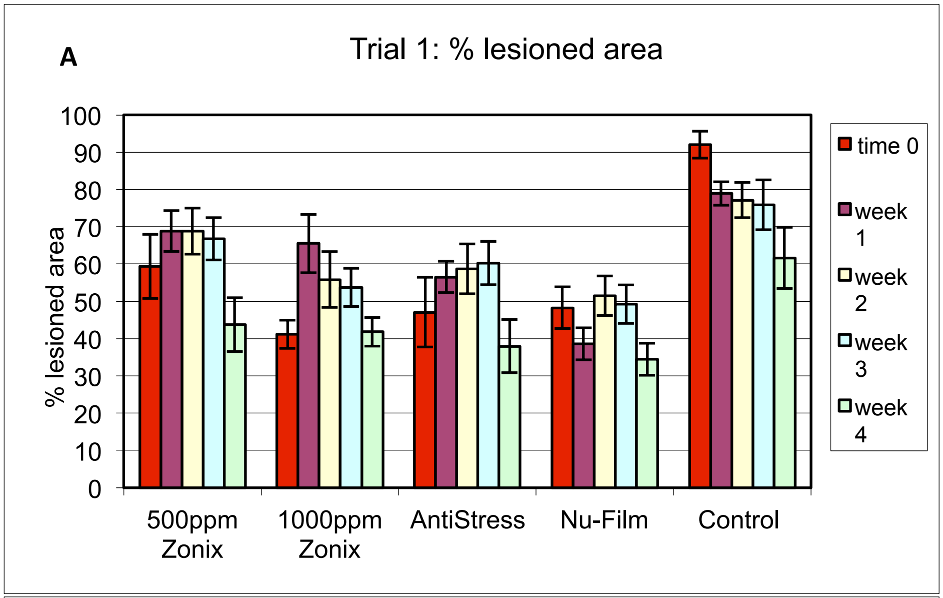


Figure 3. Average percent lesioned area for leaves treated with 4 different anti-transpirants at a 5% concentration, and an untreated control. Anti-Stress, Nu-Film, and Vapor Gard significantly reduced lesion area compared to the control. Bars represent standard error.

Objective 2: Effects of surfactants and anti-transpirants on whole plants in outdoor trials: Data on the efficacy of foliar treatments of surfactants and anti-transpirants is shown both as percent lesioned area and lesion area as a percent of the control. The effect of time upon lesion size differed between the two trials for most treatments, therefore each trial was analyzed separately. In trial 1, it appeared that several foliar treatments provided some protection initially, but were less effective over time, however there was no statistically significant change in lesion size over the 4 weeks for any treatment (Figure 4).



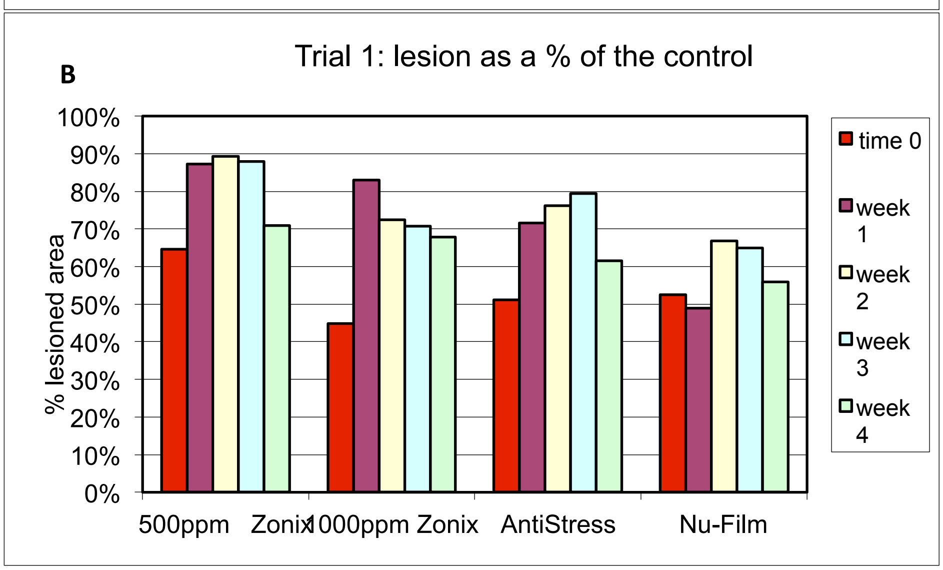


Figure 4. Disease severity on detached leaves from whole plants treated outdoors with surfactants and anti-transpirants. Trial 1 (Sept. 19-October 17). **A:** Average percent lesion area. **B:** Lesion area as a percent of the control for each sampling period.

In trial 2, efficacy significantly declined over the 4-week period for all treatments except for 1000ppm Zonix. Both Nu-film alone and the mix of Nu-film and Zonix appeared to provide the best overall protection, although there was a considerable reduction in efficacy from the Time 0 to Week 1 (Figure 5).

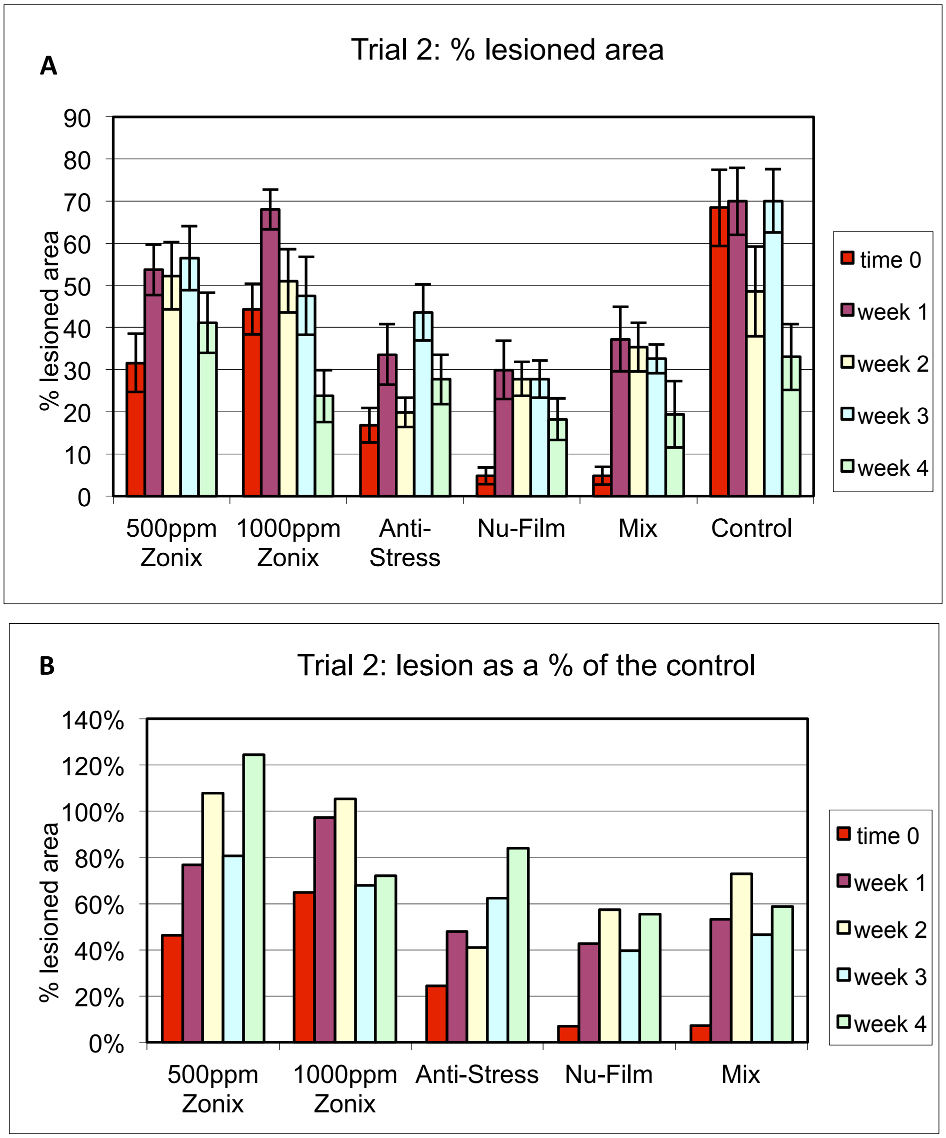


Figure 5. Disease severity on detached leaves from whole plants treated outdoors with surfactants and anti-transpirants. Trial 2 (Oct. 10-Nov. 7). **A:** Average percent lesion area. **B:** Lesion area as a percent of the control for each sampling period.

Objective 3: Effects on sporulation by Phytophthora ramorum. Surfactants and anti-transpirants were evaluated for their effects on sporulation on leaves that were already infected with *P. ramorum*. Several products significantly reduced the production of sporangia by *P. ramorum*. As compared to the untreated controls (75 sporangia per leaf), treatment with Zonix at 1000 ppm or 500 ppm resulted in 10 or 5 sporangia per leaf, respectively; the mix of Nu-film and Zonix resulted in 5 sporangia per leaf, and treatment with Anti-Stress yielded 3 sporangia per leaf. Only

Nu-film (average 60 sporangia per leaf) failed to reduce sporangia production relative to the untreated control (Figure 6).

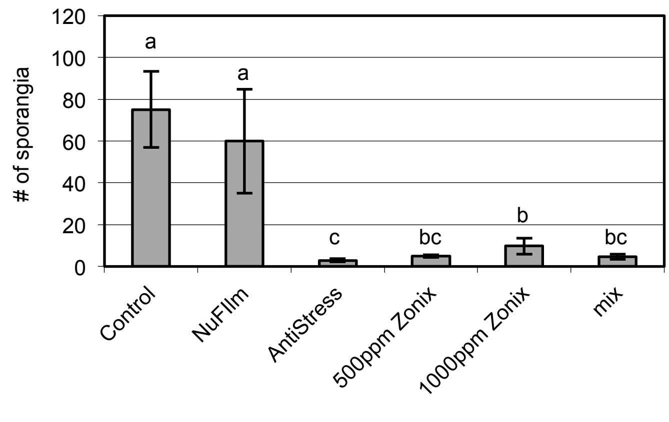


Figure 6. Average number of sporaniga produced from leaves treated with either Nufilm, AntiStress, 500ppm or 1000 ppm Zonix, or a mixture of Zonix and Nu-film 10 days after inoculation.

Interestingly, none of the products applied to the leaves after inoculation with *P. ramorum* reduced lesion development (p=0.382) as compared to the untreated control (Figure 7).

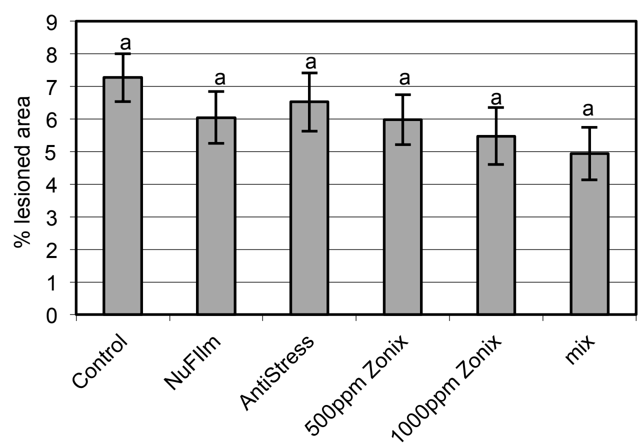


Figure 7. Average percent lesion area on detached leaves inoculated with P. ramorum and then treated with surfactants or anti-transpirants.

Discussion

Several surfactants and anti-transpirants evaluated in this study reduced foliar infection and disease development on leaves subsequently inoculated with *P. ramorum.* The surfactants Zonix and Tergitol, and the anti-transpirants Nu-Film, Anti-Stress, and VaporGard, all reduced lesion development on leaves inoculated with *P. ramorum* in lab assays. Subsequent trials in which a subset of surfactants and anti-transpirants were sprayed onto foliage of rhododendrons, and then detached leaves inoculated with the pathogen, revealed that some of these materials (Zonix, Anti-Stress, Nu-film, or a mixture of Nu-film and Zonix) have the potential to provide protection for commercially grown plants. However, differences in product efficacy and persistence between two trials with outdoor-grown plants suggest that some products may be washed off leaves with heavy rainfall as occurred during the second trial. Further testing would be necessary to optimize the timing of application, rates, and possible combinations of treatments to protect against foliar infection during periods of heavy rain.

The reduction in sporulation by certain surfactants and anti-transpirants is an important finding with potential application to commercial nurseries where *P. ramorum* is detected or suspected. Anti-Stress, Zonix, and the mixture of Nu-film and Zonix applied to *P. ramorum*-infected leaves with visible lesions substantially reduced the number of sporangia produced on those lesions. If host plants with symptoms are detected in nurseries, foliar application with one of these materials should drastically reduce the chance that *P. ramorum* will reproduce and spread. A prophylactic treatment could thus prevent disease spread while a lab diagnosis is confirmed and the USDA Certified Nursery Protocol is carried out. None of the materials applied to previously infected leaves reduced lesion development, therefore there is little likelihood that symptoms would be "masked", confounding investigations to delimit pathogen presence.

The mechanism by which surfactants protect plants against *Phytophthora* infection is attributed to lysis of membrane-bound zoospores (Stanghellini and Miller, 1997; Nielson et al., 2006). Physical disruption of the infectious propagule represents an alternative strategy for protecting foliage of highly susceptible plants without applying traditional fungicides that could result in the development of pathogen resistance. The mechanism(s) by which anti-transpirants protect plants against infection and reduce sporulation is not known, but could involve either chemical damage to the pathogen or physical barriers to infection and sporulation. The anti-transpirants tested in this study include resin-derived terpenic polymers (VaporGard, NuFilm P) and synthetic coatings made of acrylic polymers (Anti-Stress), ethylene glycol monobutyl ether (Nature Shield), or bicyclic oxazolidines (Moisturin). When applied to plant foliage, the anti-transpirants form a thin coating that reduces evaporative water loss but does not prevent photosynthesis and respiration. It is interesting that Nu-film was one of the most effective treatments for reducing leaf infection, but was the least effective in reducing sporulation.

From our studies, it appears that several surfactants and anti-transpirants are good candidates for reducing infection and sporulation as part of a multi-faceted approach to disease prevention and management. Many of the materials are already used in the nursery industry, although not labeled for use as pesticides. The surfactant Zonix is labeled for use as a biofungicide on ornamentals and vegetable crops in most states. The mixture of Nu-film with the surfactant Zonix might be a good choice for both reducing infection and reducing sporulation, in that two potentially different mechanisms are contributing to disease control. Nu-Film should also help prevent the washing off of Zonix from plant foliage by rainfall or overhead irrigation.

Further research is needed to scale up the field trials to determine if the most promising of these materials reduces pathogen sporulation and spread during epidemics under simulated nursery conditions. Because we cannot conduct field tests with *P. ramorum* in Oregon, we propose to conduct experiments with *Phytophthora plurivora*, the most common species of foliar-infecting *Phytophthora* spp. among Oregon nurseries tested (Parke et al., 2014). We hope to test the efficacy of Zonix, Anti-Stress, and NuFilm in protecting rhododendron plants against foliar infections by *P. plurivora* from two different sources of inoculum that occur naturally in nurseries: a) aerial spread from infected plants, or b) exposure to infested surface water, and demonstrate a reduction in the capacity of rhododendron plants treated post-infection to act as inoculum sources causing infection by *P. plurivora* on adjacent plants.

NOTE: This report includes information concerning experimental use of unregistered pesticides or unregistered uses of pesticides. Experimental results should not be interpreted as recommendations for use. Use of unregistered materials or use of any registered pesticide inconsistent with its label is against both Federal and State law.

Trade-name products and services are mentioned as illustrations only. This does not mean that Oregon State University endorses these products or that they intend to discriminate against products and services not mentioned.

References

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Stanghellini, M.E., and R.M. Miller. 1997. Biosurfactants: their identity and potential efficacy in the biological control of zoosporic plant pathogens. Plant Dis. 81(1):4–12.