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. Pathogen spread within nurseries occurs via plant to plant contact, splash dispersal between adjacent plants, through inoculum splashing from ground water films, or when plants are tipped into zoospore-containing puddles. Fungicides such as Ridomil, Subdue Maxx or Aliette do not kill Phytophthora spp., but merely slow the development of the pathogen. Alternative treatments could aid the detection and management of P. ramorum and other foliar Phytophthora species.

Research by Parke et al. (2013) on surfactants, and work by Peterson and Kelsey on anti-transpirants (unpublished) showed that these materials may protect plants from infection using different modes of action: by direct mechanical damage through lysing of zoospores, or by creating physical barriers to infection and sporulation. When applied post-infection, anti-transpirants may also reduce sporulation while allowing for symptom development on infected leaves, a characteristic that could be useful during evaluations of quarantined plants.

In the first year of this research, we tested several surfactants (AGAE, Tergitol, and Zonix) and anti-transpirants (Anti-Stress 2000, Moisturin, Nu-Film P, and VaporGard) in detached leaf experiments for reduction of foliar lesion development and sporulation capacity. In this current work, we selected the most effective of these materials (Zonix, Anti-Stress and Nu-Film) for additional tests to evaluate their potential to reduce pathogen spread during epidemics under simulated nursery conditions.

Project objectives:
Objective 1.

Demonstrate the efficacy of Zonix, Nu-Film P, Anti-Stress 2000, or a Zonix & Nu-Film combination to protect rhododendron plants against foliar Phytophthora infections from two different sources of inoculum naturally occurring in nurseries: a) exposure to infested surface waters, or b) aerial spread from infected plants.
Methods:

Objective 1a.

Potted 2-gal. rhododendron cv. Roseum Elegans were sprayed with DI-water, 5% Anti-Stress, 5% Nu-film, a 2.5% Nu-film & 500 ppm Zonix mixture, or a 5% Nu-film & 1000 ppm Zonix mixture, and were allowed to dry for 18 hours overnight. Fourteen branches per treatment were removed and dipped in a *P. ramorum* zoospore suspension for 4 minutes. Branches were incubated for seven days at 20°C, and lesion area was measured. This experiment was repeated once.

Objective 1b.

In February 2016, potted 2-gal. rhododendron cv. Roseum Elegans were sprayed with DI-water, 5% Anti-Stress, 5% Nu-film, a 2.5% Nu-film & 500 ppm Zonix mixture, or a 5% Nu-film & 1000 ppm Zonix mixture and allowed to dry for 18 hours overnight. Plants were arranged in groups on pallets under shade cloth in a container yard. At this time, detached leaves infested with *P. plurivora* in the laboratory were clipped to flags and placed within the upper canopy of plants to act as a primary inoculum source. *P. plurivora*, the most common species of foliar-infecting *Phytophthora* spp. among tested Oregon nurseries (Parke et al., 2014), was used as a surrogate species because we cannot perform field tests with *P. ramorum* in Oregon. Plants were overhead irrigated to supplement precipitation.

As no lesions were apparent by mid-March 2016, an additional set of *P. plurivora*-infested leaves were added to the site. We also placed new untreated, field-inoculated rhododendron plants in the center of each group of test plants to act as an inoculum source: agar plugs containing mycelia of *P. plurivora* were placed over wound sites on 12 leaves per plant; each plug was covered with a plastic cap containing moistened cotton fiber, which was then secured in place with a hair clip.

Plants and inoculum were left in the container yard until mid-May, after which we collected all symptomatic leaves and plated them in selective media to confirm infection by *P. plurivora*.

Results

Objective 1a.

Plants treated with DI-water (control) had the greatest number of *P. ramorum*-positive branches (Fig. 1a), and the greatest lesion area (Fig. 1b). 5% Anti-Stress, 5% Nu-film, and the two Nu-film and Zonix mixtures significantly reduced average lesion area relative to controls (Fig. 1b). The most effective treatment was the 5% Nu-film & 1000ppm Zonix combination.

Objective 1b.

Overall, infection rates for test plants in the container yard were low. Plants treated with DI-water (control) had the greatest number of infected leaves per plant (average: 3 per plant); the least amount of infection was observed on plants treated with 5% Anti-stress, a 2.5% Nu-film & 500 Zonix mixture, or a 5% Nu-film & 1000 Zonix mixture (Fig. 2).
Fig. 1. No. of *P. ramorum* positive branches (of 14/rep, for 28 branches total; a), and average % lesioned area per branch (b) for branches treated with 5% Anti-Stress, 5% Nu-film, a 2.5% Nu-film & 500 ppm Zonix mixture, or a 5% Nu-film & 1000 ppm Zonix mixture. Branches were dipped in a zoosporangial solution for 4 minutes then incubated for 7 days.

Fig. 2. Average no. of infected leaves per plant (3 per treatment) for plants sprayed 12 February 2016 and assessed for infection on 10 May 2016. Treatments included 5% Anti-Stress, 5% Nu-film, a 2.5% Nu-film & 500 ppm Zonix mixture, or a 5% Nu-film & 1000 ppm Zonix mixture. Plants were exposed to *P. plurivora*-infected leaf inoculum over the 3 month exposure period before plating in selective media.
Discussion

Several surfactants and anti-transpirants evaluated in this study reduced foliar infection and disease development on leaves inoculated with *P. ramorum* or *P. plurivora*. The surfactants Zonix and Tergitol, and the anti-transpirants Nu-Film P, Anti-Stress 2000 and VaporGard, all reduced lesion development on leaves inoculated with *P. ramorum* in lab assays. Differences in product efficacy and persistence in container yard trials necessitated further testing to optimize treatments to protect against foliar infection during times and pathways of greatest risk of spread.

In this most recent work, we chose to continue to test the efficacy of the anti-transpirants Anti-Stress 2000 and Nu-film P, and a combination of Nu-film and the biosurfactant, Zonix. Of the treatments, Nu-film provided the least amount of protection against infection by foliar *Phytophthora* spp. when branches were exposed to pools of inoculum and in the simulated nursery trial. As a stand-alone treatment Anti-stress performed well, however the best protection was observed in both assays when utilizing the Nu-film & Zonix mixture.

In previous trials, both Anti-Stress and the Nu-film & Zonix combination, though notably not Nu-film alone, were additionally able to significantly reduce sporulation when applied post-infection. If host plants with symptoms are detected in nurseries, foliar application with these materials should drastically reduce the chance that *P. ramorum* will reproduce and 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 these 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.

From our studies, it appears that either an Anti-Stress 2000 or a Nu-film P & Zonix combination are good candidates for reducing infection and sporulation as part of a multi-faceted approach to disease prevention and management. These materials are already used in the nursery industry, although the anti-transpirants are not labeled for use as pesticides. The surfactant Zonix is labeled for use as a biofungicide on ornamentals and vegetable crops in most states. Nu-Film should
also help prevent the washing off of Zonix from plant foliage by rainfall or overhead irrigation.

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.

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References

