An Evaluation of *Phytophthora ramorum* Preventative Treatments for Nursery and Forest Understory Plants in British Columbia, Canada[1]

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Abstract

Foliar treatments were applied to potted *Rhododendron* sp. cv 'Cunningham's white' plants over a 3-week period and evaluated for their ability to protect the leaves from *Phytophthora ramorum* infection. Treatments included several chemical and biological control products and systemic acquired resistance (SAR) elicitors. Excised leaves from treated plants were challenged by application of *P. ramorum* sporangia. Our preliminary results indicated that the most effective treatments were the two products registered for prevention of *P. ramorum* in Canada, Aliette® and Subdue Maxx®. Biocontrol treatments Rhapsody® and Actinovate® and SAR elicitors 3-aminobutyric acid (BABA) and Actigard® conferred a lesser degree of protection to the leaves and lowered the incidence of *P. ramorum* infection. Treatment with Sonata® was not significantly different than that with water. The chemical BABA did not inhibit *P. ramorum* growth *in vitro.* Plant trials are ongoing.

Introduction

On the west coast of British Columbia, Canada, several nurseries have reported *Phytophthora ramorum*-infected plants, but we have no indication whether the pathogen has spread beyond these points of entry. Plants in our forests and wildlands, as well as in our nursery industry and gardens, however, are vulnerable to this pathogen. As part of a collaborative research project among the Canadian Forest Service, Pacific Forestry Centre, the Canadian Food Inspection Agency and Agriculture and Agri-Food Canada (AAFC), we are evaluating treatments that may protect susceptible plants from infection, to be included in an integrated management approach.

Several commercially available biocontrol products, Rhapsody® and Sonata® (containing *Bacillus* spp.), and Actinovate® (*Streptomyces* sp.), were previously tested *in vitro* by using dual culture and treatments to detached leaves (Elliott and

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others 2008, in press). In the present study, we applied these treatments to whole plants and evaluated the response to *P. ramorum* infection on excised leaves of the treated plants. This plant trial also included treatments with chemical agents that may induce systemic acquired resistance (SAR) responses: Actigard® (benzothiodiazole), and 3-aminobutyric acid, (BABA) (table 1). We also included the chemical fungicides Subdue Maxx® (metalaxyl-m) and Aliette® (fosetyl-A), which are registered for use on *P. ramorum* host plants in Canada.

Methods

Plant Trials

Host plants were 1.5-year-old rooted cuttings of *Rhododendron catawbiense* cv. 'Cunningham's white' in gallon pots. Plant health was assessed before and after the trial. Eight foliar spray treatments were applied, as per manufacturers' label instructions or as noted for BABA (table 1), to five plants each. All treatments were applied once-a-week for 3 weeks, except for Aliette® and Subdue Maxx®, which were applied on a 2-week interval. Seven days after the last application of all treatments, 12 leaves per plant were excised and brought to the lab for challenge with *P. ramorum*. Sporangia were produced by growing *P. ramorum* isolate PFC 5073 (RHCC23), lineage NA2, in V8 broth for 4 days, then cultures were washed and media was replaced by water for 2 more days to induce sporangia formation. Cultures were poured through cheesecloth and the resulting purified sporangia solution was diluted to 10 000/ml. Half of the excised leaves from each treated plant (six) were sprayed with *P. ramorum* sporangia. All leaves were incubated for 14 days in sealed plastic boxes containing moist vermiculite, then disease response was assessed by counting the number of leaves with lesions as a percent of total leaves challenged with *P. ramorum* per treatment.

Table 1—Active ingredients and rates of foliar treatments to plants

Name, source	Active ingredient	Trial rate	PPM
Actinovate® SP, Natural Industries, Inc.	*Streptomyces lydicus* * added Silwet L-77	0.9g/litre	901ppm + 156ppm Silwet
Rhapsody® ASO, Agraquest	*Bacillus subtilis*	20ml/litre	20,000
Sonata® ASO, Agraquest	*Bacillus pumilus*	10ml/litre	10,000
Actigard®, Syngenta	benzothiadiazole	0.09g/litre	90.1
BABA, Aldrich	3-aminobutyric acid	1g/litre	1000
Aliette® WDG, Bayer	fosetyl-A	5g/litre	5006
SubdueMaxx®, Syngenta	metalaxyl-m	156µl/litre	156
Water			

Dose Response *In Vitro*

To evaluate the response of *P. ramorum* to BABA, 200µl cultures were initiated from 1000 zoospores per well in 96-well plates. Final concentrations of BABA ranged from 0.1 ppm to 1000 ppm. Plates were incubated at 20 °C. To estimate the growth of *P. ramorum* cultures, the optical density (OD) (650 nm) was measured every 24 hours for 3 days.

Results and Discussion

Our preliminary results of one plant trial indicated that the most effective foliar treatments for the protection of healthy rhododendrons from infection by *P. ramorum* were the two chemical fungicides registered for this use in Canada: Subdue Maxx® and Aliette® (fig. 1). Treatment of plants with the SAR elicitors BABA and Actigard® and biocontrol treatments Rhapsody® and Actinovate® resulted in some degree of protection to the leaves against *P. ramorum* and reduced the number of foliar lesions. Treatment effects of the biocontrol Sonata®, were not significantly different than treatment of plants with water (fig. 1). No treatments had any apparent phytotoxic effects. More assessments were made of this experiment than are presented here, including size of lesions and effects of leaf age. These data will be presented along with the results of the repeat of this experiment.

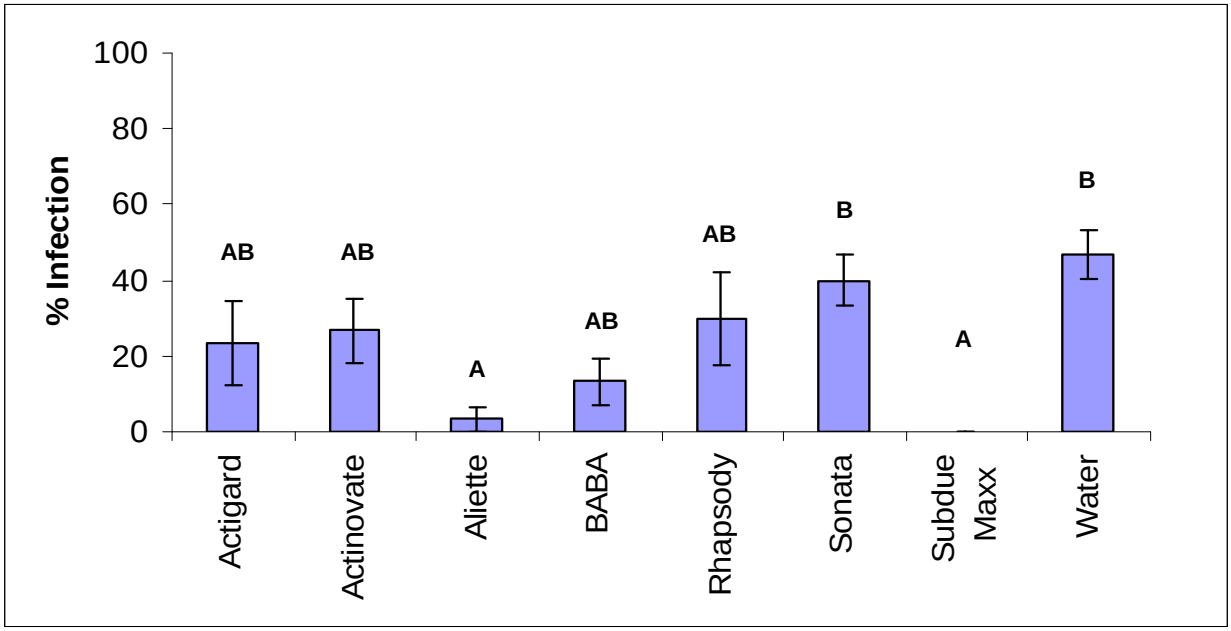

Figure 1—Disease response on excised leaves of treated plants. The number of leaves with lesions is given as a percent of total, per treatment. A one-way ANOVA comparing treatment means was performed. Treatments with the same letter were not significantly different according to the Tukey test at $P = 0.05$.

The dose response of *P. ramorum* to BABA was assessed *in vitro* over a wide range of concentrations. There was no significant difference in growth after 3 days among the cultures in different concentrations (0.01 to 1000 ppm) of BABA, showing equivalent results to growth in 0 ppm (P = 0.165) (data not shown). The chemical BABA did not

inhibit the growth of *P. ramorum* in liquid culture in concentrations up to 1000 ppm. This was the concentration used in foliar spray treatments (table 1).

More plant trials are underway on rhododendrons and other nursery and forest plants known to be hosts to *P. ramorum*. Root treatments are also being tested. One objective of these trials is to test whether resistance responses in treated whole plants can be detected in excised leaves challenged with *P. ramorum*. Infection of intact leaves on treated plants by *P. ramorum* will be performed in AAFC containment facilities and will be compared with the infection of excised leaves. Further *in vitro* screening is ongoing to identify other candidate treatments with suppressive effects against *P. ramorum* growth, including other microbials, plant extracts, and surfactants, as well as disinfectants for greenhouse use.

Literature Cited

Elliott, M.; Shamoun, S.F.; Sumampong, G.; James, D.; Briere, S.C.; Masri, S. and Varga, A. 2008. Identification of control agents and factors affecting pathogenicity of *Phytophthora ramorum*. In: Frankel, S.J.; Kliejunas, J.T.; Palmieri, K.M., tech. coords. 2008. Proceedings of the sudden oak death third science symposium. Gen. Tech. Rep. PSW-GTR-214. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station: 347–350.

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