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Evaluation of Chemical Agents for the Control of *Phytophthora ramorum* and Other Species of *Phytophthora* on Nursery Crops

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Abstract

Phytophthora diseases occur frequently in nurseries, and the recent incidence of ramorum blight, caused by *P. ramorum*, on nursery crops has underscored the need for improved management strategies against all *Phytophthora* diseases. We evaluated several chemicals that target Oomycete pathogens, inoculating detached rhododendron or lilac leaves removed from plants previously treated with various chemicals, or chemically-treated leaves on intact plants. Results indicated that Subdue MAXX (drench or foliar application) was the most effective chemical in suppressing infections caused by all species of *Phytophthora* tested (*P. ramorum*, *P. citricola*, *P. citrophthora*, and *P. nicotianae*) except *P. citrophthora*; with *P. ramorum*, it was active for at least 6 weeks after spray application. More chemicals were effective when sporangial rather than mycelial plug inoculum of *P. ramorum* was used, including Aliette, Ranman, Stature DM, and Fenamidone. All chemicals tested were fungistatic, not fungicidal. These tests indicate that several materials inhibit infection by *Phytophthora* species, and that the detached leaf test is effective in evaluating efficacy of chemical agents for the suppression of *Phytophthora* pathogens from nurseries.

Introduction

Species of *Phytophthora* are known to cause many leaf, shoot, and root diseases of a wide range of nursery crops. The discovery of *P. ramorum* (16,19) killing trees and infecting shrubs in the forests of California (2) and Oregon (4) and causing ramorum blight on nursery or landscape plants in several European countries (3) and in nurseries in the United States (9,13,14), underscores the threat and risk that these pathogens pose to the nursery industries. Ramorum blight affects numerous plant genera, especially members of the Ericaceae (such as *Rhododendron*, *Kalmia*, *Pieris*, and *Vaccinium*) (18), Fagaceae (*Quercus*, *Lithocarpus*, and *Fagus*), Theaceae (*Camellia*), and Caprifoliaceae (*Viburnum*). The reported symptoms caused by *P. ramorum* on rhododendrons are remarkably similar to those caused by other *Phytophthora* pathogens known to exist in the nursery industry, especially rhododendron blight, caused by *P. syringae* (Kleb.) Kleb. (6) and others (1). Surveys in many areas (5,9,10,13,14) indicate that there are many species of *Phytophthora* active in the nursery industry and not being controlled by current management practices. That fact, and the occurrence and reoccurrence, even under quarantine restrictions, of ramorum blight in nurseries, suggests the need for a careful evaluation of control strategies, including application of chemical agents (11,12,17).

The purpose of these studies, therefore, was to evaluate a range of chemicals that are specifically targeted to Oomycete pathogens (*Pythium* and *Phytophthora* species) for their capacity to suppress infections by a selection of *Phytophthora* species. Included was *P. ramorum* and several other species that also cause foliar blights on rhododendrons; a detached leaf assay was used (9).

Application of Chemical Agents

Chemical agents were applied by spraying foliage or drenching roots of container rhododendron plants maintained outdoors or maintained under greenhouse conditions. Plants were originally obtained from commercial nurseries with the knowledge that they had not been treated with fungicides prior to our purchase. Except for the residual time studies, plants were not reused in subsequent tests. Fungicides were applied to separate blocks of 4.4-dm^3 container-grown rhododendron plants in an outdoor raised bed setting. A control block with no fungicide application was included. Each block (approximately 1 m^2) contained 10 plants, and blocks were separated by 0.6 × 1.5 m of open space. Foliar fungicides were applied at label or recommended rates (Table 2) to runoff using a hand sprayer during early morning hours to minimize spray drift. Eight to 14 days after application, leaves were randomly collected from among the treated plants from each block and immediately brought indoors for inoculation.

Preparation of Inoculum, Inoculation Procedures, and Measurement of Infection

All *Phytophthora* isolates were prepared by growing each on dilute V8 juice agar medium (9,15) for 14 days. All species or isolate cultures used were originally recovered from infected plants in Oregon. Culture isolates and their origins are shown in Table 1.

Table 1. Source of *Phytophthora* isolates used in the inoculation studies. All were isolated from plants in Oregon. Isolates of *P. ramorum* were of the A1 (European) or A2 (North American) mating types.

***Phytophthora* spp. (isolate no.)**	**Source**	**Host plant**
P. cactorum (25-4-3)	P. Reeser	*Rhododendron* sp.
P. citricola (Pc 98-517)	P. Reeser	*Rhododendron* sp.
P. citrophthora (Pc 01-02)	P. Reeser	*Rhododendron* sp.
P. nicotianae (= *parasitica*) (Pc 96-1513)	P. Reeser	*Daphne* sp.
P. ramorum (2027) (A2)	E. Hansen	*Lithocarpus densiflorus*
P. ramorum (03-74-D12-A) (A1)	N. Osterbauer	*Viburnum plicatum tomentosum* 'Mariesii'
P. ramorum (03-74-N10-A) (A2)	N. Osterbauer	*Rhododendron* sp.

Leaves to be inoculated were wounded with a needle probe. Unless otherwise noted, each rhododendron leaf was wounded in four spots, two on either side of the midvein. In studies with lilacs, each leaf was wounded only once, but replicate leaves were used. A 4-mm mycelial plug was placed upper-surface down onto each wound site on the adaxial (upper) leaf surface. Because of the variation and generally low sporulation among the *Phytophthora* species, mycelial plugs from the margins of the colonies were used instead of sporangia in early studies comparing efficacy against different *Phytophthora* species, even though sporangia would be the likely inoculum in nurseries. In studies with only *P. ramorum*, sporangia were used as the inoculum. Control leaves were treated with agar plugs or water, depending on the experiment.

Inoculated leaves were placed in 20 × 20-cm resealable polyethylene bags, hand-misted with deionized water as the chambers were closed, and misted 2 to 3 times as needed during incubation. The bags were maintained in a dark incubator at 20°C for 8 or 14 days, and then inoculated leaves were examined for severity of lesions. Lesion area, as a percentage of each total leaf area, was assessed quantitatively from digital photographs using ASSESS software (American Phytopathological Society, St. Paul, MN).

Spring Application of Chemical Agents to Rhododendron Plants Maintained Outdoors

In our initial studies in May 2004, fungicides (Table 2) were applied to 1-gal-container-grown rhododendron plants. Isolates of *Phytophthora cactorum* (Lebert & Cohn) Schrot, *P. citricola* Sawada, *P. citrophthora* (R. E. Sm. & E. H. Sm.), *P. nicotianae* Breda de Haan (= *P. parasitica* Dastur), and two isolates of *P. ramorum* (Werres et al.) were wound-inoculated onto detached leaves as described above. A second trial was conducted in July 2004.

Table 2. Chemical/fungicide products applied as foliar spray (S) or drench (D) to *Rhododendron* 'Nova Zembla' and *Syringa vulgaris* 'Monge' for the suppression of leaf infections by species of *Phytophthora*.

Treatment, product name	Product description	Rate of product / liter*	Application method
Agri-Fos	46% mono- and di-potassium phosphite Agrichem Mfg. Ind., Queensland, Australia	3.9 ml	S
Aliette	80% fosetyl-Al BayerCropScience LP, Research Triangle Park, NC	6.0 g	S
Banol	66.5% propamocarb hydrochloride BayerAG, Montvale, NJ	2.0 ml	S
Biophos (Lexx-a-phos)	22.7% di-potassium phosphate; 22.4% di-potassium phosphonate Foliar Nutrients, Inc., Cairo, GA	10.0 ml	S
Fenamidone	98.5% fenamidone (technical) BayerCropScience LP, Research Triangle Park, NC	1.1 ml	S
Fosphite	53% mono- and di-potassium phosphite J.H. Biotech Inc., Ventura, CA	10.0 ml	S
Heritage	50% azoxystrobin Syngenta Crop Protection, Greensboro, NC	0.3 g	S
Insignia	20% pyraclostrobin BASF Corp., Research Park Triangle, NC	2.1 g	S
Ranman	40% cyazofamid ISK Biosciences, Mentor, OH	1.0 ml	S
StatureDM	50% dimethomorph Sepro Corp., Carmel, IN	0.5 g	S
Subdue MAXX	22% mefanoxam Syngenta Crop Protection, Greensboro, NC	0.2 ml	DS
Tanos 50WG	25% famoxadone, 25% cymoxanil DuPont, Wilmington, DE	4.5 g	S
Truban 30WP	30% terrazole Scotts-Sierra Comp., Marysville, OH	0.5 ml	S
Control	No fungicide	—	—

* Product label recommended application rate.

Lesion percentage data were transformed to arcsine-square root values prior to analyses to normalize variances. Data from the two repeated trials were combined since variance among trials was homogenous by Bartlett's test. ANOVA was used to determine significance of main effects and interactions (Systat 11.0, Systat Software Inc., Richmond, CA), and means were separated by Fisher's protected least significant difference (FPLSD) at $P = 0.05$. Orthogonal contrasts were used to compare specific treatments of interest (Table 3).

Table 3. Lesion areas on leaves from rhododendron 'Nova Zembla' plants treated with chemical agents and inoculated with different species of *Phytophthora*, relative to untreated control lesion areas.

Chemical product	Leaf lesion area (%)[x]					
	P. cactorum	*P. citricola*	*P. citrophthora*	*P. nicotianae*	*P. ramorum* 2027	*P. ramorum* D-12A
Agri-Fos	4.7 bc	19.5 c	18.3 ef	9.0 f	7.4d e	42.5 bc
Aliette	3.5 c	25.2 bc	15.5 f	14.4 cde	11.9 abc	31.4 fg
Banol	4.3 bc	17.0 c	18.6 c-f	6.6 f	13.6 ab	33.1 efg
BioPhos	5.6 bc	31.3 a	26.3 a	23.6 a	14.0 a	46.0 abc
Fenamidone	5.6 bc	30.9 a	20.4 b-e	16.6 bcd	14.7 a	49.9 a
Fosphite	4.6 bc	19.4 c	18.3 def	17.2 bc	5.8 e	29.3 g
Insignia	6.7 b	20.0 c	21.7 b-e	11.9 e	6.7 de	37.1 de
Ranman	4.3 bc	18.9 c	21.2 b-e	6.4 f	8.8 cd	42.1 cd
Stature	5.9 bc	20.5 c	25.7 a	20.5 ab	14.2 a	35.9 ef
Subdue MAXX	0.4 d	8.8 d	24.1 ab	0.9 g	0.7 f	0.9 h
Tanos	5.2 bc	29.0 ab	23.5 abc	19.8 b	11.5 abc	43.7 bc
Truban	21.8 a	28.1 ab	22.1 a-d	16.3 bcd	9.7 bcd	46.4 ab
Untreated control	5.9 bc	24.8 bc	18.0 def	13.2 de	11.9 abc	35.6 ef
Chemical mean	6.1	22.4	21.3	13.6	9.9	36.5
Linear contrasts of treatments[y]						
1. Aliette vs. all others	**	**	**	NS	**	**
2. Subdue vs. all others	**	**	**	**	**	**
3. Control vs. all chemicals	NS	**	**	NS	**	NS

[x] Each value is the mean of 6 replicate leaves from two different field trials. Data are percentages of the total leaf area exhibiting lesions. Untransformed means are presented; two-way analysis of variance was performed on arcsine-transformed data. Numbers in each column followed by a different letter are significantly different ($P = 0.05$) according to Fisher's protected least signficant difference (FPLSD) test.

[y] Orthogonal contrasts were significant at $P \leq 0.01$ (**) or not significant (NS) at $P = 0.05$.

Both fungicide and pathogen isolates contributed significant effects ($P \leq 0.001$) to lesion area results, as did their interaction. In general, fungicides significantly reduced leaf lesion areas compared to untreated leaves for *P. citricola* and *P. ramorum* 2027, but not so for *P. cactorum*, *P. nicotianae*, or *P. ramorum* D12A (Table 3). Some fungicides also significantly increased lesion areas of leaves inoculated with all species of *Phytophthora* except *P. ramorum* 2027. By orthogonal contrasts, Subdue MAXX significantly reduced lesion area compared to other chemical agents for all pathogens except *P. citrophthora*, where it significantly increased lesion area. Agri-Fos, BioPhos, Fenamindone, Fosphite, Ranman, Stature DM, Tanos, and Truban increased lesion areas relative to controls in 5 of the 6 pathogen treatments. No single fungicide reduced lesion areas, relative to controls, across all pathogens, although Subdue MAXX application caused the greatest, most consistent reduction. When we made isolations from wound-sites with no or restricted lesions, we recovered the pathogens, indicating that chemicals were fungistatic and not fungicidal.

Detached and Intact Leaf Assays of Fall Chemical Applications to Rhododendron and Lilac Plants

In the fall of 2004, fungicides were again applied to blocks of 4.4-dm^3-container-grown rhododendrons and 0.6-dm^3-container-grown lilacs maintained in a greenhouse environment. Because of our results in the spring

trial showing no efficacy, as well as other unreported studies, Banol and Tanos were removed from the choice of chemicals. However, Heritage was added, as well as a foliar application of Subdue MAXX. These coincided more closely with fungicides currently used in the regional nursery industry. Control blocks with no fungicide were also included, and all blocks contained nine plants. As in the earlier spring trials, foliar fungicides were applied, at label or recommended rates, to runoff using a hand sprayer. Fourteen days after application, plants were inoculated with only *Phytophthora citricola, P. ramorum* D12A, or *P. ramorum* 2027. There were three replicate plants randomly selected for each isolate inoculation.

Each rhododendron plant had three leaves removed for a detached inoculation, and three leaves selected on the plant for intact inoculation with a pathogen using four mycelial plugs per leaf. Each lilac plant had three leaves removed for detached leaf inoculation, and three leaves on the plant were chosen for intact inoculation, but due to their small size, each leaf was inoculated with a single mycelial plug. Wounding and inoculation of leaves was as described above.

Inoculated plants were hand-misted and enclosed in 4.4-dm^3 clear polyethylene bags, and incubated in a large walk-in incubator at 20°C under lights for 8 days. Detached leaves were hand-misted and placed in moist, sealable containers, and incubated in the same environment as the intact plants. Lesion area, as a percentage of each total leaf area, was assessed quantitatively from digital photographs using ASSESS software as described above.

Data for each plant species were examined independently. Lesion percentage data were transformed to arcsine-square root values prior to analyses to normalize variances. ANOVA was used to determine significance of main effects and interactions (Systat 11.0 software). Since highly significant interactions were apparent between main effects (Table 4), means were compared in Figure 1 using 95% confidence interval bars, as this seemed more appropriate to nursery applications. Overlapping bars indicate insignificant differences.

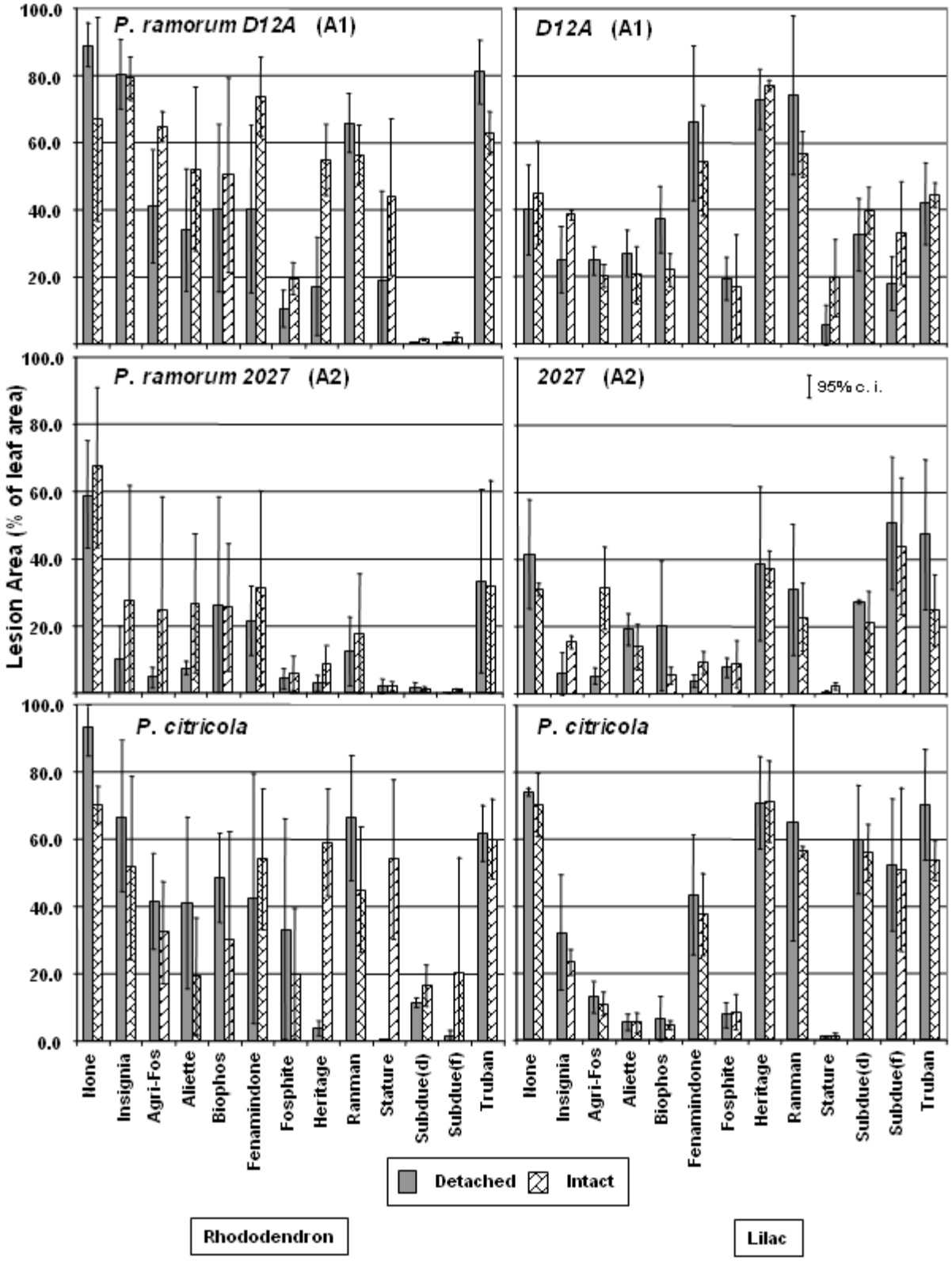

Fig. 1. Detached and intact leaf assays of chemical agent applications to rhododendron and lilac plants. Complete product tradenames are listed in Table 1. Each column is the mean of 3 replicate leaves wound-inoculated with mycelial plugs of a *Phytophthora* isolate in 4 spots. Overlapping 95% confidence interval bars are not significantly different.

Table 4. Analysis of variance (ANOVA) to compare detached vs intact leaf assay method, fungicide, and isolate effects on lesion areas of rhododendrons and lilacs (Experiment 2).*

Source		df	Sum of square	Mean square	*F* value	*P*
Rhodo-dendron	Method	1	0.30	0.30	8.83	0.003
	Fungicide	2	14.62	1.22	35.36	0.001
	Isolate	12	4.82	2.41	69.91	0.001
	Method × fungicide	12	1.78	0.15	4.31	0.001
	Method × isolate	2	0.05	0.03	0.79	0.458
	Fungicide × isolate	24	1.86	0.08	2.25	0.002
	Method × fungicide × isolate	24	1.66	0.07	2.01	0.006
	Error	156	5.37	0.03	—	—
Lilac	Method	1	0.05	0.05	3.11	0.080
	Fungicide	2	9.94	0.83	52.74	0.001
	Isolate	12	1.64	0.82	52.06	0.001
	Method × fungicide	12	0.35	0.03	1.85	0.050
	Method × isolate	2	0.08	0.04	2.55	0.080
	Fungicide × isolate	24	3.46	0.15	9.17	0.001
	Method × fungicide × isolate	24	0.63	0.03	1.66	0.036
	Error	156	2.45	0.02	—	—

* ANOVA of arcsine-square root transformed perscentage values of leaf lesion areas.

In three-way ANOVAs using method, fungicide, and isolates as factors, the method effect remained insignificant in lilac bioassays, and the other two parameters of fungicide and isolate were highly significant (Table 4). However, in rhododendron assays, three-way ANOVAs showed high significance ($P \leq 0.003$) of all three parameters, as well as several interactions, indicating the effects of any given parameter were not consistent.

Both foliar and drench forms of Subdue MAXX applications were effective in reducing lesion areas of rhododendron leaves for all three *Phytophthora* isolates (Fig. 1) relative to other fungicides. Other fungicides showed mixed efficacy with the different isolates. Heritage was effective on detached leaves against all isolates, but marginally effective on intact leaves. Fungicide efficacy was not similar for rhododendrons and lilacs.

On lilacs, Stature DM was more effective at reducing lesion size than either form of Subdue MAXX application across all *Phytophthora* isolates on detached leaves (Fig. 1). Heritage, Ranman, and Truban offered no protection against any isolate, and in some cases, increased lesion area. In general, there was little significant protection against *P. ramorum* D12A by most fungicides.

Residual Effects of Foliar Application of Subdue MAXX to Rhododendrons

Since previous experiments indicated that Subdue MAXX provided the most control of *Phytophthora* species on rhododendrons, we conducted another experiment to determine the length of residual effects of the chemical before it lost efficacy. In early spring of 2005, Subdue MAXX was applied to the foliage of large 66.1-dm^3 container-grown 'Nova Zembla' rhododendrons at a rate of 0.2 ml/liter. Three replicate plants were used for chemical treatment and three for water controls. Two weeks after treatment, nine random leaves were collected from each plant on a weekly basis for 8 weeks. Three leaves were inoculated with

mycelial plugs of *P. ramorum* D12A, *P. ramorum* 2027, or *P. citricola*. Wounding, inoculation, storage, and lesion assessment of all leaves was as described above.

Data were analyzed as a repeated-measure design using Systat 11.0 software. The week of inoculation was the repeated variable (not a regressor); chemical treatment and *Phytophthora* isolate were the fixed effects. Interactions and singular effects of the chemical and isolate factors were all very highly significant ($P \leq 0.001$); 95% confidence intervals were chosen to distinguish significant treatments.

Subdue MAXX reduced disease symptoms of the *Phytophthora* isolates tested for 6 weeks after treatment, compared to the water controls (Fig. 2), although not statistically significant ($P \leq 0.05$) for *P. ramorum* 2027. At that time, symptoms caused by *P. ramorum* 2027 or *P. citricola* were reduced by Subdue MAXX, but by 8 weeks, neither pathogen was controlled. However, with *P. ramorum* D12A, symptoms were still suppressed at 8 weeks. These results relate to quarantine regulations specifying that 6 weeks without fungicide application are necessary for the masking effects from previous treatments to have dissipated enough for symptoms from latent infections to appear.

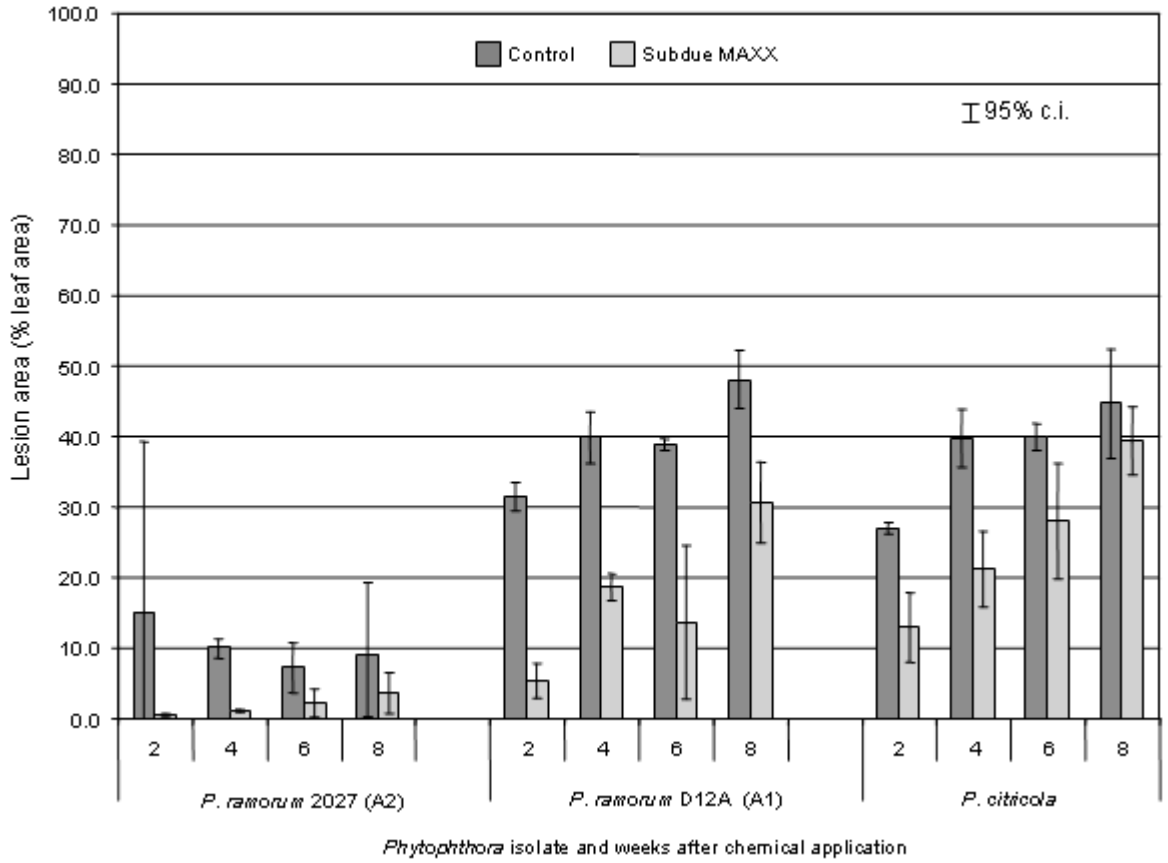

Fig. 2. Residual control by Subdue MAXX against *Phytophthora* isolates. Each column is the mean of 3 replicate leaves wound-inoculated with mycelial plugs of a *Phytophthora* isolate on 4 spots per leaf. Overlapping 95% confidence interval bars are not significantly different.

Lesion Development on Rhododendron Leaves Inoculated with *P. ramorum* Sporangia after Foliar Applications of Chemical Agents

In trials comparing lesion sizes on rhododendron leaves inoculated with mycelial plugs versus sporangia of *P. ramorum* isolates D12A and N10A, we found more severe symptoms induced by the plug method across a variety of foliar chemicals (*data not shown*). In addition, there were no significant differences in the lesion symptoms induced by the two isolates in plug

inoculations. Since wound-infections by sporangia or zoospores are more likely to occur under field conditions, we conducted subsequent *P. ramorum* studies inoculating with only sporangia of the N10 isolate.

In August 2006, 4.4-dm^3-container-grown 'Catawbiense Boursault' rhododendrons maintained outdoors were sprayed with selected chemical products (Table 5) seven days before inoculation with *P. ramorum* isolate N10A. Five replicate plants were arranged in separate treatment blocks for chemical application, and three random leaves were picked from each plant one hour before inoculation. Each leaf was wounded as described earlier and inoculated with 10 µL of N10A sporangia (containing 152 sporangia/ml as determined from dilution plate counts). Sporangia were produced on dilute V8 juice agar plates, starting from a sporangial suspension spread on the plates, and incubated at 20° C for 10 days. Just prior to inoculation, the plates were flooded with 5 ml of sterile distilled water and the surface of the agar gently scraped with the edge of a spatula. The aqueous suspension of cauducous sporangia was then poured into a beaker and gently swirled using a magnetic stirrer. Inoculated leaves were placed on moist vermiculite in polyethylene incubation bags and allowed to sit for 4 h before receiving a light misting as each bag was sealed. Leaves were incubated as described earlier, followed by lesion assessment at 8 days.

Table 5. Chemical products applied to *Rhododendron* 'Catawbiense Boursault' prior to inoculation with *Phytophthora ramorum* (2006).

Treatment, product name	**Product description**	**Rate of product / liter**
Aliette WDG	80% fosetyl-Al BayerCropScience LP, Research Triangle Park, NC	0.8 g
Alude	45.8% mono- and di-potassium salts of phosphorus acid Cleary Chemical Corp., Dayton, NJ	0.2 ml
BioPhos (Lexx-a-phos)	22.7% di-potassium phosphate; 22.4% di-potassium phosphonate Foliar Nutrients, Inc., Cairo, GA	19.2 ml
Captan 50WP	50% Captan Micro Flo Company, LLC; Memphis, TX	2.5 g
Fenamidone	98.5% fenamidone (technical) BayerCropScience LP, Research Triangle Park, NC	High: 1.1 ml Low: 0.5 ml
Heritage	50% azoxystrobin Syngenta Crop Protection, Greensboro, NC	0.3 g
Insignia (BAS 500)	20% pyraclostrobin BASF Corp., Research Park Triangle, NC	0.6 g
Magellan	53.6% mono- and di-basic sodium, potassium, ammonium phosphites Nufarm Americas, Burr Ridge, IL	0.2 ml
Multiguard	75% furfural Agriguard Co., LLC, Cranford, NJ	1.0 ml
Ranman	40% cyazofamid ISK Biosciences, Mentor, OH	High: 0.5 ml Low: 0.3 ml
StatureDM	50% dimethomorph Sepro Corp., Carmel, IN	1.0 g
Subdue MAXX	22% mefanoxam Syngenta Crop Protection, Greensboro, NC	0.3 ml
Terrazole 35WP	35% etridiazole Crompton Uniroyal; Middlegury, CT	0.5 g
Control	No fungicide	—

The study was conducted twice, once in 2005, once in 2006. Data from the two trials were combined for analysis since variance among trials was homogeneous according to Bartlett's test (Systat 11.0). Means were separated by Fisher's protected least significant difference (FPLSD) at $P = 0.05$.

Analysis of arcsine-transformed data indicated that chemical treatment effect was very highly significant ($P \leq 0.001$). Aliette, Fenamindone (high rate), Stature DM, and Subdue MAXX demonstrated significant reduction in lesion size relative to no chemical application, but only Subdue MAXX was able to effectively control the pathogen. Blight symptoms actually worsened when Heritage and Insignia were applied (Fig. 3).

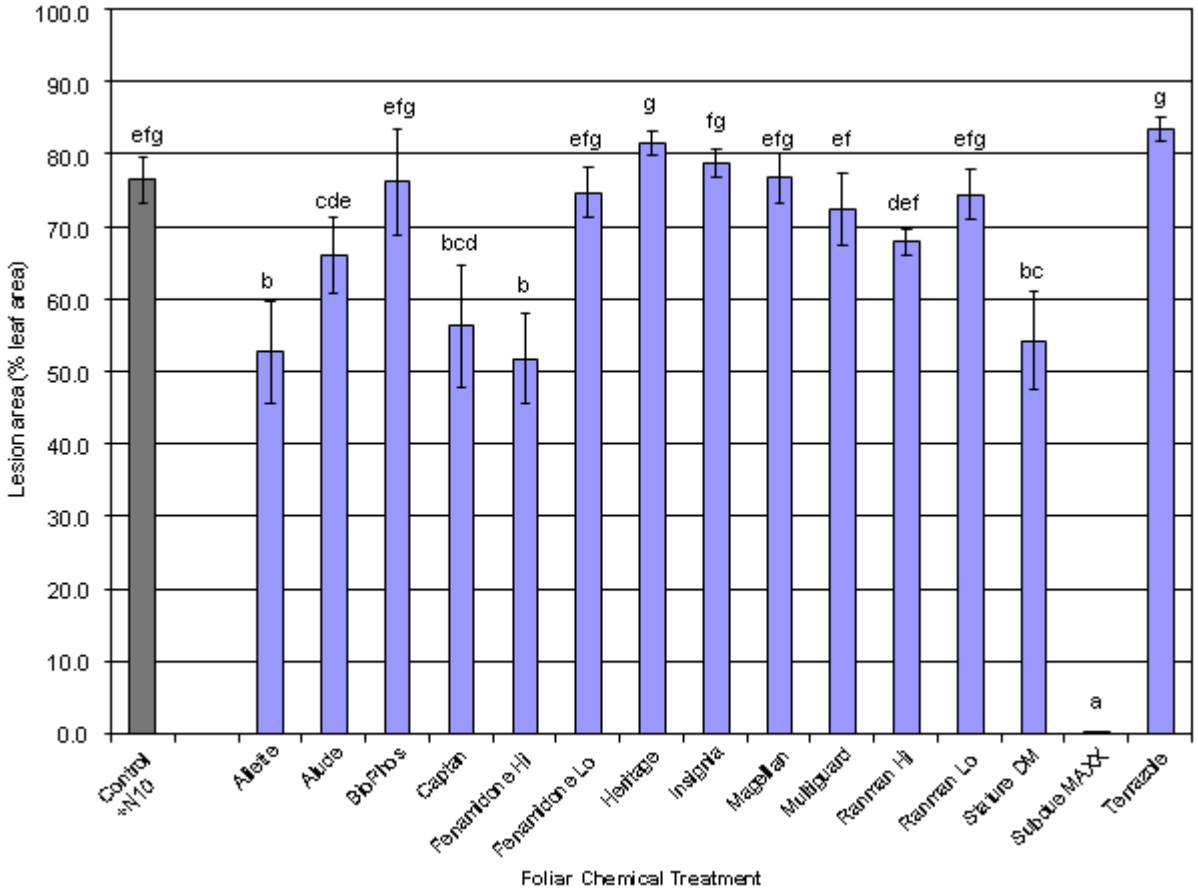

Fig. 3. Lesion development on detached rhododendron leaves inoculated with *P. ramorum* N10A sporangia after foliar application of chemical agents. Complete product tradenames are listed in Table 5. Mean lesion areas for each chemical treatment are based on 4 inoculated spots per leaf; 8 replicate leaves from two combined trials (± SEM). Columns with the same letter designator are not signficantly different at $P = 0.05$.

Fig. 4. Response of rhododendron 'Nova Zembla' leaves treated with Subdue MAXX (right pair) compared to the water control (left pair) and inoculated at four wound sites per leaf with *Phytophthora ramorum* isolate 2027 (A2 mating type).

Summary and Discussion

Our studies strongly indicate the superior chemical suppression of species of *Phytophthora* by Subdue MAXX, applied either as a drench or to the foliage. The systemic movement and persistence of the chemical was also demonstrated, and it remained significantly effective against several species even 6 weeks after application. While other chemicals used showed statistically significant reductions in symptom development, none was comparable to Subdue MAXX. However, we have experienced considerable variability in efficacy in the many experiments conducted, suggesting that environmental, seasonal, and physiological effects played undetermined roles. For example, we often observed some replicate leaves that, when inoculated, were clearly protected from infection, while others were not. Similarly, of the four inoculation sites used per leaf, at lower rates or after long residual periods, efficacy was different among the four inoculation sites on a single leaf. We suspect that there was variation in the movement of the chemicals within the plant tissues. Furthermore, the random selection of leaves from a sprayed plant could account for variation in efficacy if some leaves were treated more thoroughly than others.

In our early studies, comparing various *Phytophthora* species with different sporulation potentials, we needed to use mycelial plugs for comparative purposes. However, in our later studies focusing on *P. ramorum* it was more appropriate to use sporangial inoculum. Furthermore, in an unpublished study comparing mycelial plug versus sporangial inoculum of *P. ramorum*, we found that chemicals were less effective in reducing lesion size induced by the plug method. We found effective lesion development on rhododendron leaves with fewer than 10 sporangia per drop, applied to a single needle wound. This method has given consistent lesion development and closely simulates infections that could occur in a nursery. This method was also used successfully in other studies (7,8).

Phosphonate materials, such as Aliette, generally were not very effective against *P. ramorum*, at least not compared to Subdue MAXX. However, this was highly pathogen isolate and host dependent. These Oomycete-targeted chemicals are not equally effective against all species or isolates of *Phytophthora*. Furthermore, host differences, such as between rhododendron and lilac, also played some role. Thus, we caution growers and manufacturers that chemicals that are effective on one species of *Phytophthora* may not be effective against another species on the same or another host.

We observed that some chemicals slightly increased the size of lesions caused by *Phytophthora* species. This could be related to the detached leaf assay itself, although our results in general indicate that the assay does accurately reflect the potential for a chemical to suppress the disease. *P. citrophthora* was one pathogen that seemed to react that way to some chemicals, including Subdue MAXX (Table 3). However, we determined from the grower where that pathogen had been isolated that metalaxyl had been used in the nursery for some 15 years, suggesting that the lack of control could be due to it having developed resistance to the chemical.

The fact that all the materials used were only fungistatic, not fungicidal, leads to the concern that application of chemical control agents can mask symptoms. Reducing the size of lesions with chemical regimes could make their detection by inspectors very difficult. Furthermore, extended exposure to those chemicals, including Subdue MAXX, could hasten the development of resistance by the pathogens. This has long-term significance since most chemicals were only partially effective in suppressing *P. ramorum*, making resistance management difficult.

Acknowledgments and Disclaimers

We acknowledge the excellent assistance from Bryan Beck, Amber Wierck, and Kenneth Rolfe in this project. Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the United States Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that also may be suitable.

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