

# Effectiveness of Fungicides in Protecting Conifers and Rhododendrons From *Phytophthora ramorum*<sup>1</sup>

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## Abstract

The effectiveness of 19 fungicides in protecting noble fir, grand fir, and *Rhododendron x* 'Nova Zembla' foliage from *P. ramorum* was tested. The tops of conifer seedlings with newly emerging shoots and mature rhododendron leaves were collected from treated plants 7 days after drench applications or 1 day after foliar applications. Detached seedling tops and leaves were then placed on moistened filter paper in Petri dishes and inoculated. Zoospores from three European genotype isolates of the A1 mating type (EU1 lineage) and three NA1 lineage isolates of the A2 mating type were mixed to create two inoculum sources representing the A1 and A2 mating types for the conifer tests. Detached rhododendron leaves were inoculated with suspensions of zoospores from a EU1 A1 mating type rhododendron isolate and a North American genotype NA1 A2 mating type rhododendron isolate. The rhododendron leaves were inoculated by pipetting three 10 ul drops of zoospore suspension onto the lower leaf surface on each side of the leaf midrib. The leaf tissue was injured beneath 3 drops on one side of the leaf midrib using an insect pin. The tissues beneath the drops on the other side of the leaf were left unwounded. Checks included inoculated and non-inoculated conifer tops and rhododendron leaves that had been treated with plain water. Following inoculation, the Petri dishes were incubated for 7 days at 19°C in plastic tubs. Fungicide efficacy on the conifers was quantified by calculating the percent of visibly diseased shoots on each seedling top. Fungicide efficacy on the rhododendron leaves was quantified by counting the number of inoculation sites that developed symptoms and the area of each resulting lesion using ASSESS. No disease developed on any of the non-inoculated conifer or rhododendron checks. On the conifers, ANOVA analysis indicated that genotype and treatment ( $P < 0.0001$ ) were the only variables that had a significant affect on infection. Overall, the seedling tops inoculated with the A1 mating type had significantly fewer infected shoots than the seedlings inoculated with the A2 mating type. Applications of Dithane, Gavel, Stature, Ranman, Maneb, Polyram, Fenstar, Daconil Ultrex, V-10161, and Insignia were the most effective fungicides in reducing infection of the conifer shoots by both the A1 and A2 mating types of *P. ramorum*. On rhododendron, ANOVA analysis indicated that wounding and treatment ( $P = 0.001$ ) were the only variables that had significant effects on the number of infected inoculation sites and lesion size. Overall, fewer fungicides were effective in reducing disease development on the wounded leaf surfaces. Maneb, Gavel, Subdue MAXX, Ranman (3 oz), Stature DM and Dithane were the most effective treatments in controlling disease development on both the wounded and non-wounded rhododendron leaves.

Key words: *Phytophthora ramorum*, disease management, fungicides, control, conifers, rhododendrons.

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## Introduction

The exotic plant pathogen, *Phytophthora ramorum*, has the potential to infect a number of commonly grown nursery crops in the Pacific Northwest, which is a major production area for ornamental and conifer nursery stock. Nurseries currently utilize a number of cultural practices, such as isolation of production blocks, drainage, treatment of recirculated water, and irrigation management, to prevent various *Phytophthora* diseases. There are a number of systemic and contact fungicides that are also often used to help control *Phytophthora* diseases on nursery crops.

Limited information is available regarding the effectiveness of many of these fungicides in protecting plants from *P. ramorum*. During 2005, we conducted two trials to evaluate the effectiveness of 19 fungicides in protecting noble fir (*Abies procera*), grand fir (*A. grandis*), and *Rhododendron* x 'Nova Zembla' foliage from infection by *P. ramorum* (table 1).

**Table 1—List of fungicides tested**

Trade name	Common name	Rate of product/100 gal	Application method <sup>1</sup>
Aliette	Fosetyl AL	5 lbs	F
Alude	Phos. acid gen.	1 qt	D
Biophos	Phos. acid gen.	2%	D
Champ Formula 2F	Copper hydroxide	1.33 pts	F
Daconil Ultrex	Chlorothalonil	1.4 lbs	F
Dithane 75 DF	Mancozeb	2 lbs	F
Fenstar	Fenamidone	14 & 28 oz	F
Gavel 75 DF	Mancozeb + zoxamide	2 lbs	F
Insignia 20W	Pyraclostrobin	16 & 40 oz	F
Magellan	Phos. acid gen.	80 fl oz	D
Maneb 75 DF	Maneb	2 lbs	F
Polyram 80 DF	Metiram	2 lbs	F
Ranman	Cyazofamid	3 & 6 oz	F
Rhapsody	Bacillus subtilis	1, 1.5, & 2%	F
Stature DM	Dimethomorph	6.4 & 12.8 oz	F
Subdue MAXX	Mefenoxam	2 fl oz	D
TM-473	Fluoxastrobin	5 oz	F
V-10161	Potassium phosphite	1.4 oz ai	F
Vital	Phos. acid gen.	4 pt	D

<sup>1</sup>Application method: F = foliar and D = drench.

## Materials and Methods

### Conifer Test

Frozen field-grown noble fir (size 1-0) and container-grown grand fir seedlings (Styro 2A plugs) were transplanted into Ray Leach “Conetainers”™ and stored in a cooler at 3°C. In order to force bud break, seedlings were placed in a warm greenhouse until the new shoots at the top of the seedling had reached 1 to 2 cm in length. Drench treatments were applied 7 days prior to inoculation. The foliar fungicide treatments were applied with a backpack sprayer to seedling tops 6 days after the drench applications. The following day, tops of both the drenched and foliar-treated seedlings were cut off and placed on moistened filter paper (Whatman #2) in a Petri dish. The detached noble fir and grand fir seedling tops had an average of 6.7 and 6.9 emerging shoots per top, respectively.

Two zoospore suspensions, representing the European (EU1 lineage A1 mating type) and North American (NA1 lineage A2 mating type) genotypes of *P. ramorum* were prepared and used to inoculate the seedling material in each petri plate. Three isolates of each genotype were mixed to provide inoculum. The EU1 A1 isolates used were 03-74-1 (from Oregon, host *Pieris japonica*), 03-74-2 (Oregon, host *Viburnum x bodnantense* ‘Dawn’) and 03-74-N11-A (Oregon, host *Rhododendron* x ‘Unique’). The NA1 A2 isolates were originally from Oregon tanoak (No. 2018.1, 2027.1, and 2109). Spore counts of each suspension were calculated using a hemacytometer. The EU1 A1 spore count was 378,000 spores/ml and the A2 was 350,500 spores/ml. The spore suspensions were applied using an airbrush sprayer. The non-inoculated check tops were sprayed with water only. Following inoculation, the Petri plates were placed in sealed tubs and incubated for 7 days at 19°C.

Treatment effectiveness was determined by counting the number of diseased emerging shoots out of a maximum of eight buds on each seedling top. Isolations were done from representative symptomatic shoots in each plate onto CARP medium and then subcultured to 1/3 V8 medium for identification to ensure symptoms were associated with infection by *P. ramorum*.

This experiment was a randomized split block design with two hosts (noble and grand fir), two *P. ramorum* genotypes (A1 and A2), and 27 treatments. Treatments included inoculated and non-inoculated checks. There were three noble fir and four grand fir seedlings per treatment and genotype. Data were analyzed using a one-way ANOVA (GLM SAS). Treatment means were separated using Tukey’s Studentized Range (HSD) Test.

### Rhododendron Test

*Rhododendron* x ‘Nova Zembla’ plants grown outdoors in 3.8 l (1 gal) pots were used during this test. One day after the foliar applications and 7 days after the drench applications, two leaves were removed from each plant. These leaves were trimmed and each leaf was placed with its lower surface up on moistened filter paper in a Petri dish. A total of five plants were used per treatment.

Suspensions of zoospores from a single isolate of each genotype were prepared. The EU1 A1 isolate was 03-74-N11-A (from Oregon, host *Rhododendron* x ‘Unique’) and the NA1 A2 isolate was 03-74-N10-A (Oregon, *Rhododendron* x ‘Unique’). Spore counts were determined using a hemacytometer and were 688,000 spores/ml

and 668,000 spores/ml for EU1 A1 and NA1 A2, respectively. Three 10 ul drops of inoculum were placed on each side of the leaf midrib. An insect pin was then used to injure the leaf tissue beneath the 3 drops on one side of the leaf. The tissue beneath the drops on the other side of the leaf was not wounded. Following inoculation, the Petri plates were placed in sealed tubs and incubated for 7 days at 19°C.

Treatment effectiveness was determined by counting the number of inoculation sites that developed symptoms and measuring the area of each resulting lesion using the APS ASSESS Image Analysis software. Isolations were done from representative symptomatic lesions onto CARP medium and subcultured to 1/3 V8 medium to ensure that *P. ramorum* was the cause of infection.

This experiment was a split-split plot design with plants nested in treatments and split by mating type (EU1 A1 and NA1 A2) and wounding (wounded and non-wounded). Treatments included inoculated and non-inoculated checks. Data were analyzed using ANOVA (GLM SAS). Treatment means were separated using Tukey's Studentized Range (HSD) Test.

## Results and Discussion

### ***Conifer Test***

Genotype ( $P < 0.0001$ ) and treatment ( $P < 0.0001$ ) were the only variables that were significant in the ANOVA. Overall, the seedlings tops inoculated with the EU1 A1 genotype had significantly fewer diseased shoots than the seedlings inoculated with the NA1 A2 genotype (data not shown). No symptoms developed on any of the non-inoculated seedling tops.

For seedlings inoculated with the EU1 A1 genotype, the numbers of diseased shoots on several of the treated seedlings were not significantly different than the non-inoculated checks (table 2). These treatments included Dithane, Gavel, Stature, Ranman, Maneb, Polyram, Fenstar, Daconil Ultrex, V-10161, Insignia, and Subdue MAXX. None of the other fungicides reduced the number of diseased shoots compared to the inoculated checks.

When inoculated with the NA1 A2 genotype, the number of diseased shoots on seedlings treated with Dithane, Gavel, Stature, Ranman, Maneb, Polyram, Fenstar, Daconil Ultrex, V-10161, and Insignia (1.18 l = 40 oz) also did not differ significantly from the non-inoculated checks (table 2). None of the other fungicides reduced infections compared to the inoculated checks.

### ***Rhododendron Test***

ANOVA analysis indicated that wounding and treatments were the only variables that had significant effects on the number of inoculation sites that developed symptoms and lesion size. Since genotype had no effect on symptom development and lesion size, separate analyses were done on the wounded and non-wounded inoculation sites to assess treatment difference using the combined EU1 A1 and NA1 A2 data.

**Table 2—Effect of fungicide treatments on the average number of infected shoots on the detached noble and grand fir seedling tops**

Treatment	Rate/100 gal	Application method <sup>1</sup>	<i>P. ramorum</i> genotype	
			A1	A2
Vital	4 pt	D	3.3 abc <sup>2</sup>	5.7 a
Rhapsody	2%	F	2.9 bcde	5.6 a
Magellan	80 fl oz	D	3.0 abcd	5.6 a
Inoc. Check	n/a	n/a	4.8 a	5.6 a
Alude	1 qt	D	2.6 cdef	5.3 a
Biophos	2%	D	4.4 ab	5.1 ab
Rhapsody	1%	F	4.7 a	5.0 abc
Rhapsody	1.5%	F	4.6 ab	4.4 abcd
Insignia 20W	16 oz	F	1.3 defgh	3.3 bcde
Champ Formula 2F	1.33 pts	F	2.1 cdefg	3.0 cdef
Aliette	5 lbs	F	3.0 abcd	2.9 def
TM-473	5 oz	F	3.3 abc	2.7 defg
Subdue MAXX	2 fl oz	D	0.1 h	2.6 defgh
Insignia 20W	40 oz	F	1.4 defgh	1.7 efghi
V-10161	1.4 oz ai	F	1.0 fgh	1.6 efghi
Fenstar	14 oz	F	0.3 h	1.4 efghi
Daconil Ultrex	1.4 lbs	F	1.1 efgh	1.4 efghi
Fenstar	28 oz	F	0.0 h	1.1 efghi
Ranman	3 oz	F	0.3 h	1.1 efghi
Polyram 80 DF	2 lbs	F	0.3 h	1.0 fghi
Stature DM	6.4 oz	F	1.1 efgh	1.0 fghi
Maneb 75 DF	2 lbs	F	0.0 h	0.6 ghi
Ranman	6 oz	F	0.0 h	0.4 hi
Stature DM	12.8 oz	F	0.0 h	0.4 hi
Gavel 75 DF	2 lbs	F	0.6 gh	0.1 i
Dithane 75 DF	2 lbs	F	0.0 h	0.0 i
Non-inoc. Check	n/a	n/a	0.0 h	0.0 i

<sup>1</sup>Drench (D) or foliar (F) application.

<sup>2</sup>Numbers in columns followed by the same letter are not significantly different, P=0.05, Duncan's Multiple Range Test.

On the non-wounded inoculation sites, numbers of symptomatic inoculation sites and lesion area on leaves treated with Maneb, Fenstar, Gavel, Dithane, Polyram, Subdue MAXX, Ranman, Daconil Ultrex, Stature (363.7 ml = 12.8 oz), and Insignia (1.18 l = 40 oz) were not significantly different than the number of symptomatic inoculation sites and lesion area on the non-inoculated checks, which were zeros (table 3).

Although the lesion areas on the leaves treated with Insignia (473.2 ml = 16 oz), Stature (189.3 ml = 6.4 oz) and V-10161 were also not different than those on the non-inoculated checks, the number of symptomatic inoculation sites was higher and did not differ from the inoculated checks. TM-473 provided intermediate reduction in lesion area, but had no effect on the number of inoculation sites that developed symptoms. The other fungicides (Magellan, Rhapsody, Aliette, Vital, Biophos, Alude, and Champ) neither significantly reduced the number of inoculation sites that developed symptoms nor did they reduce lesion area compared to the inoculated checks.

**Table 3—Effectiveness of fungicides in limiting infection and lesion size on non-wounded leaves inoculated with *Phytophthora ramorum***

Treatment	Rate	Application Method <sup>1</sup>	No. infected sites <sup>2</sup>	Lesion area <sup>3</sup>
Magellan	80 fl oz	D	3.0 a <sup>4</sup>	127.5 a
Rhapsody	1.5% formula	F	2.8 ab	118.0 abc
Rhapsody	1% formula	F	2.8 ab	98.8 abcd
Inoc. Check	n/a	n/a	2.7 abc	123.0 ab
Aliette	5 lbs	F	2.7 abc	87.2 cd
Vital	4 pt	D	2.5 abc	79.9 de
Biophos	2% formula	D	2.5 abc	67.5 defg
Alude	1 qt	D	2.4 abc	94.9 abcd
TM-473	5 oz	F	2.1 bcd	48.1 efgh
Champ Formula 2F	1.33 pts	F	1.9 cde	72.3 def
Rhapsody	2% formula	F	1.5 def	89.1 bcd
Insignia 20W	16 oz	F	1.4 defg	33.7 ghi
Stature DM	6.4 oz	F	1.3 defgh	43.0 fgh
V-10161	1.4 oz ai	F	1.3 defgh	27.5 hi
Insignia 20W	40 oz	F	1.1 efghi	20.6 hi
Stature DM	12.8 oz	F	0.9 fghij	27.2 hi
Daconil Ultrex	1.4 lbs	F	0.9 fghij	20.6 hi
Ranman	3 oz	F	0.9 fghij	18.9 hi
Ranman	6 oz	F	0.7 fghij	18.0 hi
Fenstar	28 oz	F	0.6 ghij	17.7 hi
Subdue MAXX	2 fl oz	D	0.6 ghij	3.0 i
Polyram 80 DF	2 lbs	F	0.5 hij	11.9 hi
Dithane 75 DF	2 lbs	F	0.4 ij	14.9 hi
Gavel 75 DF	2 lbs	F	0.1 j	0.0 i
Fenstar	14 oz	F	0.0 j	0.0 i
Maneb 75 DF	2 lbs	F	0.0 j	0.0 i
Non-inoc. Check	n/a	n/a	0.0 j	0.0 i

<sup>1</sup>D = Drench and F = Foliar spray.

<sup>2</sup>Average number out of 3 inoculated sites per leaf.

<sup>3</sup>Average lesion area (mm<sup>2</sup>) per 3 inoculation sites per leaf.

<sup>4</sup>Numbers followed by the same letter are not significantly different, P=0.05, Duncan's Multiple Range Test.

Fewer fungicides reduced disease development on the wounded leaves (table 4). The numbers of inoculation sites that developed symptoms and lesion area on leaves treated with Maneb, Gavel, Subdue MAXX, Ranman (88.7 ml = 3 oz), Stature DM and Dithane were not significantly different than the number of symptomatic inoculation sites and lesion area on the non-inoculated checks, which were zeros. Although the lesion area on the leaves treated with Polyram, V-10161, Fenstar, Ranman (177.4 ml = 6 oz), Insignia, Daconil Ultrex, and TM-473 was not significantly different than the non-inoculated checks, these fungicides did not significantly reduce the number of inoculation sites that developed symptoms. Applications of Rhapsody, Magellan, Alude, Biophos, Aliette, Vital, and Champ neither significantly reduce the number of inoculation sites that developed symptoms nor did they reduce lesion area compared to the inoculated checks.

**Table 4—Effectiveness of fungicides in limiting infection and lesion size on wounded leaves inoculated with *Phytophthora ramorum***

Treatment	Rate	Application Method <sup>1</sup>	No. infected sites <sup>2</sup>	Lesion area <sup>3</sup>
Rhapsody	<b>1.5% formula</b>	F	3.0 a <sup>4</sup>	141.9 a
Rhapsody	1% formula	F	3.0 a	102.5 abcd
Inoc. Check	n/a	n/a	2.9 ab	131.3 ab
Magellan	80 fl oz	D	2.8 ab	119.2 ab
Alude	1 qt	D	2.8 ab	105.8 abc
Biophos	2% formula	D	2.7 abc	75.9 cdef
TM-473	5 oz	F	2.7 abc	50.0 efgh
Rhapsody	2% formula	F	2.6 abc	103.8 abcd
Aliette	5 lbs	F	2.6 abc	70.2 cdefg
Insignia 20W	16 oz	F	2.5 abc	52.1 efg
Fenstar	14 oz	F	2.4 abc	26.9 ghij
Daconil Ultrex	1.4 lbs	F	2.3 abcd	52.5 efg
Vital	4 pt	D	2.2 abcde	62.0 defg
Insignia 20W	40 oz	F	2.2 abcde	41.7 fghij
Champ Formula 2F	1.33 pts	F	2.1 abcde	91.4 bcde
Ranman	6 oz	F	1.9 bcdef	35.4 fghij
Fenstar	28 oz	F	1.7 cdefg	42.3 fghij
V-10161	1.4 oz ai	F	1.7 cdefg	34.4 fghij
Polyram 80 DF	2 lbs	F	1.7 cdefg	34.0 fghij
Dithane 75 DF	2 lbs	F	1.4 defgh	56.5 efg
Stature DM	6.4 oz	F	1.3 efgh	45.7 fghi
Stature DM	12.8 oz	F	1.1 fghi	38.7 fghij
Ranman	3 oz	F	1.1 fghi	27.1 ghij
Subdue MAXX	2 fl oz	D	0.8 ghij	3.2 ij
Gavel 75 DF	2 lbs	F	0.5 hij	6.0 hij
Maneb 75 DF	2 lbs	F	0.3 ij	4.3 ij
Non-inoc. Check	n/a	n/a	0.0 j	0.0 j

<sup>1</sup>D = Drench and F = Foliar spray

<sup>2</sup>Average number out of 3 inoculated sites per leaf

<sup>3</sup>Average lesion area (mm<sup>2</sup>) per 3 inoculation sites per leaf

<sup>4</sup>Numbers followed by the same letter are not significantly different, P=0.05, Duncan's Multiple Range Test

The results of these experiments indicate that a number of fungicides have the potential to control symptom development on conifer shoots and rhododendron leaves inoculated with *P. ramorum*. They also indicate that a number of fungicides that are effective in controlling other types of *Phytophthora* diseases provide limited or no protection against *P. ramorum* on the hosts we tested. Although a number of foliar-applied treatments were very effective in preventing symptom development, it is important to recognize that these treatments were applied under optimal conditions to ensure complete coverage of the developing conifer shoots and rhododendron leaves. It is unlikely that such complete coverage would occur in many production systems where large numbers of plants are grown in close proximity to each other, thus making it very difficult to thoroughly cover susceptible host tissues. In general, fungicides that were applied as drenches were not very effective. None of the drench treatments were effective on the conifers and the Subdue MAXX was the only effective drench treatment on the rhododendrons. It is unclear if some of these products would have worked better if there had been a longer interval between application of the fungicide and inoculation of the plant.

A better understanding of the residual activity of fungicides in prolonging disease control is needed, particularly on hosts such as rhododendron where tissue is susceptible to infection for extended periods of time. Trials that are currently in progress on rhododendron leaves indicate that the residual activity of single applications of some fungicides, such as Ranman, can persist for at least 12 weeks. The isolations we did from symptomatic tissues indicate that fungicide residues on the tissue did not adversely affect our ability to isolate *P. ramorum* (data not shown). Symptomatic tissues were not tested with ELISA or PCR, so it is unclear if any of the fungicide residues might adversely affect the ability to detect the presence of the pathogen using these techniques.

We are also conducting additional experiments to determine what effect fungicide treatments have on symptom development when they are applied to asymptomatic, infected rhododendron leaves. Many of the fungicides that were effective in these trials are contact types of materials that have no systemic activity. While systemic fungicides may have the potential to inhibit symptom development when applied to asymptomatic, infected foliage, it is unclear what if any, effect contact fungicides would have on symptom development. The results of this research will provide a better understanding of the risk that specific fungicides pose in masking symptom development on rhododendron leaves.

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