

Evaluation of Fungicides for the Control of *Phytophthora ramorum* Infecting *Rhododendron*, *Camellia*, *Pieris*, and *Viburnum*

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Tjosvold, S. A., Koike, S. T., and Chambers, D. L. 2008. Evaluation of fungicides for the control of *Phytophthora ramorum* infecting *Rhododendron*, *Camellia*, *Pieris*, and *Viburnum*. Online. Plant Health Progress doi:10.1094/PHP-2008-0208-01-RS.

Abstract

Fungicides were evaluated for pre- and post-infection control of ramorum blight, caused by *Phytophthora ramorum*, on *Rhododendron* cvs. Cunningham's White and Irish Lace, *Camellia japonica*, *Pieris japonica*, and *Viburnum tinus*. Cyazofamid, dimethomorph, mefenoxam, pyraclostrobin, and fenamidone applied as foliar sprays consistently provided preventative control as indicated by reduced lesion size compared to water controls. These fungicides provided preventative activity for at least 28 days in the tested species except in *Rhododendron* where fungicides were active for at least 14 days following application. With preventative fungicide applications, the pathogen was recovered from most fungicide-treated leaves by isolation onto selective media. Dimethomorph consistently reduced the percent recovery from diseased *Rhododendron* leaves. With post-infection treatments, the fungicides did not significantly reduce lesion growth and percent recovery of the pathogen. The pathogen was recovered from lesions consistently at least 6 weeks after fungicide application in *Rhododendron* regardless of treatment on intact and fallen diseased leaves. However, the cultures resulting from isolations of diseased tissue treated with cyazofamid and dimethomorph were significantly slower growing than those cultures from other fungicide treatments. *P. ramorum* management issues relating to fungicide use in commercial nurseries are discussed.

Introduction

Phytophthora ramorum is the causal agent of the disease known as sudden oak death (SOD). The pathogen causes trunk cankers and widespread mortality on tanoak (*Lithocarpus densiflorus*), and oak (*Quercus* spp.) (18), and leafspots and blights on numerous other native hosts in California and Oregon woodlands (7). The pathogen was described as a new *Phytophthora* species in 2001, but it was observed as early as 1993 to cause leaf blights and mortality on *Rhododendron* and *Viburnum* in nurseries and public gardens in Germany and The Netherlands (21). With the recognition that this newly identified pathogen caused SOD, intensive nursery stock and public garden inspections ensued, and *P. ramorum* was found in several European countries. In December 2000, *P. ramorum* was first discovered infecting *Rhododendron* nursery stock in California (9). By 2003, agricultural inspectors found the pathogen infecting *Rhododendron*, *Camellia*, *Viburnum*, and *Pieris* nursery stock in California, Oregon, Washington, and British Columbia, Canada. In 2004, the disease became a national concern when a large wholesale nursery in California shipped camellia plants infected with *P. ramorum* to nurseries and other customers in 40 states. Presently the Animal and Plant Health Inspection Service (APHIS) Plant Protection and Quarantine (PPQ) Agency lists 110 plant taxa as proven or associated hosts (1). Of plants on that list, *Rhododendron*, *Camellia*, *Viburnum*, and *Pieris* are some of the most commonly cultivated and important host species worldwide.

There is much concern that infected or infested nursery stock may move through the nursery trade to locations favorable for disease development and provide inoculum to infect native forest and woodland hosts. In England and The Netherlands there is evidence of inoculum moving from infected *Rhododendron* and causing mortality on *Fagus sylvatica*, several *Quercus* species (*rubra*, *sylvatica*, and *ilex*) and *Aesculus hippocastrium*. Since 2001, there have been various state, federal, and international quarantine restrictions placed on the movement of plants or plant parts of known hosts of *P. ramorum*. Official inspections are an important part of those regulations and concerns exist that fungicide use may mask symptoms in nursery stock or reduce the effectiveness of laboratory detection. In contrast, as part of a comprehensive disease control program that includes exclusion, scouting, and sanitation, fungicides may play a role in the control of *P. ramorum* in nursery stock.

We evaluated fungicides for efficacy against *P. ramorum* on four commonly grown and important ornamental nursery hosts. Our first objective was to screen a wide range of commercially-available and experimental fungicides. The second objective was to select the best performing fungicides from objective one and further test their efficacy and residual action for preventative control. Treatments were also judged by their effects on pathogen recovery when isolated from treated leaves. The third objective was to determine the eradication potential of these fungicides by applying them to active lesions and evaluating subsequent lesion growth and pathogen recovery after isolation from those lesions.

Experimental Plants and Environmental Conditions

Fungicides were evaluated on four genera of plants: In the first year, rhododendron ('Cunningham's White') and azalea (*Rhododendron* 'Irish Lace'); in the second year, camellia (*Camellia japonica* 'Elena Nobile'), Japanese pieris (*Pieris japonica* 'Whitewater'), and laurustinus (*Viburnum tinus* 'Compacta'). *Rhododendron* and *Camellia* were established in 1-gal containers and *Pieris* and *Viburnum* were grown in 3.3-inch diameter containers. 'Cunningham's White' represented a highly susceptible *Rhododendron* and 'Irish Lace' represented a moderately susceptible azalea (19). Both *Rhododendron* cultivars were used in the first experiment to screen fungicides and 'Cunningham's White' was used in all subsequent *Rhododendron* experiments. All plants were initially maintained outside and moved into a research greenhouse (Salinas, CA) one to several months before inoculation. The greenhouse was kept at a maximum day temperature of 73.5°F. and minimum night temperature of 51.0°F. The soil medium of the 'Elena Nobile' contained only peat moss, while the other species were grown in approximately equal portions of Douglas fir bark, peat moss, and perlite.

Inoculum, Wounding, and Inoculation

The *Phytophthora ramorum* isolate (Pr-52) used in all experiments originated from an infected *Rhododendron* from a commercial California nursery in Santa Cruz County and has been previously characterized (13,18,20,21). Inoculum consisted of mycelial agar plugs. Wounding of leaf tissue was accomplished by gently poking the abaxial side of the selected leaf with a circular, 6-mm diameter, fine wire brush. In the first set of experiments there was no statistical difference when comparing the treatments' effectiveness on wounded and unwounded inoculations. Since wounding increased the success of infection and increased the experiment's statistical precision, it was used after the first set of experiments. The inoculum cap was placed over the wounded or selected area on the distal (1/3) end of the leaf. The lid was held in place with a modified hair curl-clip and kept there until the resulting lesion was evaluated.

Screening Potentially Effective Fungicides

Treatments in the fungicide screen trials included fungicides that had been either commercially registered or experimentally tested to have activity on *Phytophthora* species (5,6,12,16,17). Treatments included fungicides of different

chemical classes with diverse modes of action and different movement characteristics within the plant. Upwardly mobile fungicides (mefenoxom and fosetyl-Al), in addition to being applied as foliar applications, were also applied as soil drenches 7 to 14 days before inoculation to allow for absorption and translocation to the foliage. All other fungicide treatments consisted of foliar sprays and were applied one day (both *Rhododendron* cultivars) or 7 days (*Camellia*, *Viburnum*, and *Pieris*) before inoculation. Treatment rates were selected based on the highest labeled rate, or in the case of an experimental compound, the treatment was applied at the highest recommended rate given by the chemical company's experimental protocol. To facilitate foliar wetting on these hard to wet species, all foliar spray treatments as well as the water check included a surfactant Nof foam B (CMR Inc., Fresno CA) at 0.1% (*Rhododendron*) or Silwet L-77 (Loveland Industries LTD, Cambridge, UK) at 0.05% (*Camellia*, *Viburnum*, and *Pieris*) (Table 1).

Table 1. Experimental treatments: active ingredient, application type, trade name, percent active ingredient, rate applied, and treated plants.

| Treatment (active ingredient) | Application | Trade fungicide (a.i. concentration) | Treatment rate (100 gal) | Treated plants |
|---|-------------|--------------------------------------|--------------------------|----------------|
| azoxystrobin | Foliar | Heritage 50% | 2.0 oz | C,V,P |
| copper sulphate pentahydrate | Foliar | Phyton 27 21.36% | 65 fl oz | R |
| cuprous oxide | Foliar | Nordox 83.9% | 21.3 oz | R |
| cyazofamid | Foliar | Segway 34.5% | 6.0 fl oz | C,V,P |
| dimethomorph | Foliar | Stature DM 50% | 12.8 oz | R,C,V,P |
| fenamidone | Foliar | Fenstar 44.4% | 14 fl oz | R,C,V,P |
| fosetyl-Al | Soil | Aliette WDG 80% | 12.8 oz | R |
| fosetyl-Al | Foliar | Aliette WDG 80% | 5.0 lb | R,C,V,P |
| mancozeb | Foliar | Dithane 75% | 24 oz | C,V,P |
| mefenoxam | Soil | Subdue Maxx 22% | 2.0 fl oz | R |
| mefenoxam | Foliar | Subdue Maxx 22% | 2.0 fl oz | R,C,V, P |
| mono-di potassium salts of phosphorous acid | Foliar | Alude 45.8% | 64 fl oz | C,V,P |
| pyraclostrobin | Foliar | Insignia 20% | 16 oz | R,C,V,P |
| zoxamide | Foliar | Zoxium 80% | 4.0 oz | C,V,P |
| zoxamide + mancozeb | Foliar | Gavel 8.3% + 6.7% | 24 oz | C,V,P |
| water check | Foliar | — | — | R,C,V,P |

Rate given is for the formulated product. Foliar treatments applied until spray just began to drip from foliage. All foliar spray treatments as well as the water check included a surfactant Nof foam B @ 0.1% (*Rhododendron*) or Silwet L-77 @ 0.05% (*Camellia*, *Viburnum*, and *Pieris*). Soil treatment volume was 5 fl oz (just allowing some leachate from the bottom of 1-gal container). Treated Plants: 2 *Rhododendron* cultivars = R, *Camellia* = C, *Viburnum* = V, *Pieris* = P.

Determining Residual Fungicidal Action

Treatments in the residual action trials included fungicides chosen from the most efficacious fungicides in the screening experiments. Fungicide treatments were applied 28, 14, 7, and one day before the inoculation date for each experiment. Evaluation dates were dependent on the relative lesion growth rate and propensity for infected leaves to drop. This percentage was measured 6, 10, 14, and 16 days after inoculation, respectively, for *Pieris*, *Camellia*, *Rhododendron* and *Viburnum*.

Determining Post-Infection, Eradicative Action

Treatments in the eradication trials included those treatments in the residual action experiments. Fungicides were applied two weeks (*Rhododendron*) or four days (*Camellia*, *Viburnum*, and *Pieris*) after inoculation. These application times were chosen to allow sufficient time for the leaf lesions to develop before leaf abscission occurred.

Evaluation of Fungicide Effectiveness

Fungicide effectiveness was evaluated by removing treated leaves and measuring the development of leaf lesions caused by *P. ramorum*. In the *Rhododendron* screening experiment, the size of the lesion was quantified 14 days after inoculation by measuring the lesion's widest diameter. In subsequent experiments, digital images of the lesions were analyzed to determine the lesion area per total leaf area (a percentage) with ASSESS image analysis software (American Phytopathological Society, St. Paul, MN).

Isolation of *Phytophthora ramorum* from Lesions on Leaves Treated for Prevention and Eradication

In residual and eradication action experiments, lesions were evaluated on how well the pathogen was recovered from infected tissue via plating on selective media. Three infected leaves were generally sampled from each treatment replication. Infected leaves were washed three times in distilled water and a portion of the margin of the lesion was removed and placed on plates of selective PARP media (8) and incubated in the dark at 68°F. Approximately 7 to 14 days after plating, the presence or absence of *P. ramorum* was determined by examining plates for characteristic mycelial growth and chlamydospores. For *Rhododendron* in the eradication experiments, full complements of leaves were not available for all plants after the first week of evaluation because some inoculated leaves abscised from the plants before they could be selected. Isolations were also made from lesions on these fallen leaves.

Experimental Design and Statistics

Experiments were laid out on greenhouse benches in a randomized complete block design with four to six replications with one plant in each replicate. There were 3 to 5 inoculated leaves per plant, depending on the experiment, and these leaves were sampled and treated as statistical subsamples. For experiments with multiple species, species were main plots and fungicides were subplots in a split plot design. ANOVA was performed with treatments, species, and time as factors; interactions were performed if appropriate. When treatments were significantly different, the means of treatments were separated by Fisher's LSD at 95% confidence ($P \leq 0.05$) (Statgraphics Pro 5.0, Statistical Graphics Corp., Herndon, VA). If the experimental design did not support ANOVA, then pairwise comparisons between treatments were made and significance was determined with a t test, significance level at $P \leq 0.05$ with Bonferroni adjustment with SAS (SAS Institute Inc., Cary, NC).

Rhododendron

Screening potentially effective fungicides. There was a significant statistical interaction between lesion development and fungicide treatment (Table 2). In both 'Cunningham's White' and 'Irish Lace,' the treatments that significantly inhibited lesion development and therefore the best preventative control were mefenoxam (foliar), dimethomorph, fenamidone, and pyraclostrobin. Cuprous oxide was ineffective on both cultivars and was omitted from subsequent experiments. Fosetyl-Al applied as a soil drench was not effective, but the foliar treatment was marginally better than the untreated check. Fosetyl-Al as a foliar treatment was still included in subsequent experiments to see if additional time for metabolism and movement might result in better efficacy. Plants treated with copper sulphate pentahydrate developed significant phytotoxicity on both cultivars after 7 days from its application, and since the chemical was not one of the more effective treatments, it was removed from subsequent experiments.

Table 2. Effect of fungicide treatments on lesion diameter when applied before inoculation of leaves of two *Rhododendron* cultivars with *P. ramorum*

| Fungicide | Application | Mean lesion diameter (mm) | |
|------------------------------|-------------|---------------------------|----------------------|
| | | 'Irish Lace' | 'Cunningham's White' |
| copper sulphate pentahydrate | Foliar | 0.81 bc | 5.63 bc |
| cuprous oxide | Foliar | 2.85 ef | 9.36 c |
| dimethomorph | Foliar | 0.14 a | 0.19 a |
| fenamidone | Foliar | 0.31 ab | 0.20 a |
| fosetyl-Al | Foliar | 1.80 de | 3.05 b |
| fosetyl-Al | Soil | 3.08 f | 9.15 c |
| mefenoxam | Foliar | 0.30 ab | 0.25 a |
| mefenoxam | Soil | 0.95 cd | 3.93 b |
| pyraclostrobin | Foliar | 0.10 a | 0.67 a |
| water check | Foliar | 2.91 ef | 2.99 b |

Foliar treatments applied one day before inoculation and wounding (*Rhododendron* 'Irish Lace') or without wounding (*Rhododendron* 'Cunningham's White'). Soil treatments applied 7 days before inoculation. Evaluation 14 days after inoculation. There was a single plant replicate, with 3 leaves subsampled per replicate in each of 5 treatment blocks. Mean separation by LSD ($P \leq 0.05$). Means followed by unlike letters are significantly different.

Determining residual fungicidal action and recovery of pathogen.

All tested fungicide treatments were effective in reducing lesion development if applied 1, 7, and 14 days before inoculation, but not if applied 28 days before inoculation (Table 3). There were no differences in effectiveness of the tested fungicides, which reconfirmed the effectiveness of these products as determined in the screening experiments. The pathogen was re-isolated from a high percentage (median of 95%) of leaves from most fungicide treatments. However, recovery was consistently lower in those leaves that were treated with dimethomorph over all treatment-time regimes. Treatment with foliar mefenoxam provided the lowest recovery (5%) at the 14 day timepoint (Table 3)

Table 3. Effect of fungicide treatments and their timing on lesion area per leaf area (%) and pathogen recovery (%) when treatments are applied before inoculation of *Rhododendron* 'Cunningham's White' with *P. ramorum*.

| Fungicide | Mean lesion area per leaf area (%) | | | | Mean recovery from leaf lesions (%) | | | |
|-------------------|------------------------------------|-------------|------------|-----------|-------------------------------------|-------------|------------|-----------|
| | 28 days TBI | 14 days TBI | 7 days TBI | 1 day TBI | 28 days TBI | 14 days TBI | 7 days TBI | 1 day TBI |
| dimetho-morph | 12 ns | 7 a | 6 a | 8 a | 60 a | 40 b | 15 a | 40 a |
| fenamidone | 27 ns | 12 a | 8 a | 10 a | 100 b | 95 c | 85 bc | 95 b |
| fosetyl-Al foliar | 22 ns | 7 a | 10 a | 6 a | 100 b | 100 c | 100 c | 100 b |
| mefenoxam foliar | 18 ns | 5 a | 6 a | 4 a | 100 b | 5 a | 75 b | 90 b |
| pyraclo-strobin | 17 ns | 9 a | 9 a | 10 a | 95 b | 100 c | 90 bc | 95 b |
| water check | 22 ns | 22 b | 22 b | 22 b | 100 b | 100 c | 100 c | 100 b |

TBI = Treated before inoculation (days). All are foliar treatments made before inoculation. All inoculations are at the same date. Lesion area per total leaf area = mean lesion area covered relative to the leaf area measured 14 days after inoculation. Recovery = mean recovery success rate (%) of *P. ramorum* from isolation made 14 days after inoculation. There was a single plant replicate and 5 subsampled leaves per replicate in each of 4 treatment blocks. One global mean used for water check. Mean separation with LSD, $P \leq 0.05$. Means in columns followed by unlike letters are significantly different. ns = ANOVA is not significant, $P \leq 0.05$.

Determining eradication action and recovery of pathogen. Some leaves began to fall 7 days after infection, with the majority falling between 7 and 21 days after treatment (21 and 35 days after inoculation). Post-infection fungicide treatments did not inhibit lesion development on intact or abscised diseased leaves. When sampled up to 6 weeks after fungicide treatment, intact leaves had lesions that covered 13.0 to 32.0% of the leaf with a mean coverage of 23.0%. Fallen leaves had lesions that covered 44.0 to 88.0% of the leaf with a mean of 61.0%. For intact and fallen leaves, there were no statistically significant differences in lesion development between treated and water-check treated lesions. Recovery of the pathogen from diseased leaves was generally successful, declined with time, and was less successful on fallen leaves (Table 4).

Table 4. Effect of fungicide treatments and subsequent sampling time on pathogen recovery from lesions on intact and fallen leaves of *Rhododendron* 'Cunningham's White' when treatments are applied after inoculation with *P. ramorum*.

| Fungicide | Application method | 7 DAT | | 28 DAT | | 42 DAT | |
|-------------------------------|--------------------|-------|----------------|--------|----------------|--------|----------------|
| | | N | % recovery | N | % recovery | N | % recovery |
| Intact leaves | | | | | | | |
| dimethomorph | foliar | 18 | 100 | 18 | 100 | 12 | 83 |
| fenamidone | foliar | 18 | 100 | 18 | 72 | 14 | 64 |
| fosetyl-Al | soil | 18 | 100 | 18 | 94 | 10 | 90 |
| fosetyl-Al | foliar | 18 | 100 | 18 | 89 | 16 | 81 |
| mefenoxam | soil | 18 | 100 | 18 | 89 | 15 | 80 |
| mefenoxam | foliar | 18 | 94 | 18 | 94 | 17 | 35 |
| pyraclostrobin | foliar | 18 | 100 | 15 | 100 | 13 | 92 |
| water check | foliar | 18 | 100 | 18 | 89 | 9 | 56 |
| Overall mean (standard error) | — | — | 99.3 (SEM=2.8) | — | 90.9 (SEM=3.1) | — | 71.0 (SEM=7.7) |
| Fallen leaves | | | | | | | |
| dimethomorph | foliar | 3 | 100 | 13 | 23 | 0 | |
| fenamidone | foliar | 6 | 100 | 4 | 0 | 4 | 25 |
| fosetyl-Al | soil | 4 | 100 | 8 | 75 | 1 | 100 |
| fosetyl-Al | foliar | 6 | 100 | 7 | 57 | 0 | |
| mefenoxam | soil | 2 | 100 | 3 | 100 | 1 | 0 |
| mefenoxam | foliar | 3 | 100 | 4 | 0 | 1 | 100 |
| pyraclostrobin | foliar | 3 | 67 | 5 | 80 | 6 | 17 |
| water check | foliar | 6 | 83 | 12 | 83 | 2 | 0 |
| Overall mean (standard error) | — | — | 93.8 (4.4) | — | 52.3 (13.9) | — | 40.3 (19.3) |

N = number of leaves sampled from all inoculated leaves (18 intact leaves sampled if available). If inoculated leaves fell then they were also sampled. There was a single plant replicate with 6 inoculated leaves in each of 6 treatment blocks. Fungicide treatments applied 14 days after inoculation. DAT= Days after fungicide treatment at which designated leaves were sampled from plants. Recovery = isolation success. No-Foam B surfactant used in all treatments, including water check.

Camellia, Viburnum, and Japanese Pieris

Screening potentially effective fungicides. There was a significant statistical interaction between species and fungicide treatment. The treatments had similar relative effects, but lesions of each species had different growth rates, with lesions on *Pieris* having the largest percentage coverage regardless of fungicide treatment (Fig. 1). When data were pooled for the 3 species, the treatment effects could be fully realized. Many fungicides had a statistically significant affect on lesion development. Dimethomorph, pyraclostrobin, fenamidone, and mefenoxam provided better preventative control than the other fungicides, confirming the results with the fungicide screen on *Rhododendron*. In addition, zoxamide and cyazofamid provided preventative control on the tested species. There was less control with zoxamide + mancozeb and no significant control with mancozeb, azoxystrobin, mono- and dipotassium phosphorous acid, and fosetyl-Al. (Table 5).

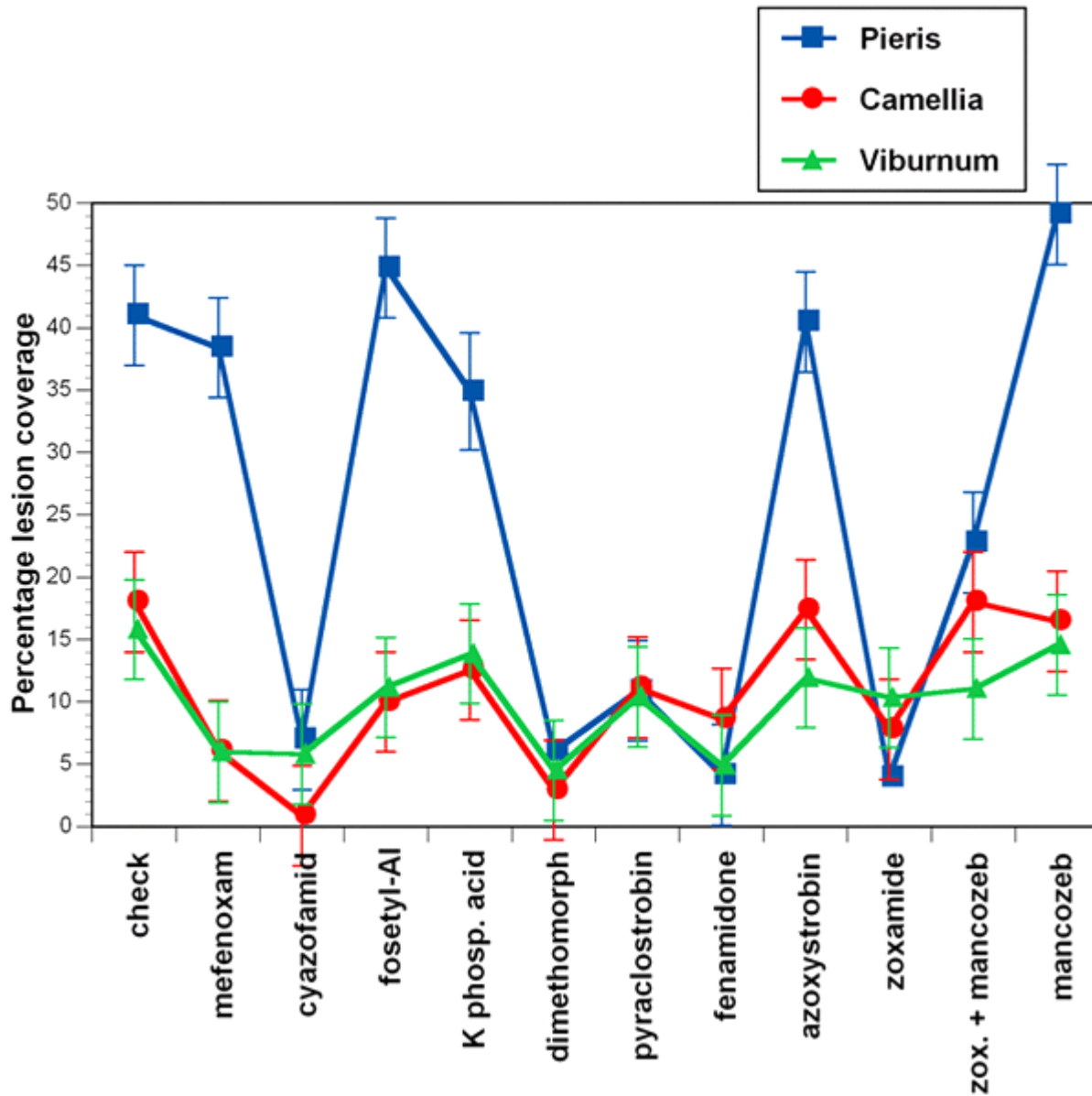


Fig. 1. Interaction of lesion development, species, and fungicide treatments. Mean lesion area per leaf area (%) and associated SEM.

Table 5. Effect of fungicide treatments on lesion area per leaf area (%) when applied 7 days before inoculation of leaves of *Camellia*, *Pieris*, and *Viburnum* with *P. ramorum*

| Fungicide | Lesion area per total leaf area (%) |
|---|-------------------------------------|
| azoxystrobin | 23.3 cd |
| cyazofamid | 4.6 a |
| dimethomorph | 4.5 a |
| fenamidone | 5.9 a |
| fosetyl-Al foliar | 22.0 cd |
| mancozeb | 26.7 d |
| mefenoxam foliar | 16.8 bc |
| mono-di potassium salts of phosphorous acid | 20.4 cd |
| pyraclostrobin | 10.8 ab |
| zoxamide | 7.3 a |
| zoxamide + mancozeb | 17.3 bc |
| water check | 25.0 d |

Data pooled for all species to help summarize and analyze results. Mean lesion area covered relative to the total leaf area measured 6, 10, and 16 days after inoculation respectively for *Pieris*, *Camellia*, and *Viburnum*. There was a single plant replicate, with 3 leaves subsampled per replicate in each of 5 treatment blocks. Mean separation with LSD., $P \leq 0.05$. Means in columns followed by unlike letters are significantly different.

Determining residual fungicidal action and recovery of pathogen.

There was a statistically significant interaction between species and fungicide. For *Camellia*, several fungicides controlled lesion development for at least 14 days. In general, efficacy was not as strong at 28 days as indicated by weaker statistical significance ($P < 0.06$), although cyazofamid and dimethomorph were clearly efficacious at 4 weeks (Table 6). For *Pieris* and *Viburnum*, generally dimethomorph, fenamidone, and cyazofamid controlled lesion development for up to 28 days. To a lesser extent, mefenoxam and pyraclostrobin controlled lesion development (Table 6). The relationship of species and fungicide activity may be due to the size of the plant as well as other inherent characteristics of plant metabolism and morphology. There were generally lower pathogen recovery rates with dimethomorph and cyazofamid, but these values were not statistically different (Table 7).

Tables 6. Effect of fungicide treatments and treatment time on lesion area per leaf when applied before inoculation of leaves of *Camellia*, *Pieris*, and *Viburnum* with *P. ramorum*

| Treatment | Mean lesion area per total leaf area (%) | | | | | | | | |
|----------------|--|-------------|------------|---------------|-------------|------------|-----------------|-------------|------------|
| | <i>Camellia</i> | | | <i>Pieris</i> | | | <i>Viburnum</i> | | |
| | 28 days TBI | 14 days TBI | 7 days TBI | 28 days TBI | 14 days TBI | 7 days TBI | 28 days TBI | 14 days TBI | 7 days TBI |
| cyazofamid | 4.7 a | 7.0 a | 15.8 bc | 27.9 ab | 13.0 a | 32.1 ab | 2.7 ab | 1.9 a | 1.7 a |
| dimethomorph | 5.8 ab | 15.5 bc | 20.6 bc | 27.0 a | 36.4 ab | 19.5 a | 2.8 ab | 1.5 a | 3.1 ab |
| fenamidone | 18.9 bc | 9.7 ab | 18.4 bc | 31.0 bc | 27.5 ab | 21.6 a | 2.4 a | 1.0 a | 1.8 a |
| mefenoxam | 20.5 bc | 7.2 a | 6.2 a | 40.8 bc | 47.1 bc | 41.9 abc | 8.1 bc | 6.2 b | 5.6 c |
| pyraclostrobin | 19.9 bc | 15.0 bc | 12.8 ab | 40.0 bc | 22.4 ab | 63.5 bc | 9.9 c | 5.7 b | 4.1 bc |
| zoxamide | 23.7 c | 19.4 cd | 25.6 cd | 52.2 c | 68.9 cd | 46.2 abc | 9.2 c | 6.6 b | 8.6 d |
| water check | 24.8 c | 25.3 d | 33.5 d | 71.0 c | 72.7 d | 69.6 c | 10.1 c | 10.6 b | 10.1 d |

TBI = Treated before inoculation (days). All inoculations on same date. Lesion per leaf area = Percent Mean lesion area covered relative to the leaf area measured 10 days after inoculation. There was a single plant replicate and 5 subsampled leaves per replicate in each of 4 treatment blocks. Mean separation in columns with LSD, $P \leq 0.05$, except for 28 days / *Camellia*. $P \leq 0.05$. ANOVA on square root transformed data. Means in columns followed by unlike letters are significantly different.

Table 7 Effect of pre-inoculation fungicide treatments on recovery success of *Phytophthora ramorum* from infected lesions for given species

| Fungicide vs water check | <i>Pieris</i> | <i>Camellia</i> | <i>Viburnum</i> |
|--------------------------|---------------|-----------------|-----------------|
| | P value* | | |
| mefenoxam | 0.7267 | 1.000 | 1.000 |
| cyazofamid | 0.0988 | 0.4035 | 0.0038 |
| dimethomorph | 0.1779 | 0.0233 | 0.0339 |
| pyraclostrobin | 0.7267 | 0.4036 | 1.000 |
| fenamidone | 1.000 | 1.000 | 0.0693 |
| zoxamide | 0.4879 | 1.000 | 1.000 |

Pair-wise comparison of recovery success rate of fungicide treatments as compared to the water check. Significance would indicate a reduction of isolation success.*P value for t test. Significance at 0.05 with Bonferroni adjustment is at ≤ 0.00012 . All data transformed before analysis with arsine square root. There was a single plant replicate and 3 subsampled leaves per replicate in each of 4 treatment blocks. Treatments applied 7, 14, and 28 days before inoculation. Data pooled for all timepoints to help summarize and analyze results.

Zoxamide efficacy was dependent on the species on which it was applied. When data were pooled for the fungicide screen for *Camellia*, *Pieris*, and *Viburnum*, zoxamide was ranked in the top group of effective fungicides. However, if the data for the fungicide screen were analyzed for each individual species, the fungicide worked significantly better for *Pieris* and not the other two species. This is also the case for the residual action test: zoxamide efficacy was better with *Pieris* but was not efficacious with the other two species. Mancozeb provided no significant protective efficacy alone. This compound works in a similar manner to that of copper by releasing toxic metal ions to control fungi. Only when mancozeb was combined with zoxamide in the formulated product (Gavel) was there significant activity. Considering the relatively greater activity of zoxamide, the efficacy of this combination of active ingredients was probably due primarily to zoxamide. Soil applied mefenoxam gave significantly greater control in *Rhododendron* 'Irish Lace' over that of untreated plants. Soil applied

fosetyl-Al had no effect on either cultivar. Metalaxyl and fosetyl-Al applied as soil drenches provided very good control of the soil-borne *Phytophthora cinnamomi* on azalea (3) and with shoot infections of *Phytophthora heveae* (2). However, to obtain control of *Phytophthora heveae* with metalaxyl, required the application of the equivalent of 20 times the active ingredient as that applied for mefenoxam in this study considering the differences in the activity of the isomers and soil volume. Fosetyl-Al (tested on all species) and mono- and dipotassium salts of phosphorus acid (tested on all except *Rhododendron*) showed consistently weak activity. Salts of phosphorous acid would not be expected to have quick activity since it has relatively weak *in vitro* activity on *P. ramorum* (11) and other metabolic mechanisms might be involved in its fungicidal activity (10). In our experiments, we allowed for this possible delay in activity but still did not measure highly efficacious control. Azoxystrobin was not effective at the tested rate, while pyraclostrobin was highly effective in these experiments, although both chemicals are strobilurins. Pyraclostrobin may be a more active compound; however, it was applied at nearly 5 times the concentration of azoxystrobin.

Determining Eradicative Action

Post-infection fungicide treatments did not impede lesion development on infected leaves when lesion size was evaluated 14 days after fungicide treatment. There were no significant differences in lesion size. Lesion diameter varied with species, with mean diameters for *Pieris*, *Camellia*, and *Viburnum* measuring 33.6, 19.8, and 11.1 mm, respectively. In addition, *P. ramorum* was commonly isolated from lesions, with no significant differences between fungicide treatments and the water-check. Pathogen recovery varied from 86 to 100%. Even though isolations were highly successful, recovered isolates were slower growing when they came from plants treated with cyazofamid and dimethomorph (2.4 and 8.3 mm mean, respectively) versus the untreated check (18.8 mm) (SEM, 1.33) (Fig. 2).

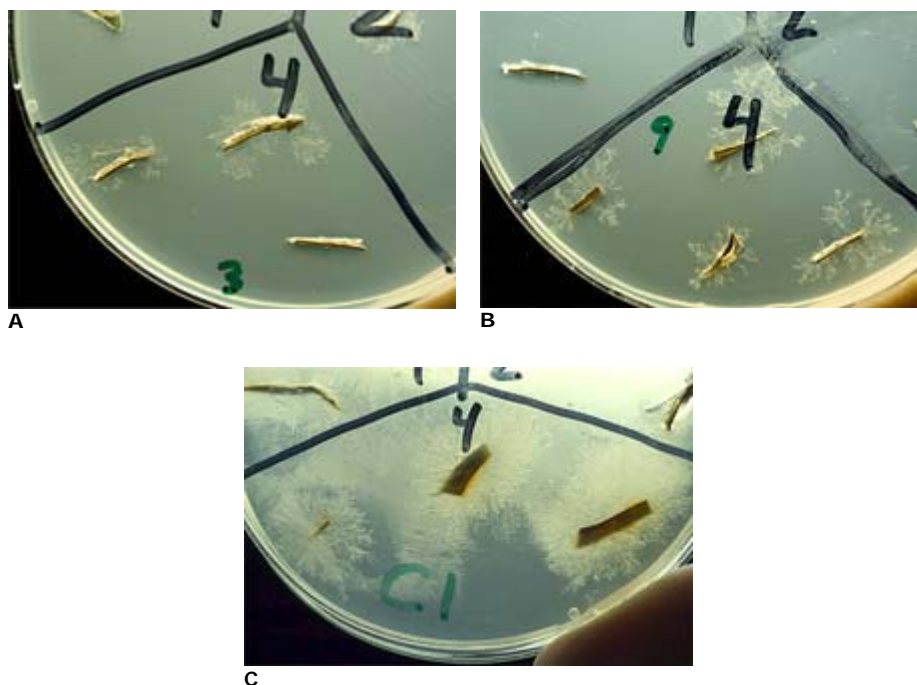


Fig. 2. Although recovery of the pathogen from isolations were mostly successful with all treatments, isolations with cyazofamid and dimethomorph developed slower growing cultures. Isolations from camellia after 10 days on PARP selective media: (A) Treated with cyazofamid, post infection (B) Treated with dimethomorph, post infection (C) Treated with water check, post infection.

Summary

The fungicides mefenoxam, dimethomorph, pyraclostrobin, and fenamidone applied to foliage were the most effective in reducing lesion development on *Rhododendron*. In addition to these fungicides, cyazofamid was effective for reducing lesion development on *Camellia*, *Pieris*, and *Viburnum*. Their performance as fungicides under commercial field and greenhouse conditions may be different.

Residual fungicide activity as measured by this experiment varied from a minimum of 14 to more than 28 days. In practice, nursery operators could reasonably expect that applications of these fungicides at 14 to 28 day intervals would provide disease control for a variety of species. Some chemical labels may dictate longer spray intervals, however, because of the risk of phytotoxicity.

Given that lesion development may be slowed with fungicide applications, it is reasonable to suspect that scouts or inspectors could find that it is more difficult to see lesions on fungicide treated leaves because of their limited size and slower growth. However, if lesions are found, sampled, and analyzed, it is likely that the pathogen will be detected using standard laboratory isolation procedures. Isolations from diseased leaves that were inoculated before and after fungicide treatments resulted in a high percentage of *P. ramorum* recovery. Only dimethomorph and cyazofamid significantly reduced recovery of *P. ramorum* from lesions in the tested species. Recovery success declines as lesions age, so inspectors should locate leaves that have recently formed lesions for isolation. Tested fungicides were not effective in killing the pathogen in leaf lesions. Infected plants will need to be destroyed in quarantine situations.

Disease Management Recommendations with Fungicides

As part of a comprehensive disease control program that includes exclusion, scouting, and sanitation, fungicides may play a role in the control of *P. ramorum* in nursery stock. Preventative applications of fungicides may already be used for control of other diseases and could also possibly control *P. ramorum*. On *Rhododendron* there are other diebacks and blights caused by several *Phytophthora* species including *P. cactorum*, *P. citricola*, *P. parasitica*, *P. syringae*, among others (4,15). On *Pieris* there is a foliar *Phytophthora* disease caused by *P. citricola* (14). Moreover, applications of fungicide soil drenches may already be applied to prevent infection of root pathogens such as *Pythium* and *Phytophthora*. Some systemic fungicides applied as soil drenches may move upward from roots to foliage and prevent foliar infection by *Phytophthora*.

Whether or not to use *Phytophthora*-specific fungicides for the sole control of *P. ramorum* is a complex question. The dedicated use of a fungicide for the exclusive purpose of preventing *P. ramorum* infection should be reserved for special cases, for example, where a nursery is exposed to local inoculum sources such as surrounding infected native hosts or when the nursery has no choice but to use irrigation water that could contain *P. ramorum* inoculum. When fungicide applications are made to control other *Phytophthora* species, it may be appropriate to select fungicides and application intervals that can also control *P. ramorum*. Regardless of the reasons to use fungicides, these chemicals should only be used after other management strategies and preventative steps have been fully implemented. Although fungicides might help prevent the spread of the disease if infested plants are introduced in the nursery, they might also hinder the detection of the disease. In addition, *Phytophthora*-specific fungicides used continuously may lead to fungicide resistance. When devising fungicide application programs, therefore, alternate or tank mix fungicides that have different modes-of-action. Finally, the masking of symptoms due to fungicide use might eventually lead to the sale and movement of infected plants to nurseries or customers in non-infested areas. Such an occurrence might circumvent the quarantine program that intends to limit the spread of Sudden Oak Death.

Fungicides should be applied to provide good coverage over the foliage, and a wetting agent might be needed to prevent significant run-off and loss of fungicides on the hard-to-wet leaves of certain plant species. Fungicide registration and labeling may preclude the use of some of these treatments. Not all these fungicides are presently registered for control of *Phytophthora* species

on ornamental plants. Before using any of these products, check product labels and local regulatory agencies for application instructions and restrictions.

Acknowledgment

This research was financially sponsored in part by the USDA Forest Service, Sudden Oak Death Research Program.

The authors wish to acknowledge the assistance of Kat Kammeijer and Patrick Headley (UC Cooperative Extension, Salinas, CA), Sylvia Mori, USDA Forest Service Statistician, Albany, CA, and Sibdas Gosh and his students (Dominican University of California, San Rafael, CA) for assistance with the intensive greenhouse inoculations. Also for the generous donations of plant material from Suncrest Nursery (Watsonville, CA), Tiedemann Nursery (Soquel, CA), Knudsen Nursery (Manteca, CA), and Floraland Nursery (OR).

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