Fungicide Control of *Phytophthora ramorum* on Rhododendron[1]

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Abstract

Commercial rhododendron plants have been the most important hosts of *Phytophthora ramorum* in Europe. As part of the European Union (EU) emergency phytosanitary measures 2002/757/EU and 2004/426/EU all commercial rhododendron-growing premises are surveyed for *P. ramorum*. Detection of *P. ramorum* leads to quarantine measures, including destruction of plants, and could lead to considerable financial damage for the companies involved. Growers are taking all possible measures to avoid *P. ramorum*, including preventive fungicide treatments. However, because little information is available on the effect of the different oomycete fungicides on *P. ramorum*, there is a danger of conducting sub-optimal protective fungicide treatments. The objective of this research was to evaluate the efficacy of different oomycete fungicides against *P. ramorum*. Active ingredients from most of the oomycete fungicides on the Belgian market were screened for their *in vitro* effect on the mycelial growth of four *P. ramorum* isolates. Based on the results from the *in vitro* experiments, a selection of these fungicides was tested against *P. ramorum* on rhododendron plants. Application time and application method were included as variables. A wide range of *in vitro* fungicide activity was observed. Metalaxyl, dimethomorph, and benthiavalicarb-isopropyl showed complete inhibition of mycelial growth at 1 µg ml^{-1} or less. Cymoxanil, etridiazole, and mancozeb caused complete growth inhibition at 1 to 100 µg ml^{-1}. Chlorothalonil, Cu-oxychloride, famoxadone, fluazinam, and cyazofamid did not completely inhibit growth at 100 µg ml^{-1}. Fosetyl-Al and propamocarb did not cause growth inhibition at 100 µg ml^{-1}. Fungicide effects were independent of the strain of *P. ramorum* used, except for one strain, which showed a decreased sensitivity to metalaxyl. A subset of the fungicides was tested on plants. Overall, fungicides that performed best on plants were metalaxyl, cyazofamid, and benthiavalicarb-isopropyl. Dimethomorph and fosetyl-Al had intermediate effects. Cymoxanil and mancozeb were the least effective of the products tested. Protective effects were best

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when the lower surface of the leaf was covered with the fungicide, consistent with the observation that infection of non-wounded leaves inoculated with zoospores takes mostly place through the lower surface of the leaves. Curative fungicide treatments 2 days after zoospore inoculation were much less effective than protective treatments (1 day before zoospore inoculation). This research shows that protective applications of specific fungicides can contribute to effective control strategies of *P. ramorum* on rhododendron. Considering that we identified a strain with decreased activity against metalaxyl, it seems advisable to limit the number of consecutive uses of products with the same mode of action. However, growers may face few options in alternating fungicides due to the limited number of products with use permits on rhododendron.

Introduction

In 1993, a new species of *Phytophthora* was found responsible for an aggressive twig and leaf blight of rhododendron and a stem blight of viburnum in German and Dutch nursery settings (Werres and Marwitz 1997, Werres and others 2001). Starting in 1995, extensive mortality of native oak species such as tanoak (*Lithocarpus densiflorus*) and coast live oak (*Quercus agrifolia*) occurred in California and in one part of Oregon, caused by a disease called sudden oak death (SOD) (Goheen and others 2002, Rizzo and others 2002). This same new *Phytophthora* species that was responsible for the diseases on the European ornamentals was found to be responsible for SOD. It has since been designated *P. ramorum* (Werres and others 2001). In the United States, an increasing number of over 30 natural hosts have been identified, mostly in natural settings (APHIS 2004). Depending on the host, symptoms vary from leaf spots, leaf blight, twig dieback, to cankers, and ultimately plant mortality. In Europe, the pathogen has been found in Belgium, Denmark, France, Germany, Ireland, Italy, Netherlands, Norway, Poland, Slovenia, Spain (Galicia, Islas Baleares: Mallorca), Switzerland, Sweden and the United Kingdom (EPPO 2005), but almost exclusively in nursery settings. A limited number of findings took place on trees in England and in the Netherlands, but each time it was found in the vicinity of infected rhododendron plants (DEFRA 2003a, DEFRA 2003b, PD Nederland 2003). There are genetic differences between the United States and the European populations of *P. ramorum* (Ivors and others 2004, Kroon and others 2004) but the pathogenicity is not substantially different. The detection of the pathogen in an increasing number of European countries and the potential danger for spread from nurseries to natural oak and beech populations has prompted the EU to install emergency phytosanitary measures (directives 2002/757/EU and 2004/426/EU). These measures include inspections on all nurseries where rhododendron, viburnum or camellia are grown. The most important host of *P. ramorum* in Europe is rhododendron. Although the

percentage of positive sites is comparable to that of its neighboring countries, the impact of the EU measures on the Flemish rhododendron industry is considerable because Flanders (Northern part of Belgium) is one of largest rhododendron-producing areas in Europe. Therefore, rhododendron growers are taking all possible measures to avoid *P. ramorum*, including use of preventive fungicide treatments. The objective of this research was to do an initial evaluation of the efficacy of the different oomycete fungicides on the Belgian market against *P. ramorum* on rhododendron.

Materials and Methods

In vitro experiments

Four *Phytophthora ramorum* isolates from four different Flemish nurseries were used in this study. Isolates 02-880 and 02-1658 were from rhododendron; isolates 02-2084 and 02-2086 were from viburnum. The isolates were maintained on V8 agar and CMA at 4ºC and transferred periodically. *In vitro* evaluation of fungicide activity was based on inhibition of mycelial growth only. Fungicides used were benthiavalicarb-isopropyl (non-commercial; 15 percent a.i.), chlorothalonil (Clortosip; 50 percent a.i.), Cu-oxychloride (Cupravit Forte; 50 percent a.i.), cyazofamid (non-commercial; 40 percent a.i.), cymoxanil (non-commercial; 96 percent a.i.), dimethomorph (non-commercial; 50 percent a.i.), etridiazole (Aaterra; 35 percent a.i.), famoxadone (non-commercial; 96 percenta.i.), fluazinam (Shirlan; 50 percenta.i.), fosethyl-Al (Aliette; 80 percenta.i.), mancozeb (Dithane WG; 75 percent a.i.), metalaxyl (Ridomil 5G, 5 percent a.i.), and propamocarb (Previcur N; 72.2 percent a.i.). Except for benthiavalicarb-isopropyl, the active ingredients listed were available in at least one commercial product on the Belgian market at the time this research was initiated. However, inclusion in this study does not imply they are registered for use against *P. ramorum* at this time. Stock solutions of the fungicides were made in DMSO or sterile water and added to V8 agar to a final concentration of either 0, 0.001, 0.01, 0.1, 1, 10, or 100 µg active ingredient ml^{-1}. Rifampicin was also added at a final concentration of 10 µg ml^{-1} to suppress potential development of bacteria originating from the fungicide formulations. For each *P. ramorum* isolate used, separate plates were inoculated with a mycelial plug from an actively growing culture. Plates were incubated at 20°C in the dark until the colony margin of the cultures in the control treatment almost reached the edge of the plate. Growth inhibition in the fungicide treatments was calculated based on the proportional decrease in radial growth to the control plates. A minimum of two replicate plates were used for every combination of fungicide concentration and fungal isolate. All experiments were conducted twice.

In planta experiments

A subset of the fungicides from the *in vitro* experiments was selected based on the extent of their *in vitro* effect. These selected fungicides were tested in three separate *in planta* experiments. Fungicides used were benthiavalicarb-isopropyl (non-commercial formulation, 15 percent a.i.), cyazofamid (Ranman A+B; 40 percent a.i.), cymoxanil (Cymopur; 35 percent a.i.), dimethomorph (Paraat; 50 percent a.i.), mancozeb (Dithane WG; 75 percent a.i.), and mefenoxam (non-commercial formulation, 48 percent a.i.). Although results with fosethyl-Al (Aliette; 80 percent) were poor in the *in vitro* experiments, it was included in the *in planta* experiments based on its expected activity in plants. Fungicide solutions were prepared at the following rates and applied to run-off: benthiavalicarb-isopropyl (75 ppm), cyazofamid (80 ppm), cymoxanil (119 ppm), dimethomorph (150 ppm), fosethyl-Al (2000 ppm), mancozeb (1575 ppm), and mefenoxam (192 ppm). Rates are expressed as active ingredient and were based on label (or company) recommendations. Cyazofamid was used in trials two and three only.

Potted rhododendron plants were obtained from a commercial nursery. Plants within each trial originated from the same batch. In trial one and two, cultivar "Percy Wiseman" was used. In trial three, cultivar "Germania" was used. To avoid residual effects from preventive fungicide applications at the rhododendron nursery, plants from trials two and three were maintained in our greenhouse for at least 6 weeks before use in the experiments. All experiments took place in a climatized greenhouse during the months October through April with a day temperature setting of 20°C and a night temperature setting of 15°C. Pots were placed in individual saucers and watered from below. Plants received natural light only.

The pathogen inoculum consisted of an equal mixture of zoospores of *P. ramorum* isolates 02-1658, 02-2084, and 02-2086. A $5x10^4$ ml^{-1} zoospore suspension was applied at 20 ml per plant. The zoospores were sprayed onto the lower side of all leaves with a modified syringe. The syringe was modified in such a way that a sufficiently fine droplet size was obtained to get good distribution but without causing the zoospores to encyst by the spraying process. Post inoculation, each plant was individually covered with a large plastic bag to ensure high humidity conditions. Bags were removed one week after inoculation. To produce zoospores, sporangia from each isolate were separately harvested from 10-day-old cultures on diluted (10 percent clarified juice) V8 agar with a total of 20 ml sterile MilliQ water per plate. Sporangia solutions were chilled in a refrigerator and zoospores allowed to emerge over a period of 2 to 3 hours. Zoospores were separated from sporangia by filtering through a nylon mesh (10 μm) and their concentrations were determined with a

Bürker chamber. Equal numbers of zoospores from the three strains were mixed and diluted to the desired concentration with chilled MilliQ water. Zoospore solutions were kept on ice until application. At the end of the experiment, the mobility of the remainder of the zoospores was verified microscopically. No decrease in zoospore mobility was observed.

In the first *in planta* experiment, fungicides were applied in a preventive manner only, but with different application methods: (1) to the upper side of the leaves (1 day before pathogen inoculation), (2) to the lower side of the leaves (1 day before pathogen inoculation), or (3) systemically, to the potting medium (7 days before pathogen inoculation). Systemic application was not made with mancozeb and cymoxanil, as those are contact fungicides only. Applications via the potting medium (and roots) consisted of adding 100 ml of fungicide solution to the saucers in which the pots were placed. The liquid was absorbed within 8 hours for all pots. An equal amount of water was applied in the same manner to the other treatments. In the second and third experiment, fungicides were applied to the upper side of the leaves only, but either preventive (1 day before pathogen inoculation) or curative (2 days post pathogen inoculation). There were five replicate plants per treatment in trial one, and six replicates in trials two and three. The experimental setup was a randomized complete block, with blocks consisting of location in the greenhouse and pathogen inoculation order. There was no contact between individual plants. Non-inoculated controls and non-fungicide treated controls were included.

Plants were scored for symptoms on a weekly basis until no new symptoms developed. The number of leaves with lesions and number of stems with lesions were counted. No distinction was made between the size of the lesions. Stem lesions were evaluated in experiments one and two. Leaf lesions were evaluated in experiments two and three. In the first experiment, a part of each lesion was plated on selective medium (PARP) to confirm the cause of the lesion. In later experiments only lesions that were not typical were plated. Analysis of variance or Kruskal-Wallis tests were performed on the lesion data where appropriate, using Minitab 12.1. To summarize the effects of the fungicides in the different experiments, the results of each fungicide were put in one of four different categories within each experiment. Based on cut-off levels of the number of lesions, the following categories were established: 1 = excellent control, 2 = good control, 3 = some control, 4 = inadequate control.

The plants from experiment two were maintained in the greenhouse for 6 months after the end of the experiment (no new lesions), under stressful conditions in terms of temperature (high) and water availability (low). At the end of this 6-month period, mortality was assessed and correlated with previous infection level.

Results

In vitro experiments

A wide range of *in vitro* fungicide activity was observed. The effects were separated in four groups. Fungicides in group one (metalaxyl, dimethomorph, and benthiavalicarb-isopropyl) showed 100 percent growth inhibition at 1 µg ml^{-1} or less (*Fig*. 1A). Fungicides in group two (cymoxanil, etridiazole, and mancozeb) showed 100 percent growth inhibition at 1 to 100 µg ml^{-1} (*Fig.* 1B). Fungicides in group three (chlorothalonil, Cu-oxychloride, cyazofamid, famoxadone, and fluazinam) did not reach 100 percent growth inhibition at 100 µg ml^{-1} (*Fig*. 1C). Lastly, fungicides in group four (fosetyl-Al and propamocarb) did not show any *in vitro* activity (*Fig*. 1D). The fungicide effects were independent of the strain of *P. ramorum* used, except for strain 02-880, which showed a decreased sensitivity to metalaxyl. The smallest concentration at which inhibitory activity was observed was 0.001 µg ml^{-1} with cyazofamid (23 percent growth inhibition). Interestingly, growth inhibition with this compound leveled off at approximately 80 percent starting at 1 µg ml^{-1}.

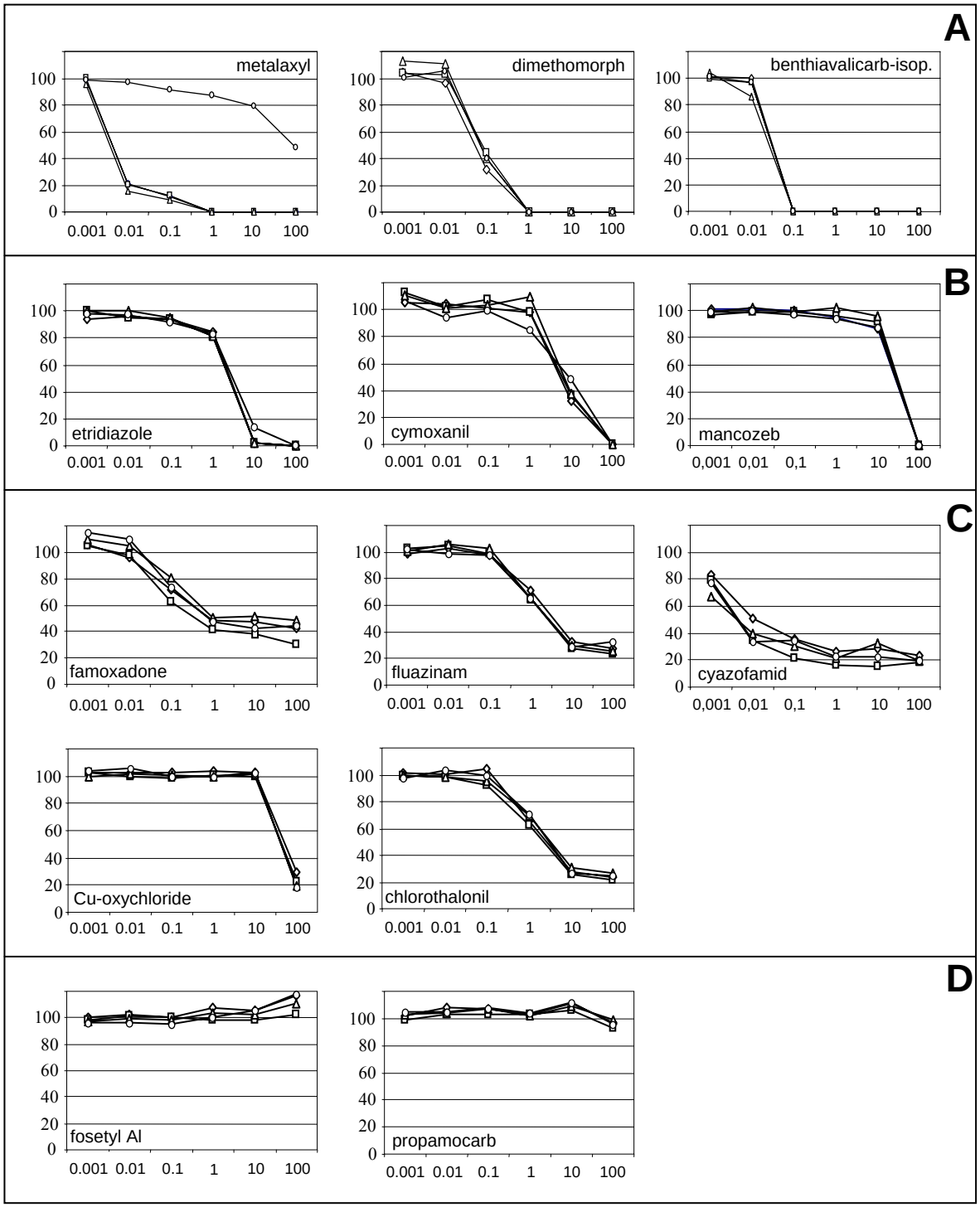

Figure 1— Mycelial growth of *P. ramorum* in agar medium amended with fungicides, expressed as a percentage (Y-axes) of the growth in the control plates (=100 percent). Each graph is labeled with the corresponding fungicide and fungicide concentrations are given in ppm (X-axes). Each graph contains the results of four *P. ramorum* isolates: 02-880 (circles), 02-1658 (squares), 02-2084 (diamonds), 02-2886 (triangles). The four subparts of the graph (A through D) correspond with the four efficacy classes (groups 1 through 4), as indicated in the results section of the text.

In planta experiments

No new primary leaf lesions developed after 14 days. Secondary leaf lesions, which only occurred if the pathogen spread via the stem, were not counted. Stem lesions took longer to develop, mostly between 4 and 10 weeks post inoculation.

Results from the first *in planta* experiment are presented in *table 1*. The overall level of infection was lower than expected based on preliminary experiments. Therefore, only the total number of lesions in the five replicates are presented for this first experiment. Residual effects from fungicides applied at the nursery where the plants originated were probably responsible for the limited number of lesions. To prevent this effect, plants from nurseries were maintained in the greenhouse for at least 6 weeks in all later experiments. Even though the level of infection was low in trial one, some important trends were clearly visible. The application method played a major role in the level of infection: application of the fungicides to the lower side of the leaves, where the infection with zoospores takes place, gave the best protection. Even the contact fungicides (mancozeb and cymoxanil) were very effective with this application method. Applying the fungicides via the potting medium was least effective. Overall, mefenoxam, benthiavalicarb-isopropyl, and dimethomorph (except when applied systemically) gave the best results.

Table 1—*Total number of stems with infections due to* Phytophthora ramorum *in the first* in planta *experiment. Fungicides were applied either systemically, onto the upper side of the leaves, or onto the lower side of the leaves.*

Fungicide treatment	Fungicide application mode		
	via roots	upper leaf side	lower leaf side
no pathogen control[2]	0	0	0
no fungicide control[2]	9	9	9
mefenoxam	5	1	0
fosetyl-Al	5	3	1
benthiavalicarb-isopropyl	5	0	0
dimethomorph	12	0	0
mancozeb	NA[1]	5	2
cymoxanil	NA	8	0
mean±stdev[3]	6.8±3.5	2.8±3.2	0.5±0.8

[1] NA = Not Applicable (contact fungicides)

[2] Considering controls did not involve a fungicide treatment, they were the same for each application mode

[3] Mean ± standard deviation of application method, excluding the control treatments

Results from the second *in planta* experiment are presented in *table 2*. The overall level of infection was much higher in this experiment than in the first one, hence the evaluation of leaf infections as well as stem infections. As expected, the average level of infection was significantly higher in the curative treatment than in the preventive

treatment ($p<0.0001$). This was clear in the average number of infected leaves per plant (4.95 versus 0.67 infected leaves on 5.86 versus 2.00 plants out of 6) and the average number of stem lesions per plant (1.26 versus 0.12 infected stems on 3.43 versus 0.29 plants out of 6). There was no significant block effect when evaluating leaf ($p=0.35$) or stem ($p=0.52$) lesions. Effects on stems and leaves were similar for most fungicides, except for dimethomorph and cymoxanil, which were considerably better on stems than on leaves in preventive treatments, and cyazofamid, which was considerably better on stems than on leaves in the curative treatment.

Table 2—*Mean+/-stdev number of leaves and stems with lesions per plant in the second* in planta *experiment. Fungicides were either applied 1 day before (preventive) or 2 days after the pathogen (curative).*

Fungicide	preventive				curative			
	leaves		stems		leaves		stems	
	lesions	pl.[1]	lesions	pl.[1]	lesions	pl.[1]	lesions	pl.[1]
no pathogen control	0.00±0.00	0	0.00±0.00	0	0.00±0.00	0	0.00±0.00	0
no fungicide control	8.50±3.94	6	2.83±1.60	6	8.00±3.03	6	1.50±1.52	4
fosetyl-Al	0.67±0.61	3	0.00±0.00	0	5.67±4.03	6	1.83±2.56	3
mefenoxam	0.50±0.55	3	0.17±0.41	1	3.17±2.56	6	1.00±1.55	3
benthiavalicarb-isopropyl	0.00±0.00	0	0.00±0.00	0	4.83±3.97	5	0.83±2.56	4
cyazofamid	0.00±0.00	0	0.00±0.00	0	4.50±2.74	6	0.00±0.00	0
dimethomorph	1.67±3.61	2	0.00±0.00	0	5.33±2.34	6	1.17±0.75	5
cymoxanil	1.33±1.51	3	0.00±0.00	0	4.67±2.25	6	1.33±1.97	3
mancozeb	0.50±0.55	3	0.67±1.63	1	6.50±2.51	6	2.67±1.03	6
mean±stdev[2]	0.67±0.63	2.00	0.12±0.25	0.29	4.95±1.04	5.86	1.26±0.83	3.43

[1] number of replicate plants (out of 6) with at least one lesion
[2] mean (± standard deviation) within each column, excluding the control treatments

The effects of the fungicides could be divided in different groups. When averaged over effects on leaves and stems, excellent control was obtained with cyazofamid and benthiavalicarb-isopropyl for the preventive treatments. For the curative treatments, cyazofamid, mefenoxam, and benthiavalicarb-isopropyl resulted in the smallest number of lesions. These three fungicides were the only ones that were significantly different from the positive control according to the Kruskal-Wallis test and comparison of the average ranks with the Dunns test (leaf data, averaged over preventive and curative treatments). Using the same test and when averaged over leaf and stem data, these same three fungicides were significantly different from the positive control in the preventive treatments. In the curative treatments, no fungicide

was significantly different from the control (averaged over stem and leaf lesions). Fungicides with intermediate effects were fosetyl-Al and dimethomorph. Fungicides with the smallest effects were cymoxanil and mancozeb, although mancozeb still showed reasonable effect in the preventive treatment.At the end of the second *in planta* experiment, which contained 108 plants total, 12 plants were heavily infected (three or more stem lesions) and 23 were moderately infected (one or two stem lesions). After 6 extra months, during which the plants were stored under stressful conditions, 50 percent of the heavily infected plants and 35 percent of the moderately infected plants had died. Mortality was 10 percent among the plants without stem lesions. Both ratios are significantly different from the control ratio ($p=0.006$ for heavy infection and $p=0.017$ for moderate infection) based on χ^2 tests. Within the infection classes, no obvious relationship was present between mortality and previous fungicide treatment.

Results from the third *in planta* experiment are presented in *table* 3. In this last experiment a different rhododendron cultivar was used. A higher level of leaf infections was observed with this cultivar: an average of 2.71 versus 0.67 infected leaves per plant in the preventive treatments and 10.31 versus 4.95 infected leaves in the curative treatment. The number of plants on which leaf lesions were observed was also higher in the preventive treatment: 4.86 versus 2.00. In contrast to the large number of leaf infections, hardly any stem infections were observed over the entire third experiment and therefore not reported. As in the previous experiments, a major difference was observed between the preventive and the curative treatments ($p<0.001$): the average number of infected leaves per plant was 2.71 versus 10.31 in the respective groups. In the curative treatment, no fungicide gave good control and no significant difference was obtained between the fungicide treatments (except for the negative control) using the Kruskal-Wallis and the Dunn tests. In the preventive treatments, benthiavalicarb-isopropyl, mefenoxam and cyazofamid gave the best control, but only benthiavalicarb-isopropyl was significantly different from the positive control (Kruskal-Wallis and the Dunn tests). Mancozeb, fosetyl-Al, and dimethomorph gave intermediate effects, and cymoxanil did not give acceptable control.

An interpreted summary of the results from the three experiments is presented in *table* 4. It consists of the effects on stem lesions in experiments one and two and leaf lesions in experiments two and three. Benthiavalicarb-isopropyl, cyazofamid, and mefenoxam were the best products for the preventive treatments (summarized over stem and leaf). Those same products also were the best for the curative treatments, be it in a different order and at an overall less favorable score. In the curative treatments, a few products ranked worse than the positive control.

Table 3—*Mean +/-stdev number of leaves with lesions per plant in the third* in planta *experiment. Fungicides were either applied 1 day before (preventive) or 2 days after the pathogen (curative).*

Fungicide	preventive		curative	
	lesions	plants[1]	lesions	plants[1]
no pathogen control	0.00±0.00	0	0.00±0.00	0
no fungicide control	9.33±4.80	6	6.33±1.86	6
fosetyl-Al	3.83±4.17	5	5.83±5.31	6
mefenoxam	1.33±0.82	5	7.00±5.93	5
benthiavalicarb-isop.	0.83±0.98	3	12.50±6.28	6
cyazofamid	1.33±1.21	4	10.00±8.92	6
dimethomorph	3.50±2.81	5	8.33±3.88	6
cymoxanil	5.50±3.08	6	13.33±5.09	6
mancozeb	2.67±1.86	6	15.17±9.77	6
mean±stdev[2]	2.71±1.68	4.86	10.31±3.48	5.86

[1] number of replicate plants (out of 6) with at least one leaf lesion
[2] mean (± standard deviation) within each column, excluding the control treatments

Table 4—*Average category of fungicide effects in the three* in planta *experiments, based on the following categories: 1 = excellent control, 2 = good control, 3 = some control, 4 = inadequate control. Within each group, fungicides with the same score were sorted based on the actual number of lesions observed.*

timing of fungicide application	leaf		stem		leaf + stem	
	rank	fungicide	rank	fungicide	rank	fungicide
preventive	1.0	no pathogen	1.0	no pathogen	1.0	no pathogen
	1.5	benthiavalicarb	1.0	benthiavalicarb	1.3	benthiavalicarb
	1.5	cyazofamid	1.0	cyazofamid	1.3	cyazofamid
	2.0	mefenoxam	1.0	dimethomorph	1.8	mefenoxam
	2.5	fosetyl-Al	1.5	mefenoxam	2.0	fosetyl-Al
	2.5	mancozeb	1.5	fosetyl-Al	2.0	dimethomorph
	3.0	dimethomorph	2.0	cymoxanil	2.5	mancozeb
	3.5	cymoxanil	2.5	mancozeb	2.8	cymoxanil
	4.0	no fungicide	4.0	no fungicide	4.0	no fungicide
curative	1.0	no pathogen	1.0	no pathogen	1.0	no pathogen
	2.0	mefenoxam	1.0	cyazofamid	2.0	cyazofamid
	2.5	fosetyl-Al	2.0	benthiavalicarb	2.5	mefenoxam
	3.0	cyazofamid	3.0	mefenoxam	2.8	benthiavalicarb
	3.0	dimethomorph	3.0	dimethomorph	3.0	dimethomorph
	3.0	no fungicide	3.0	cymoxanil	3.0	no fungicide
	3.5	benthiavalicarb	3.0	no fungicide	3.3	fosetyl-Al
	3.5	cymoxanil	4.0	fosetyl-Al	3.3	cymoxanil
	4.0	mancozeb	4.0	mancozeb	4.0	mancozeb

Discussion

Prevention of the introduction of *Phytophthora ramorum* and its spread within the EU is the objective of the current European phytosanitary measures. Prevention of the spread via infected plants is in part accomplished through a plant passporting system involving surveys, eradication of infected plants within a 2 m radius, and quarantine measures of the remaining plants within a 10 m radius. The potential threat from the pathogen and the impact of the quarantine measures has caused growers to also take preventive initiatives. They try to prevent the introduction and spread within their nursery through sanitation, reduction of disease conducive conditions such as wounding and high moisture conditions, avoidance or protection of susceptible cultivars, and preventive fungicide applications. Curative fungicide applications, or applications in the 10-m quarantine zone, are not allowed under the EU phytosanitary measures. Our data tend to support that eradication through curative application of fungicides is extremely difficult under optimal conditions for pathogen development. However, preventive use of fungicides was very effective with selected fungicides, even under the conditions of high inoculum dose used, and may be encouraged as a protective measure. Therefore, our data can contribute to the more effective use of fungicides.

Our approach was to pre-select fungicides based on their *in vitro* activity. In most cases this approach was effective as the fungicides with excellent *in vitro* effect such as metalaxyl (and mefenoxam) and benthiavalicarb-isopropyl were among the fungicides with the best *in planta* effects. Cyazofamid, which also had excellent *in planta* effects, did not show complete growth inhibition *in vitro.* When using *Phytophthora infestans* as the test organism, 100 percent growth inhibition was obtained at 0.1 ppm cyazofamid (data not shown). Although cyazofamid did not give 100 percent growth inhibition *in vitro* against *P. ramorum*, it was the fungicide with activity at the lowest concentration. This concentration effect may have been sufficient to give superior results on plants. Alternatively, the *in planta* effect of cyazofamid may have been the result of an effect on zoospores instead of on mycelial growth. Effects on zoospores were not tested in the *in vitro* experiments. This could also explain why the curative effect of cyazofamid on leaves was less extensive, as that kind of effect mostly involved activity on mycelial growth. Dimethomorph, which gave excellent effects *in vitro*, only gave intermediate overall results *in planta*. However, this was mostly due to its inferior results on leaf lesions. Results on stem lesions with dimethomorph were comparable to those of mefenoxam. Differences in results on leaves and stems could indicate that the formulation and/or (semi)-systemic characteristics of the fungicide may also play a role in the effect on plants.

The *in vitro* fungicide effects were independent of the strain of *P. ramorum* used, except for strain 02-880, which showed a decreased sensitivity to metalaxyl. The risk of rapid development of resistance against metalaxyl is well known (Gisi 1992). We can only speculate that this strain had been exposed to metalaxyl in the field. The three most effective fungicides in our experiments (mefenoxam, cyazofamid, benthiavalicarb-isopropyl) are single target fungicides, indicating a higher risk for the development of resistance. Even though most of them are commercialized in a mixture with a broad spectrum fungicide like mancozeb, alternating their use seems highly advisable.

Although there were differences in the details of each of the three *in planta* experiments, no major differences were observed in the relative efficacy of the fungicides throughout the three experiments. An apparent difference was the smaller effect of dimethomorph in experiment two as compared to experiment one, but this was due to the smaller protective effect of this compound on leaves in experiment two. In experiment one, only stem lesions were taken into account. In the curative treatment of experiment three, a somewhat different result was obtained with fosetyl-Al (better) and with benthiavalicarb-isopropyl (worse) as compared to the other experiments. However, under the intense disease pressure of the curative part of experiment three, none of the treatments were performing particularly well. Several fungicide treatments even performed worse than the control. This is in part because the score for the positive control for the curative treatment of experiment three was better than expected. However, this negative effect of some fungicides in the curative treatment was also observed in experiment two (stem lesions). It is possible that lesion development was enhanced if the plants were stressed by the fungicide applications. Overall, when averaged over the three experiments, mefenoxam, cyazofamid and benthiavalicarb-isopropyl performed the best. Fosetyl-Al and dimethomorph gave intermediate results, and mancozeb and cymoxanil gave poorer protection. Some fungicides such as mefenoxam also had a considerable effect on lesion size. Those data are not presented in this paper, as only presence or absence of lesions was recorded, independent of lesion size. Lesion size is relevant in terms of level of pathogen growth and probably in terms of the amount of secondary inoculum that can be produced. However, when the Plant Health Inspection Service takes samples, lesion size does not affect the level of consequences when samples are found positive.

The difference in the results with the two rhododendron cultivars was rather striking: on cv. Percy Wiseman, a moderate number of leaf lesions and moderate number of stem lesions where observed, while on cv. Germania, a large number of leaf lesions was observed, but hardly any stem lesions. Differences in the number and extent of

leaf and stem lesions was in correspondence with results from a study comparing the susceptibility of many rhododendron cultivars (De Dobbelaere and others 2005). One reason for the smaller number of stem infections in cv. Germania could be that a large proportion of its infected leaves dropped from the plant before the lesion spread extensively within the leaf. This prevents the spread of the pathogen from infected leaves into the stem.

The application method of the fungicides played a major role in the effect, with application to the lower part of the leaves as the most effective. This was not surprising, considering the zoospores were also applied to that surface. Preliminary experiments showed repeatedly that infection of non-wounded leaves inoculated with zoospores almost exclusively takes place through the lower side of the leaves (Heungens and others 2003). So even if the zoospores had been applied to both sides of the leaves, application of the fungicides to the lower leaf surface would still be expected to be significantly better than the other application modes. The excellent control effect of the preventive fungicide applications to the lower leaves also shows that the application of the zoospores in itself did not substantially wash the fungicide from the leaves, which could have happened with the contact fungicides. At nurseries, fungicides are usually applied through overhead irrigation systems. Application methods which help reach the lower side of the leaf, such as higher pressure application or application with air support could potentially increase the protective effect. Application via the roots gave a smaller protective effect than expected, even with compounds that have proven systemic activity such as mefenoxam. This could be due to the time of year (reduced evapotranspiration or reduced translocation) or to a suboptimal concentration of the fungicide.

Progression of *P. ramorum* twig infections often seems to halt when conducive conditions (high moisture) stop. Although anecdotal and not the primary goal of this research, we considered it interesting to verify this assumption and observe the mortality data of plants with different levels of infection, several months after inoculation. If there is progression of disease then mortality is expected to be larger in infected plants than in control plants. Our results did indeed indicate that mortality of plants was correlated with *P. ramorum* infection level under the stressful conditions used. However, we can not exclude that the increased level of mortality is in part due to the already weakened status of the plant instead of the continued activity of the fungus.

One of the concerns with the use of fungicides is that symptoms may be suppressed without completely killing the fungus, thus facilitating its latent spread. In our experiments, no problems were experienced with the isolation of *P. ramorum* on

PARP medium from any of the lesions. Based on our results, the risk for missing the detection of the pathogen through false negative plating results of suspected material thus seems limited. Of course, finding suspected material can become problematic if symptoms are considerably fewer or smaller.

Until recently, reports on the activity of fungicides against *P. ramorum* were limited and mostly involved trees. Phosphonates were one class of compounds able to reduce *P. ramorum* lesions and canker size on trees (Garbelotto and others 2002, Garbelotto and others 2003, Schmidt and others 2002). One such compound (fosetyl-Al) was included in our studies. Although this fungicide has interesting systemic properties and has a good reputation for its activity against *Phytophthora* species, it only gave intermediate effects in our experiments. This could in part be due to the limited physiological activity of the plants during the period of the experiment. Other compounds tested on trees and woodland shrubs are metalaxyl, copper sulfate pentahydrate and dimethomorph+mancozeb (Garbelotto and others 2002, Goheen and others 2005). Especially metalaxyl and dimethomorph+mancozeb had an effect, but rarely the complete suppression of the pathogen. At the SOD Science Symposium II, several new reports were presented on fungicide activity against *P. ramorum* in horticultural settings (Chastagner and others 2005, Linderman and Davis 2005, Tjösvold and Chambers 2005, Turner and others 2005). In general, similar results were reported as to the effect of the different fungicides. Overall, metalaxyl and cyazofamid were among the better products. However, cyazofamid did not have a consistent effect on all strains tested in one report (Linderman and Davis 2005). Compounds with good results on plants that were tested in other studies but not in ours were azoxystrobulin (Turner and others 2005), fenamidone (Tjösvold and Chambers 2005, Turner and others 2005), and pyraclostrobin (Tjösvold and Chambers 2005). Future experiments are needed to evaluate their activity under our test conditions.

This research shows that protective applications of specific fungicides can contribute to effective control strategies of *P. ramorum* on rhododendron. Considering that we identified a strain with decreased activity against metalaxyl, it seems advisable to limit the number of consecutive uses of products with a single target site. However, depending on the specifics of each country, growers may face few options in alternating fungicides due to the limited number of products with use permits on rhododendron.

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References

APHIS. 2004. ***Phytophthora ramorum***. http://www.aphis.usda.gov/ppq/ispm/sod.

Chastagner, G.A.; Hansen, E.M.; Riley, K.L.; and Sutton, W. 2006. **Effectiveness of fungicides in protecting Douglas-fir shoots from infection by *Phytophthora ramorum***. In: The Proceedings of the Sudden Oak Death Second Science Symposium; January 18-21, 2005; Monterey, CA, This volume.

De Dobbelaere, Isabelle; Heungens, Kurt; and Maes, Martine. 2005. **Susceptibility levels of *Rhododendron* species and hybrids to *Phytophthora ramorum***. In: The Proceedings of the Sudden Oak Death Second Science Symposium; January 18-21, 2005; Monterey, CA, this volume.

DEFRA. 2003a. **First infected oak found in Sussex**. http://www.defra.gov.uk/news/2003/031105b.htm.

DEFRA. 2003b. ***Phytophthora ramorum*: more resources and new disease confirmations**. http://www.defra.gov.uk/news/2003/031204c.htm.

EPPO. 2005. ***Phytophthora ramorum***. http://www.eppo.org/QUARANTINE/Alert_List/fungi/oak_death.htm.

Garbelotto, M.; Rizzo, D.M.; and Marais, L. 2002. ***Phytophthora ramorum* and Sudden Oak Death in California: IV. Chemical control**. In: Standiford, R.; McCreary, D.; eds. General Technical Report. 5th Symposium on California Oak Woodlands. U.S. Forest Service. p. 811-818.

Garbelotto, M.; Schmidt, D.J.; Tjösvold, S.; and Harnik , T.Y. 2003. **Chemical treatment strategies for control of sudden oak death in oaks and tanoaks**. Phytopathology 93: S28 (Abstract).

Gisi, U. 1992. **FRAC methods for monitoring the sensitivity of fungal pathogens to phenylamide fungicides developed by the Phenylamide Fungicide Resistance Action Committee (PA-FRAC) of GIFAP.** Bulletin OEPP 22(2): 297-322.

Goheen, E.M.; Hansen, E.M.; Kanaskie, A.; McWilliams, M.G.; Osterbauer, N.; and Sutton, W. 2002. **Sudden oak death caused by *Phytophthora ramorum* in Oregon**. Plant Disease 86: 441.

Goheen, E.; Kanaskie, A.; Parke, J.; Roth, M.; Osterbauer, N.; and Trippe, A. 2005. **Applications of Fungicides to Protect Four Hosts from Foliar Infection by *Phytophthora ramorum* in Curry County, Oregon**. In: The Proceedings of the Sudden Oak Death Second Science Symposium; January 18-21, 2005; Monterey, CA, this volume.

Heungens, Kurt; Crepel, Caroline; Inghelbrecht, Sven; and Maes, Martine. 2003. ***Phytophthora ramorum* in Belgium: 2002 survey results and research efforts**. Communications in Agricultural and Applied Biological Sciences 68(4b): 589-598.

Ivors, K.L.; Hayden, K.J.; Bonants, P.J.M.; Rizzo, D.M.; and Garbelotto, M. 2004. **AFLP and phylogenetic analyses of North American and European populations of *Phytophthora ramorum***. Mycology Research 108(4): 378-392.

Kroon, L.P.N.M.; Verstappen, E.C.P.; Kox, L.F.F.; and Bonants, P.J.M. 2004. **A rapid diagnostic test to distinguish between American and European isolates of *Phytophthora ramorum***. Phytopathology 94(6): 613-620.

Linderman, R.G. and Davis, E.A. 2005. **Evaluation of Chemical and Biological Agents for Control of *Phytophthora* species on Intact Plants or Detached Rhododendron Leaves**. In: The Proceedings of the Sudden Oak Death Second Science Symposium; January 18-21, 2005; Monterey, CA, this volume.

PD Nederland. 2003. ***Phytophthora ramomum* in Amerikaanse eik. Persmededeling Plantenziektenkundige Dienst.** Wageningen: Plantenziektenkundige Dienst.

Rizzo, D.M.; Garbelotto, M.; Davidson, J.M.; Slaughter, G.W.; and Koike, S.T. 2002. ***Phytophthora ramorum* as the cause of extensive mortality of *Quercus* spp. and *Lithocarpus densiflorus* in California**. Plant Disease 86: 205-214.

Schmidt, D.; Harnik, T.; Rizzo, D. M.; and Garbelotto, Matteo. 2002. **Chemical treatment strategies for control of sudden oak death in oaks and tanoaks**. The Proceedings of the Sudden Oak Death Second Science Symposium; January 18-21, 2005; Monterey, CA, this volume.

Tjösvold, S.A and Chambers, D.L. 2005. **Evaluation of Fungicides for the Control of *Phytophthora ramorum* Infecting Rhododendron, Camellia, Viburnum and Pieris**. In: The Proceedings of the Sudden Oak Death Second Science Symposium; January 18-21, 2005; Monterey, CA, this volume.

Turner, J.; Jennings, P.; McDonough, S.; Liddell, D.; and Stonehouse, J. 2005. **Chemical control of *Phytophthora ramorum* causing foliar disease in hardy nursery stock**. In: The Proceedings of the Sudden Oak Death Second Science Symposium; January 18-21, 2005; Monterey, CA, this volume.

Werres, S. and Marwitz, R. 1997. **Triebsterben an rhododendron: unbekannte *Phytophthora***. Deutscher Gartenbau 21: 1166-1168.

Werres, S.; Marwitz, R.; Man in 't Veld, W.A.; De Cock, A.W.; Bonants, P.J.M.; De Weerdt, M.; Themann, K.; Ilieva, E.; and Baayen, R.P. 2001. ***Phytophthora ramorum* sp. nov: a new pathogen on Rhododendron and Viburnum**. Mycological Research 105(10): 1155-1165.