Crop Protection 67 (2015) 136–144

Contents lists available at ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

Effects of systemic and contact fungicides on life stages and symptom expression of *Phytophthora ramorum in vitro* and *in planta*

Marianne Elliott [a], Simon F. Shamoun [b, *], Grace Sumampong [b]

[a] Washington State University, Puyallup Research and Extension Center, Puyallup, WA 98371, USA
[b] Natural Resources Canada, Canadian Forest Service, Pacific Forestry Centre, Victoria, BC V8Z 1M5, Canada

ARTICLE INFO

Article history:
Received 2 May 2014
Received in revised form
15 October 2014
Accepted 16 October 2014
Available online

Keywords:
Chemical control
Nursery crops
Phytophthora

ABSTRACT

Nine isolates of *Phytophthora ramorum* Werres, de Cock & Man in't Veld were screened using a variety of systemic and contact fungicides *in vitro* for mycelial growth inhibition and zoospore germination inhibition, and *in planta* for suppression of lesion expansion on rhododendron foliage. Three isolates from each of the major clonal lineages, NA1, NA2, and EU1 were used. Systemic fungicides were the most effective at preventing mycelial growth and zoospore germination of *P. ramorum*, and the results from testing on host plants at the labeled rate supported the *in vitro* results. Development of resistance to some chemicals used for routine control of *P. ramorum* in the nursery should be monitored, especially in the EU1 and NA2 populations. Metalaxyl-M had the lowest EC_{50} for both mycelial growth inhibition and zoospore germination inhibition for all isolates. EC_{50} was higher for zoospore germination inhibition of the EU1 isolates by two strobilurin fungicides, indicating possible cross-resistance in this group.

1. Introduction

International trade and travel have facilitated the spread of invasive alien pathogens around the world, and human-mediated movement of plants and plant products is now generally accepted to be the primary mode of introduction of plant pathogens (Liebhold et al., 2012). Several species of *Phytophthora*, including introduced species, cause diseases that result in devastating losses to a wide variety of plants. These diseases, including root and crown rots, cankers, foliar blights, and fruit rots, affect food and fiber crops, forest trees, and a variety of ornamental plants (Agrios, 2005; Erwin and Ribeiro, 1996; Brasier, 2008). One of the most notorious is *Phytophthora ramorum* Werres, de Cock & Man in't Veld. It has been associated with twig blight of nursery *Rhododendron* and *Viburnum* in Germany and the Netherlands since the early 1990s and was first described in 2001 (Werres et al., 2001). Later, the same species was found to cause a canker disease of oak forests along the central coast of California (Rizzo and Garbelotto, 2003). This disease is commonly known as Sudden Oak Death (SOD) and has resulted in widespread mortality on tan oak (*Notholithocarpus densiflorus* (Hook. & Arn.) Manos, Cannon & S.H.Oh), coast live oak (*Quercus agrifolia* Neé), California black oak (*Quercus kellogii* Newb.), and Shreve's oak (*Quercus parvula var shrevei* (C.H.Mull.) Nixon) in the coastal regions of northern California and southwestern Oregon, USA, and is a serious threat to the native forests of North America (Rizzo and Garbelotto, 2003; Rizzo et al., 2002). Most recently, *P. ramorum* has been associated with a destructive disease of Japanese larch (*Larix kaempferi* (Lamb.) Carr.) in the United Kingdom. Symptoms included widespread dieback and mortality of mature and juvenile larch plantations. This devastating disease was identified as Sudden Larch Death (SLD) (Brasier and Webber, 2010).

The pathogen (*P. ramorum*) is believed to have been introduced to Europe and North America from an unknown geographic origin. Molecular data indicate that there are four distinct clonal lineages of *P. ramorum*, one originally discovered in Europe, but also found in western North America (EU1), a new lineage recently detected in Europe (EU2), and two lineages only present in North America (NA1 and NA2) (Grünwald et al., 2012; Van Poucke et al., 2012; Elliott et al., 2009). The known host range of *P. ramorum* is very broad (more than 100 host plants) and includes species such as rhododendrons, viburnum, beech, Oregon grape, salal, arbutus, and other woody ornamentals. Many of these host species are currently present in forested and urban areas in the west coast of the US and Canada. They are primarily foliar hosts that can serve as potential reservoirs for *P. ramorum* inoculum. Establishment of *P. ramorum* on these hosts increases the risk of disease spread to more susceptible

* Corresponding author. Tel.: +1 250 298 2358.
E-mail address: Simon.Shamoun@NRCan-RNCan.gc.ca (S.F. Shamoun).

http://dx.doi.org/10.1016/j.cropro.2014.10.008

hosts in other locations, especially through nursery trade operations (APHIS-PPQ, 2013; Kristjansson and Miller, 2009; Grünwald et al., 2008).

The Canadian Food Inspection Agency (CFIA) first confirmed the presence of *P. ramorum* in plants from a number of retail garden centers in the Vancouver, British Columbia (BC) area in 2004 and eradication procedures were conducted several times over the past nine years (Kristjansson and Miller, 2009). While all three major clonal lineages (NA1, NA2, EU1) of *P. ramorum* have been detected in BC nurseries, the most common has been NA2 (Goss et al., 2011). Because *P. ramorum* is not established in Canada, the situation is much like that of the eastern US states. Establishment of *P. ramorum* in BC nurseries and landscapes could result in large economic losses and limitations to trade in ornamental plants and threats to biodiversity and sustainability of forest ecosystems, if any Canadian forest species prove to be highly susceptible to *P. ramorum* infection. While Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) is a known host for *P. ramorum* and is an important forest product of the US and Canada, the symptoms caused by *P. ramorum* on this host are minor (Chastagner et al., 2013).

P. ramorum spreads through airborne deciduous sporangia formed on the surface of infected leaves or twigs that are locally splash-dispersed or spread over long distances by wind and wind-driven rain. Motile zoospores are released from sporangia, and upon contact with susceptible host tissue they encyst, germinate, and penetrate host tissue. Sporangia can also germinate directly without releasing zoospores. *P. ramorum* colonizes host tissue by means of mycelial growth (Riedel et al., 2012). Chlamydospores are abundantly produced within infected plant tissue and allow *P. ramorum* to survive adverse environmental conditions in infected stems and leaves of the plant, in plant debris on the soil surface, or in the soil (Grünwald et al., 2012).

Phytophthora diseases of plants in agricultural, nursery and natural ecosystem settings are often managed using chemical fungicides (Cohen and Coffey, 1986; Stein and Kirk, 2002; Jeffers, 2003; Linderman and Davis, 2008; Tjosvold et al., 2008; Chastagner et al., 2008; Garbelotto et al., 2007; Guest et al., 1995; Jackson et al., 2000). Fungicides protect host plants in risky situations, such as in an existing nursery or landscape, from disease introduced on imported material, rather than eradicate the disease on an infected plant. Chemical fungicides are one tool in integrated pest management, which serves to keep diseases and pests below threshold levels. However, there is concern that use of fungicides may mask or delay symptom development on nursery crops being sold, making it more difficult to detect *P. ramorum* during an inspection.

In Canada, based on the CFIA Pest Risk Assessment Summary (Kristjansson and Miller, 2009), the likelihood for the introduction of *P. ramorum* to Canada is high, but the consequences for introduction are estimated to be of medium risk. Certification is required for movement of plants from regulated areas of the US and Europe, so the shipping nursery must be a CFIA approved pest-free production site. Host plants that are shipped from these nurseries are required to be inspected and issued a phytosanitary certificate (CFIA, 2013). To maintain certification, the shipping nursery is required to keep records, including fungicide applications, for 24 months. A buyer could potentially know when the plants were treated and quarantine them until the effects had worn off. The burden is on the shipping nursery to ensure that plants entering Canada are clean. In Canada, *P. ramorum* has only been detected in BC and at levels lower than in non-regulated US states, so the pathogen is considered to be a non-regulated quarantine pest in Canada. This means that no certification is required for movement of host material within or from Canada.

At present, there are five chemical fungicides registered for use against *P. ramorum* and other Oomycetes on nursery crops in Canada. These fungicides are dimethomorph (ACROBAT® 50 WP); propamocarb (Previcur N®); and metalaxyl-M (SUBDUE MAXX®), ammonium phosphite (Phostrol and others), and fluopicolide (Presidio) (PMRA Health Canada, 2013), but when the experiments we report on here were conducted only metalaxyl-M was registered in Canada.

The complex nature of the *P. ramorum* life cycle presents challenges to screen and test the efficacy of different fungicide formulations. There are many chemical fungicides on the market with varying modes of action and effectiveness on different life stages of *Phytophthora* spp. However, little research work has been conducted to date to evaluate the efficacy of fungicides for management of *P. ramorum* (Heungens et al., 2006; Wagner et al., 2008; Linderman and Davis, 2008; Tjosvold et al., 2008; Garbelotto et al., 2007). Furthermore, very little is known about the effects of specific fungicides on various stages of the *P. ramorum* life cycle (Turner et al., 2006; Goheen et al., 2006; Orlikowski, 2004; Jeffers, 2003). Effects of fungicides on certain life stages has been shown in other *Phytophthora* species, including *Phytophthora cinnamomi*, *P. cactorum*, *Phytophthora citricola*, *P. citrophthora*, *P. nicotianae*, and *Phytophthora infestans* (McCarren et al., 2009; Linderman and Davis, 2008; Stein and Kirk, 2002; Coffey and Joseph, 1985; Coffey et al., 1984).

The variation in sensitivity to different chemical fungicides among *Phytophthora* isolates belonging to the same species has been reported (McCarren et al., 2009; Coffey and Bower, 1984; Wilkinson et al., 2001; Ferrin and Kabashima, 1991), but such variation has yet to be explored among the clonal lineages of *P. ramorum* (NA1, NA2 and EU1) found infecting nursery plants. Our earlier results have shown that there are differences in the pathogenicity among isolates from the three lineages (Elliott et al., 2011). Monitoring *P. ramorum* populations within each lineage for resistance to a fungicide is essential for development of management strategies that can delay or prevent development of resistance to fungicides and fungicide failure.

In many European regions, some *P. ramorum* isolates belonging to the EU1 lineage obtained from nursery plants have shown resistance to metalaxyl-M (Heungens et al., 2006; Turner et al., 2008; Wagner et al., 2008; Vercauteren et al., 2010; Pérez-Sierra et al., 2011). The *P. ramorum* NA1 clonal lineage has been extensively studied with microsatellite markers and a high level of genetic diversity has been found (Goss et al., 2009). Information is not available on genetic diversity within NA2. In the EU1 clonal lineage, low genetic diversity was seen in Belgian and Spanish populations (Vercauteren et al., 2010; Pérez-Sierra et al., 2011). In these countries, metalaxyl use has decreased genetic diversity by selecting for resistant strains. The percentage of metalaxyl-sensitive isolates increased, as did genetic diversity, after metalaxyl use was discontinued in Belgian nurseries in 2005 (Vercauteren et al., 2010). Alternating the use of metalaxyl-M with other fungicides is recommended to reduce the probability of resistance development (Kliejunas, 2010).

None of the previous work on fungicide sensitivity of *P. ramorum* has tested the NA2 lineage and compared it with NA1 and EU1. In this study, we combine *in vitro* tests on two life stages of *P. ramorum* (zoospore germination inhibition and mycelial growth inhibition) using representatives from the three major clonal lineages NA1, NA2, and EU1, and also test representative isolates of these clonal lineages on rhododendron plants treated with various fungicides. Knowledge and information relevant to the sensitivity of *P. ramorum* isolates within each lineage and among the lineages to several fungicides has important ecological and environmental implications in management of sudden oak death disease. Specific objectives of this study were: 1) to determine the effects of 5 selected systemic and 3 contact fungicides on mycelial growth of 9

Table 1
Isolates[a] of *Phytophthora ramorum* used in this study.

Isolate number	Strain number	Host	Clonal lineage	Source
5038	2027	*Notholithocarpus densiflorus*	NA1	OR, USA
5039	03-74-D12-A	*Viburnum plicatum*	EU1	OR, USA
5046[a]	2339	*Notholithocarpus densiflorus*	NA1	OR, USA
5054	04-207-Q	*Pieris japonica*	NA1	OR, USA
5063	WSDA3765	*Rhododendron* cultivar	NA2	WA, USA
5073	RHCC-23	*Rhododendron* cultivar	NA2	CA, USA
5074[a]	RHCC-4	*Rhododendron* cultivar	NA2	CA, USA
5084	CSL 2266, BBA 9/95	*Rhododendron catawbiense*	EU1	Germany
5086[a]	CSL2268	*Rhododendron grandiflora*	EU1	UK

[a] Isolates used in plant tests.

isolates *in vitro* within the lineages (NA1, NA2 and EU1) of *P. ramorum*; 2) to analyze the effects of 7 selected systemic and 4 contact fungicides *in vitro* on zoospore germination on 9 isolates within the lineages (NA1, NA2 and EU1) of *P. ramorum*; and 3) to test the efficacy of 3 systemic and 2 contact fungicides *in planta* on reducing infection frequency and lesion area of a single isolate of *P. ramorum* from each of the NA1, NA2 and EU1 lineages.

2. Materials and methods

2.1. P. ramorum isolates, fungicides and plants

Nine isolates of *P. ramorum* (Table 1) were used in this study and maintained on 15% V8A (150 mL V8 juice, 1.5 g $CaCO_3$, 15.0 g bactoagar (Difco). Formulated products of the chemical fungicides were donated by the manufacturers. Stock solutions in sterile deionized water were prepared containing concentrations of active ingredient (a.i.) ranging from 100 to 100,000 μg mL^{-1} or μL L^{-1}, depending on the recommended dose for each chemical.

For the plant tests, *Rhododendron* 'Cunningham's White' cultivar plants grown in 1 gallon pots were obtained from a local nursery. A total of 144 healthy plants were selected and maintained under greenhouse conditions (21 °C day/15 °C night, 60% relative humidity (RH) and 16-h photoperiod) at least one month prior to treatment to allow plants to acclimatize to the greenhouse conditions.

2.2. Effect of fungicides on P. ramorum mycelial growth

Inoculum of each of the nine *P. ramorum* isolates (Table 1) was grown on 15% V8A in 9 cm petri plates for 14 days at 20 °C. Mycelial plugs (7 mm diameter) excised from the edge of an actively growing colony were transferred to the center of each 6 cm petri plate containing ~10 mL 15% V8A amended with the test fungicides (Table 2) at eight different concentrations that included the recommended dose in μg mL^{-1} or μL L^{-1} for each chemical. The final concentration of active ingredient ranged between 0 and 10,000 μg mL^{-1} or μL L^{-1}. Fungicides were added to the V8A media after autoclaving when the media had cooled to 55 °C. A set of plates containing V8A without fungicide was included for each isolate as a control. The plates were parafilmed, placed in a plastic container with a lid and incubated in the dark at 20 °C. For each fungicide and concentration, 3 replicate plates were used and the experiment was repeated once. This was a randomized complete block design with fungicide as the blocking factor.

Colony diameter was measured after 7 days in two perpendicular directions on each plate. The diameter of the mycelial plug inoculum was subtracted and the two diameter measurements were averaged. Percent growth inhibition for each isolate/fungicide concentration was calculated by dividing colony diameter in the treated plates by that in the control plates (no fungicide added). The values were expressed as percent radial growth inhibition relative to the control. The half-maximal effective concentration (EC_{50}) value for each fungicide was calculated for each *P. ramorum* isolate (Alexander et al., 1999).

2.3. Effect of fungicides on zoospore germination

Effects of 11 fungicides (Table 2) on zoospore germination were evaluated using methods modified from Kuhajek et al. (2003). Sporangia production was initiated from 14 day old mycelia grown on 15% V8A plates and incubated at 20 °C with 24 h continuous light. Plates with sporangia (4 plates per isolate) were then flooded with 10 mL sterile distilled water and incubated at 4–5 °C for 1–2 h, followed by at least 30 min incubation at room temperature (22 °C) to induce release of zoospores. After zoospore release, the combined liquid suspensions from all plates were poured into one 50-mL sterile falcon tube for each isolate. Zoospore concentration was quantified for each isolate using a hemocytometer, adjusted to 10^5 zoospores/mL, and 100 μL was pipetted into each well of a 96-well plate per isolate. For each fungicide, eight concentrations were chosen to determine the EC_{50} for inhibition of zoospore germination. These concentrations were 100, 10, 1, 0.1, 0.01, 0.001, 0.0001, and 0 μg mL^{-1} or μL L^{-1} using a 10,000 μg mL^{-1} or μL L^{-1} stock fungicide concentration diluted with RPMI-1640 (Sigma; cat #RH130-1L). 100 μL fungicide-RPMI solution was pipetted into each

Table 2
Some properties of chemical fungicides screened for their effects on life stages of *Phytophthora ramorum*.

	Active ingredient	Mode of action, target site[a]	Product, manufacturer	Symbol	FRAC code[a]	Label rate, mg or ml a.i./L (ppm)	Tests[b]
Systemic	Metalaxyl-M	Nucleic acid synthesis, RNA polymerase 1	Subdue Maxx (Syngenta)	SM	4	0.04 ml	m, z, p
	Azoxystrobin	Respiration, cytochrome bc1	Quadris (Syngenta)	QU	11	0.08 ml	z
	Fenamidone	Respiration, cytochrome bc1	Reason (Bayer)	RE	11	0.49 ml	m, z, p
	Pyraclostrobin	Respiration, cytochrome bc1	Cabrio (BASF)	CA	11	240 mg	z
	Cymoxanil	Unknown	Curzate (DuPont)	CU	27	135 mg	z
	Propamocarb	Cell membrane permeability, fatty acids	Previcur N (Bayer)	PR	28	1.41 ml	m, z
	Fosetyl-Al	Unknown	Aliette (Bayer)	AL	33	4000 mg	m
	Dimethomorph	Cell wall biosynthesis, cellulose synthase	Acrobat (BASF)	AC	40	459 mg	m, z, p
Contact	Etridiazole	Cell membrane, lipid peroxidation	Truban (Scotts)	TR	14	225 mg	z
	Copper hydroxide	Multi-site contact activity	Kocide (DuPont)	KO	M1	1356 mg	m, z
	Mancozeb	Multi-site contact activity	Manzate (DuPont)	MA	M3	1875 mg	m, z, p
	Chlorothalonil	Multi-site contact activity	Daconil (Syngenta)	DA	M5	1.01 ml	m, z, p

[a] FRAC Code List 2013. Fungicide Resistance Action Committee. Online, accessed 6/26/2013. http://www.frac.info/publication/anhang/FRAC%20Code%20List%202013-update%20April-2013.pdf.
[b] Tests – m = mycelial growth inhibition, z = zoospore germination inhibition, p = plant symptom suppression.

well, with 6 replicates per concentration per fungicide per isolate. This was a randomized complete block design, with isolate as the blocking factor. The experiment was repeated once. Absorbance at 650 nm was measured at 0 and 48 h after inoculation to calculate percent inhibition. EC_{50} was calculated as described above for mycelial growth inhibition.

2.4. Effect of fungicides on foliar infection by P. ramorum

2.4.1. Selection of P. ramorum isolates

For this experiment, a preliminary assessment of 13 *P. ramorum* isolates representing the NA1, NA2, and EU1 clonal lineages was performed in order to select the most suitable isolates for the *in planta* assay. Cultures of each isolate were grown on both PARP-V8 (Ferguson and Jeffers, 1999) and on 15% V8A media for 2 weeks and assessed for mycelial growth, sporangia and zoospore formation. A mycelial plug from each isolate and media combination was placed on detached rhododendron "Cunningham's White" leaves. Three replicate wounded and 3 replicate unwounded leaves per isolate per media type were used. Leaves were incubated at 20 °C for 10 days. After 10 days, leaves were photographed on a flatbed scanner and lesion size on each leaf caused by *P. ramorum* was measured using ASSESS (Lamari, 2002). Based on similarity of results for sporangia and zoospore production and pathogenicity as determined from lesion area on detached leaves (data not shown), one isolate from each genotype was selected for this experiment (Table 1).

The three representative *P. ramorum* isolates were re-isolated from inoculated detached leaves and subcultured on PARP-V8 agar for eight days at 20 °C. Actively growing mycelia were transferred to 15% V8 agar and were subsequently used to inoculate leaves detached from rhododendron plants that were treated with fungicides.

2.4.2. Fungicide treatment of rhododendron plants

For each fungicide treatment (Table 2), three rhododendron plants were randomly selected. Fungicides were applied at the label rate (Table 2). Fungicide treatment was applied as a foliar spray with a hand sprayer to runoff after which time the plants were maintained in the greenhouse for 14 days. All plants were kept in one greenhouse. This was set up as a completely randomized design where fungicide treatments were randomly assigned to test plants. Plants were hand-watered to avoid water contacting the treated foliage.

2.4.3. Inoculation on detached leaves treated with fungicides

Due to lack of space and quarantine restrictions, it was not possible to do whole-plant studies of fungicide effectiveness. Therefore, a detached leaf method was used. After 14 days, 20 leaves were harvested from each treated plant for the detached leaf assay. Ten leaves were wounded next to the midrib using forceps in order to measure the effectiveness of fungicide treatments on growth of the pathogen once it had penetrated external host defenses. Inoculum was applied to 10 unwounded leaves to evaluate protectant abilities of the fungicides. A 7 mm plug of *P. ramorum* inoculum from each isolate or blank V8A plug was placed mycelium side down over the wounded area and over a similar location on the unwounded leaf on the abaxial (underside) side of the leaf. Inoculated leaves were incubated in the dark at 20 °C for 10 days. At the end of the incubation period, lesion area was measured as described above. Lesion area was adjusted for lesion caused by wounding in the blank (no inoculum) treatments and considered to be zero if the lesion was equal to or less than that caused by wounding. Lesion area and infection frequency were determined for each treatment and compared to controls not treated with the fungicides. This experiment was repeated once.

2.5. Data analysis

Data analysis was done using the program R version 2.14.0 (The R Foundation for Statistical Computing, 2011). Data were examined for homogeneity of variance using the Fligner–Killeen test. Since the data did not follow a normal distribution and the variance was not constant, non-parametric tests were used. Differences among groups were examined with the Kruskal–Wallis test followed by Dunn's multiple comparisons when the differences were significant at $p = 0.05$. The overall difference between fungicide effects on *P. ramorum in vitro* and *in planta* was evaluated on all isolates taken together. For the *in vitro* tests, differences in sensitivity of each isolate and clonal lineage to a fungicide was evaluated when differences among isolates and lineages were significant. To examine cross-resistance among fungicides median EC_{50} values for all isolates were transformed to logarithmic values (log EC_{50}) and subjected to Spearman's rank correlation analysis (Revelle, 2013).

3. Results

3.1. Fungicide effects on mycelial growth of P. ramorum

Of the eight fungicides tested, EC_{50} for mycelial growth inhibition of all isolates was the lowest for metalaxyl-M and dimethomorph (Fig. 1). There was no inhibition of mycelial growth by fenamidone and propamocarb. Among isolates, median EC_{50} for

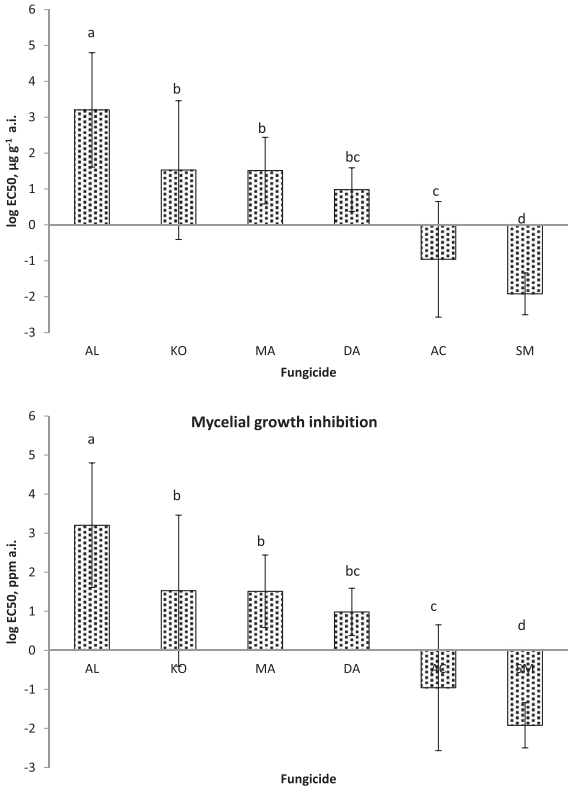

Fig. 1. Median log EC_{50} for mycelial growth inhibition *in vitro* of nine isolates of *P. ramorum* for six fungicides. Two of the fungicides tested, propamocarb (PR) and fosetyl-Al (AL) had no effect on mycelial growth inhibition and are not shown here. Error bars are ±median absolute deviation (MAD). Bars with different letters are significantly different at $p = 0.05$ (Kruskal–Wallis test, Dunn's multiple comparisons). Abbreviations for each fungicide are given in Table 2.

Table 3
Median (± median absolute deviation, MAD) EC_{50} (ppm a.i.) for mycelial growth inhibition of isolates of *Phytophthora ramorum* by six chemical fungicides.[a]

Isolate	Systemic			Contact		
	Metalaxyl-M (SM)	Fosetyl-AL (AL)	Dimethomorph (AC)	Copper hydroxide (KO)	Mancozeb (MA)	Chlorothalonil (DA)
Range, ppm	0–1.0	0–10,000	0–10	0–1000	0–1000	0–1000
EU1_5039	0.011 (0.008)	1804.24 (72.40) bc	0.085 (0.015)	35.66 ab (10.68)	33.61 (1.08) ab	9.75 (4.26) ab
EU1_5084	0.0085 (0.007)	1361.30 (300.12) b	0.095 (0.044)	30.13 ab (6.52)	18.63 (0.47) ab	4.03 (1.37) a
EU1_5086	0.007 (0.003)	1736.15 (158.28) bc	0.105 (0.022)	25.78 a (6.46)	16.24 (0.19) a	4.14 (3.76) a
NA1_5038	0.015 (0.008)	1090.38 (190.53) a	0.105 (0.007)	32.34 ab (1.41)	27.90 (3.76) ab	6.61 (2.41) a
NA1_5046	0.012 (0.007)	1306.61 (42.28) ab	0.09 (0.044)	34.90 ab (7.95)	37.49 (3.14) ab	10.58 (2.13) ab
NA1_5054	0.013 (0.006)	1484.30 (86.59) bc	0.105 (0.044)	40.90 ab (10.88)	19.18 (1.01) ab	9.51 (1.24) ab
NA2_5063	0.015 (0.009)	1955.53 (303.24) bc	0.14 (0.029)	45.10 b (9.38)	36.96 (4.66) ab	18.97 (0.75) b
NA2_5073	0.016 (0.006)	2092.21 (157.89) c	0.135 (0.052)	32.55 ab (6.58)	69.21 (11.99) b	16.32 (2.74) b
NA2_5074	0.015 (0.007)	1997.58 (164.81) bc	0.135 (0.052)	35.08 ab (5.95)	36.65 (6.14) ab	21.20 (2.38) b
P	0.3806	**<0.001**	0.1594	**0.009777**	**0.002569**	**<0.001**
Lineage						
EU1	0.007 (0.004) a	1592.53 (313.88) a	0.09 (0.044) a	30.30 (9.36) a	18.63 (3.54) a	5.30 (3.22) a
NA1	0.013 (0.007) ab	1326.73 (184.05) a	0.11 (0.029) a	33.65 (4.57) ab	27.90 (11.08) ab	8.86 (2.09) a
NA2	0.015 (0.007) b	2059.35 (205.38) b	0.14 (0.044) b	37.88 (8.29) b	40.10 (11.25) b	18.91 (2.25) b
P	**0.02162**	**<0.001**	**0.005306**	**0.01853**	**0.002501**	**<0.001**

[a] There was no inhibition of mycelial growth by the systemic fungicides fenamidone (RE) and propamocarb (PR) (data not shown). Isolate and clonal lineage EC_{50} values in columns for each chemical with different letters and *p*-values in bold are significantly different at $p = 0.05$ (Kruskal–Wallis test, Dunn's multiple comparisons). Range of active ingredient concentrations used to determine EC_{50} is shown for each fungicide.

metalaxyl-M ranged between 0.007 and 0.016 μL L^{-1} a.i., and for dimethomorph between 0.085 and 0.14 μg mL^{-1} a.i. The NA2 isolates tended to have the highest EC_{50} values for these two fungicides, but the differences among isolates were not significant (Table 3). However, there were significant differences in EC_{50} for mycelial growth inhibition between clonal lineages for all the fungicides tested, with the NA2 isolates having the highest EC_{50} values.

3.2. Fungicide effects on zoospore germination inhibition of *P. ramorum*

For most fungicides tested EC_{50} for zoospore germination inhibition was much less than that for mycelial growth inhibition in *P. ramorum*. The most effective fungicide against zoospore germination was metalaxyl-M, with EC_{50} ranging from 0.00061 μL L^{-1} a.i. to 0.0033 μL L^{-1} a.i. (Fig. 2). The quinone outside inhibitor (QoI, Fungicide Resistance Action Committee (FRAC) group 11) fungicides pyraclostrobin, azoxystrobin, and fenamidone were similar in their effectiveness at reducing zoospore germination, with fenamidone having the lowest range of EC_{50} (0.013–1.42 μL L^{-1} a.i.) (Table 4). There were no significant differences in EC_{50} among *P. ramorum* isolates for zoospore germination inhibition by the systemic fungicide dimethomorph, and the contact fungicides copper hydroxide and etridiazole (Table 4). There were significant differences in EC_{50} for zoospore germination inhibition among clonal lineages for three of the systemic fungicides (azoxystrobin, pyraclostrobin, and propamocarb) and for two of the contact fungicides (mancozeb and chlorothalonil). EC_{50} for zoospore germination inhibition of EU1 isolates by pyraclostrobin was significantly greater than that for the other two lineages (Table 4). Cross-resistance, or resistance to fungicides of the same chemical group, was seen among the group 11 chemicals (strobilurins) fenamidone and azoxystrobin ($\rho = 0.92$, $p < 0.001$).

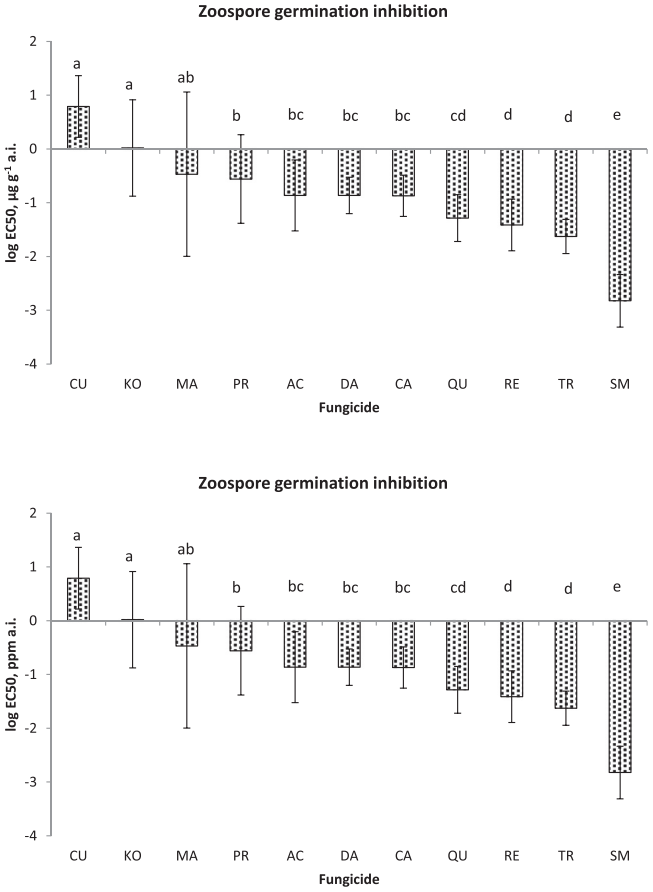

Fig. 2. Median log EC_{50} for zoospore germination inhibition *in vitro* of nine isolates of *P. ramorum* for eleven fungicides. Error bars are ±median absolute deviation (MAD). Bars with different letters are significantly different at $p = 0.05$ (Kruskal–Wallis test, Dunn's multiple comparisons). Abbreviations for each fungicide are given in Table 2.

3.3. Effects of fungicides on foliar infection by *P. ramorum*

Unwounded leaves of rhododendron treated with the systemic fungicides metalaxyl-M, dimethomorph, and fenamidone did not become infected when inoculated with *P. ramorum*. Infection frequency by two of the isolates on unwounded leaves treated with the two contact fungicides chlorothalonil and mancozeb was higher than on untreated leaves (Table 5). On leaves wounded before inoculation, average infection frequency for all isolates of *P. ramorum* was low for metalaxyl-M (0%) and dimethomorph (6%),

Table 4
Median (± median absolute deviation, MAD) EC_{50} (ppm a.i.) for zoospore germination inhibition of isolates of *Phytophthora ramorum* by eleven chemical fungicides.[a]

Isolate	Systemic							Contact			
	Metalaxyl-M (SM)	Azoxystrobin (QU)	Fenamidone (RE)	Pyraclostrobin (CA)	Cymoxanil (CU)	Propamocarb (PR)	Dimethomorph (AC)	Etridiazole (TR)	Copper hydroxide (KO)	Mancozeb (MA)	Chlorothalonil (DA)
EU1_5039	0.0033 (0.00044) b	1.16 (1.24) c	1.42 (1.48) b	51.38 (38.05) b	14.59 (9.15) b	0.37 (0.11) b	0.22 (0.032)	0.038 (0.012)	0.92 (0.19)	0.29 (0.031) ab	0.56 (0.50) cd
EU1_5084	0.0019 (0.000) ab	0.066 (0.027) abc	0.052 (0.046) ab	10.43 (15.47) ab	5.39 (1.32) ab	0.12 (0.053) a	0.076 (0.034)	0.0023 (0.0014)	0.89 (0.37)	0.36 (0.076) ab	0.063 (0.03) abc
EU1_5086	0.00079 (0.000052) ab	0.032 (0.014) abc	0.013 (0.0093) a	0.27 (0.37) ab	3.61 (2.40) a	0.12 (0.031) a	0.11 (0.011)	0.018 (0.019)	1.20 (0.12)	0.42 (0.083) b	0.076 (0.05) abcd
NA1_5038	0.0015 (0.00037) ab	0.083 (0.054) abc	0.26 (0.15) b	0.18 (0.05) ab	20.70 (4.18) b	0.36 (0.097) b	0.17 (0.098)	0.049 (0.059)	0.96 (0.47)	0.30 (0.099) ab	0.29 (0.26) bcd
NA1_5046	0.00062 (0.00059) ab	0.015 (0.0061) a	0.028 (0.011) a	0.046 (0.02) a	0.044 (0.03) a	0.17 (0.012) ab	0.14 (0.095)	0.018 (0.014)	0.54 (0.29)	0.19 (0.14) a	0.022 (0.01) ab
NA1_5054	0.00061 (0.00037) a	0.032 (0.0061) abc	0.033 (0.013) a	0.041 (0.02) a	5.58 (2.02) ab	0.23 (0.094) ab	0.12 (0.054)	0.34 (0.45)	1.54 (0.62)	0.38 (0.14) ab	0.0097 (0.01) a
NA2_5063	0.0029 (0.00096) ab	0.20 (0.11) bc	0.065 (0.015) ab	0.74 (0.52) ab	15.14 (8.52) b	0.36 (0.070) b	0.18 (0.054)	0.019 (0.01)	1.46 (0.36)	0.39 (0.071) ab	1.99 (0.79) d
NA2_5073	0.0015 (0.0012) ab	0.068 (0.042) abc	0.053 (0.018) ab	0.079 (0.05) a	4.54 (3.65) ab	0.28 (0.039) ab	0.19 (0.053)	0.014 (0.01)	0.87 (0.47)	0.36 (0.048) ab	0.27 (0.29) abcd
NA2_5074	0.00071 (0.00074) ab	0.031 (0.0082) ab	0.023 (0.0083) a	0.058 (0.04) a	4.89 (2.65) ab	0.31 (0.069) ab	0.14 (0.074)	0.26 (0.19)	1.16 (0.08)	0.30 (0.13) ab	0.15 (0.06) abcd
P	**0.004253**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.07158	0.05011	0.09755	**0.01296**	**<0.001**
Lineage											
EU1	0.0019 (0.0015)	0.090 (0.11) b	0.052 (0.065)	8.189 (12.12) b	5.62 (3.08)	0.14 (0.10) a	0.12 (0.058)	0.020 (0.026)	1.02 (0.33)	0.34 (0.92)	0.137 (0.14) ab
NA1	0.0010 (0.00074)	0.029 (0.021) a	0.035 (0.033)	0.084 (0.072) a	6.49 (9.55)	0.25 (0.13) ab	0.15 (0.086)	0.050 (0.072)	0.96 (0.69)	0.30 (0.13)	0.034 (0.045) a
NA2	0.0018 (0.0019)	0.068 (0.059) ab	0.050 (0.025)	0.104 (0.097) a	6.42 (7.70)	0.31 (0.06) b	0.17 (0.072)	0.024 (0.025)	1.16 (0.47)	0.37 (0.06)	0.307 (0.34) b
P	0.4984	**0.00818**	0.7503	**0.001147**	0.9953	**0.01968**	0.4787	0.1681	0.5444	0.09286	**<0.001**

[a] Isolate and clonal lineage EC_{50} values in columns for each chemical with different letters and *p*-values in bold are significantly different at $p = 0.05$ (Kruskal–Wallis test, Dunn's multiple comparisons).

but high for fenamidone (75%). However, on wounded plant material fenamidone provided more protection than mancozeb and chlorothalonil.

Differences in lesion size among isolates for each treatment was rarely significant. Lesion area for the two contact fungicides mancozeb and chlorothalonil did not differ from the untreated, except for the EU1 isolate, which had larger lesion area on the leaves treated with chlorothalonil than on untreated leaves (Table 6). On wounded leaves, lesion area caused by the EU1 isolate was smaller than from the NA1 and NA2 isolates in untreated and mancozeb treatments. There was no difference in lesion area among isolates in the remaining treatments. Lesion area was smaller on the unwounded leaves that became infected than on infected wounded leaves for all treatments (data not shown).

4. Discussion

4.1. Most effective fungicides for controlling P. ramorum

Best control of *P. ramorum* mycelial growth, zoospore germination, and infection of rhododendron foliage in this study was obtained with systemic fungicides. These included metalaxyl-M (also known as mefenoxam), dimethomorph, fenamidone, azoxystrobin, and pyraclostrobin. While these chemicals are very effective, they have a higher risk of resistance developing due to their single-site mode of action than do contact or protectant fungicides (Brent and Hollomon, 2007). Multi-site systemic fungicides and plant activators such as phosphite have a lower risk of resistance.

The most effective systemic fungicide for controlling *P. ramorum* was metalaxyl-M. This chemical is effective in controlling all life stages of *Phytophthora* spp., however there is a risk of resistance developing (Qi et al., 2012; Cohen and Samoucha, 1984; Gisi et al., 2000). The EC_{50} amounts for mycelial growth inhibition and zoospore germination inhibition were below the minimum recommended rate of 29 μL L^{-1} according to the product label. This chemical provided 100% control of both infection and lesion development *in planta*. Other studies have shown that metalaxyl-M is effective in reducing infection by *P. ramorum* in various nursery crops, although *P. ramorum* can be isolated from lesions (Shishkoff, 2005; Turner et al., 2006; Chastagner et al., 2010). This demonstrates that the chemical is fungistatic rather than fungicidal (Linderman and Davis, 2008; Tjosvold et al., 2008). However, this chemical should be used cautiously since metalaxyl-M resistant and tolerant isolates of *P. ramorum* have been found in the EU1 population (Wagner et al., 2008; Pérez-Sierra et al., 2011; Vercauteren et al., 2010).

Dimethomorph was almost as effective as metalaxyl-M in controlling *P. ramorum*. Median EC_{50} for all isolates was higher than for metalaxyl-M, for mycelial growth inhibition and for zoospore germination inhibition, similar to results obtained by Wagner et al. (2008). There was no difference in EC_{50} among isolates tested, showing that resistance to this chemical is not developing. On plants, dimethomorph provided a similar level of protection as metalaxyl-M, but *P. ramorum* was isolated less frequently from inoculation sites (Tjosvold et al., 2008).

There was no reduction of mycelial growth by fenamidone at any of the concentrations tested, but chlamydospore production was inhibited in cultures amended with >5 μL L^{-1} (data not shown). Two other fungicides in the strobilurin group (FRAC Group 11, quinone outside inhibitors), pyraclostrobin and azoxystrobin, were only tested for zoospore germination inhibition and had similar values to fenamidone. Two of the EU1 isolates had higher EC_{50} for zoospore germination inhibition with pyraclostrobin, suggesting that resistance to this chemical may be developing in the EU1 population (Table 4), and one EU1 and one NA2 isolate for

Table 5
Differences in infection frequency of three isolates of *Phytophthora ramorum* on detached leaves of Rhododendron 'Cunningham's White' treated with several fungicides at the manufacturer's labeled rate.[a]

	Fungicide	Unwounded			Wounded		
		EU1_5086	NA1_5046	NA2_5074	EU1_5086	NA1_5046	NA2_5074
Systemic	Metalaxyl-M (SM)	0% a	0% a	0% a	0% a	0% a	0% a
	Fenamidone (RE)	0% a	0% a	0% a	77% c	75% b	73% c
	Dimethomorph (AC)	0% a	0% a	0% a	13% b	2% a	3% b
Contact	Mancozeb (MA)	32% c	27% b	15% b	95% d	90% c	95% d
	Chlorothalonil (DA)	27% c	32% b	8% b	100% e	92% c	98% d
	Untreated	7% b	27% b	0% a	82% c	98% c	95% d
	P	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**

[a] Values for each isolate and wounding treatment with different letters in each column and *p*-values in bold are significantly different at $p = 0.05$. Chi-squared test followed by modified Tukey multiple comparisons for proportions.

azoxystrobin. In the study by Wagner et al. (2008), azoxystrobin was ineffective at the label rate on the EU1 isolates they tested, with mean EC_{50} for zoospore germination inhibition of 90.50 μL L^{-1}.

The strobilurin fungicide fenamidone also performed well on plants by preventing infection on unwounded leaves; however, wounded material became infected by *P. ramorum*. Lesion area by the EU1 isolate on wounded material treated with fenamidone was not significantly different from the untreated control leaves (Table 6). In tests on wounded rhododendron leaves, Linderman and Davis (2008) found no significant difference in lesion area for the control and the low dose of fenamidone, and the recommended rates of pyraclostrobin and azoxystrobin. They did not test unwounded leaves. There was no significant difference in lesion area between both wounded and unwounded rhododendron leaves treated with the strobilurins fenamidone and pyraclostrobin and those treated with dimethomorph and metalaxyl (Tjosvold et al., 2008).

Contact fungicides are considered protectants as they are thought to inhibit spore germination on the plant surface. The contact fungicides chlorothalonil, copper hydroxide, and mancozeb had higher EC_{50} values than the systemic fungicides for mycelial growth inhibition (with the exception of fosetyl-Al), and similar values to the systemic fungicides dimethomorph, pyraclostrobin, and propamocarb for zoospore germination inhibition. The contact fungicides tested on plants were much less effective in preventing infection and reducing lesion area by *P. ramorum* than the systemic fungicides.

4.2. EC_{50} for *P. ramorum* compared to other *Phytophthora* spp.

Overall, the EC_{50} for mycelial growth inhibition and zoospore germination inhibition of *P. ramorum* by fungicides with different chemistries/target sites was lower than for other *Phytophthora* spp. as reported in the literature (Coffey et al., 1984; Matheron and Porchas, 2000; Perez et al., 2009; Qi et al., 2012). This suggests that *P. ramorum* is a relatively new introduction to nurseries and landscapes and has not been exposed to these chemicals long enough to build up a resistant population. However, some resistance appears to be developing (Wagner et al., 2008).

For metalaxyl-M, EC_{50} of *P. ramorum* for mycelial growth inhibition was 0.013 μL L^{-1} and for zoospore germination inhibition was 0.002 μL L^{-1} (median of all isolates). None of the *P. ramorum* isolates tested in this study were resistant or tolerant to metalaxyl-M. We did not screen our entire collection for resistance to metalaxyl-M prior to this experiment, although this is the subject of a current study. Wagner et al. (2008) found the EC_{50} for mycelial growth inhibition ranged from 1.0 to >1000 μL L^{-1} and zoospore germination inhibition from 0.01 to >1000 μL L^{-1} in the population of EU1 isolates they tested. In a study of fungicide sensitivity of *Phytophthora capsici*, EC_{50} for metalaxyl-M ranged from 0.243 to 318 μL L^{-1} for mycelial growth inhibition (Qi et al., 2012). Matheron and Porchas (2000) found EC_{50} for mycelial growth inhibition for *P. capsici* to be 0.16 μL L^{-1} and for zoospore germination inhibition to be 32 μL L^{-1}. Similarly, EC_{50} for mycelial growth inhibition of *P. citrophthora* was <0.1 μL L^{-1} and 0.38 μL L^{-1} for *P. parasitica*, and zoospore germination inhibition of *P. citrophthora* was 34 μL L^{-1} and 280 μL L^{-1} for *P. parasitica*. In *P. infestans*, where metalaxyl-M resistance has been found at high rates, the EC_{50} for mycelial growth inhibition can be as high as 813 μL L^{-1} (Perez et al., 2009).

In this study, EC_{50} for mycelial growth inhibition of *P. ramorum* by fosetyl-Al was much higher than the recommended amount of 500 μg mL^{-1} a.i., so this chemical would not be effective against mycelial growth. This is not surprising since the chemical works by increasing plant defenses and not directly on the pathogen itself (Fenn and Coffey, 1984). In tests on other *Phytophthora* spp., EC_{50} for this chemical on mycelial growth inhibition was much lower than that for *P. ramorum* and ranged from 23.6 μg mL^{-1} (*P. citrophthora*) to 103 μg mL^{-1} (*P. capsici*), and from 317 μg mL^{-1} (P. capsici) to

Table 6
Median (± median absolute deviation, MAD) lesion area of three isolates of *Phytophthora ramorum* on detached leaves of Rhododendron 'Cunningham's White' treated with several fungicides at the manufacturer's labeled rate.[a]

	Fungicide	Unwounded			Wounded		
		EU1_5086	NA1_5046	NA2_5074	EU1_5086	NA1_5046	NA2_5074
Systemic	Metalaxyl-M (SM)	No infection	No infection	No infection	No infection	No infection	No infection
	Fenamidone (RE)	No infection	No infection	No infection	230.03 (130.67) c	289.82 (202.17) a	244.71 (113.69) a
	Dimethomorph (AC)	No infection	No infection	No infection	91.45 (111.94) a	133.17 (0) a	273.94 (40.38) a
Contact	Mancozeb (MA)	114.23 (130.48)	243.84 (216.98)	115.68 (147.44)	295.07 (112.60) d	309.96 (157.14) b	381.16 (128.49) b
	Chlorothalonil (DA)	148.74 (154.46)	183.49 (98.69)	296.4 (109.24)	421.43 (210.78) e	379.52 (122.88) c	389.01 (194.43) b
	Untreated	75.28 (41.05)	136.36 (70.64)	No infection	214.42 (124.98) b	463.87 (183.96) c	327.07 (157.61) b
	P	0.541	0.078	0.161	**<0.001**	**<0.001**	**<0.001**

[a] Values with different letters and *p*-values in bold in each column are significantly different at $p = 0.05$. Kruskal–Wallis test, Dunn's multiple comparison procedure.

>1000 μg mL^{-1} (*P. parasitica*) for zoospore germination inhibition (Matheron and Porchas, 2000).

We assume that infection on leaves occurred primarily through mycelial growth since inoculation was done using mycelial plugs, rather than zoospore suspensions, although sporangia and chlamydospores were also present. Greenhouse conditions were not suitable for zoospore release and germination, so it is likely that sporangia germinated directly into mycelium. EC_{50} for all isolates was below the recommended levels calculated from the label rates for mycelial growth inhibition except for fosetyl-Al, propamocarb, and fenamidone, which were ineffective in reducing mycelial growth at all concentrations tested. However, fenamidone was effective in preventing infection on unwounded plant material when applied at the recommended concentration.

4.3. Cross-resistance

Fungicide resistance is defined to be the likelihood of resistance developing to the extent that causes failure of disease control in the field, rather than detecting resistant isolates at low levels or experimentally inducing resistance (Brent and Hollomon, 2007). Still, laboratory studies and monitoring of field situations provides information about the potential for resistance to a chemical to develop in a pathogen, and better examination of the mechanism of action. The risk of resistance to chemical fungicides depends on a number of factors, including mode of action of the chemical, biology of the target organism, and patterns of usage in the nursery or field. Some chemicals, such as those that target a single site of action, have higher potential of resistance developing than others and these should be used cautiously. Furthermore, pathogens can develop cross-resistance among chemicals of the same class, and these pathogens are likely to be resistant to new chemicals in that class that are created. This was observed for *P. ramorum* in this study with the strobilurin fungicides fenamidone and azoxystrobin.

5. Conclusions

In this study, systemic fungicides were the most effective against mycelial growth and zoospore germination of *P. ramorum*, and the results from testing on host plants at the labeled rate supported the *in vitro* results. Development of resistance to some chemicals should be monitored, especially in the NA2 and EU1 populations. In addition, there is an urgent need to assess the effects of fungicides on the most recent discovery of a fourth evolutionary EU2 lineage of *P. ramorum* in the U.K (Van Poucke et al., 2012). In Canada, monitoring *P. ramorum* by the CFIA has thus far avoided establishment and spread of *P. ramorum* to natural ecosystems.

Acknowledgments

This research work was supported by the Canadian Forest Service – Forest Invasive Alien Species and Phytosanitary research program and by the Natural Sciences and Engineering Research Council of Canada (NSERC). We acknowledge the support by the Canadian Food Inspection Agency. The technical support by Robert Kowbel is greatly appreciated. Isolates of *P. ramorum* used in this study were kindly provided by Nik Grünwald, USDA ARS, Corvallis, OR. Furthermore, the authors would like to thank the reviewers for their helpful comments and suggestions of this article. Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the Natural Resources Canada, Canadian Forest Service and does not imply its approval to the exclusion of other products or vendors that also may be suitable.

References

Agrios, G.N., 2005. Plant Pathology, fifth ed. Elsevier Academic Press, New York.
Alexander, B., Browse, D.J., Reading, S.J., Benjamin, I.S., 1999. A simple and accurate mathematical method for calculation of the EC_{50}. J. Pharmacol. Toxicol. 41, 55–58.
APHIS- PPQ, 2013. *Phytophthora ramorum*/Sudden oak death. http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/index.shtml (accessed 09.08.14.).
Brasier, C.M., 2008. The biosecurity threat to the UK and global environment from international trade in plants. Plant Pathol. 57, 792–808.
Brasier, C.M., Webber, J., 2010. Sudden larch death. Nature 466, 824–825.
Brent, K.J., Hollomon, D.W., 2007. Fungicide Resistance: the Assessment of Risk. FRAC Monograph No. 2 second (revised) ed..
CFIA, 2013. D-08-04: Plant protection import requirements for plants and plant parts for planting, 2nd revision. http://www.inspection.gc.ca/plants/plant-protection/directives/imports/d-08-04/eng/1323752901318/1323753560467#c2 (accessed 09.08.14.).
Chastagner, G.A., DeBauw, A., Riley, K., Dart, N., 2008. Effectiveness of fungicides in protecting conifers and rhododendrons from *Phytophthora ramorum*. In: Frankel, S.J., Kliejunas, J.T., Palmieri, K.M. (Eds.), Proceedings of the Sudden Oak Death Third Science Symposium. US Department of Agriculure, Forest Service, Pacific Southwest Research Station, Albany, CA, pp. 325–333. General Technical Report PSW-GTR-214.
Chastagner, G.A., DeBauw, A., Riley, K., 2010. Effect of fungicides on the isolation of *Phytophthora ramorum* from symptomatic and asymptomatic rhododendron leaf tissue. In: Frankel, S.J., Kliejunas, J.T., Palmieri, K.M. (Eds.), Proceedings of the Sudden Oak Death Fourth Science Symposium. US Department of Agriculture, Forest Service, Pacific Southwest Research Station, Albany, CA, pp. 302–304. General Technical Report PSW-GTR-214.
Chastagner, G.A., Riley, K., Elliott, M., 2013. Susceptibility of larch, hemlock, Sitka spruce, and Douglas-fir to *Phytophthora ramorum*. In: Frankel, S.J., Kliejunas, J.T., Palmieri, K.M., Alexander, J.M. (Eds.), Proceedings of the Sudden Oak Death Fifth Science Symposium. U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station, Albany, CA, pp. 77–79. General Technical Report PSW-GTR-243.
Coffey, M.D., Klure, L.J., Bower, L.A., 1984. Variability in sensitivity to metalaxyl of isolates of *Phytophthora cinnamomi* and *Phytophthora citricola*. Phytopathology 74, 417–422.
Coffey, M.D., Bower, L.A., 1984. *In vitro* variability among isolates of six *Phytophthora* species in response to metalaxyl. Phytopathology 74, 502–506.
Coffey, M.D., Joseph, N.C., 1985. Effect of phosphorous acid and fosetyl-Al on life cycle of *Phytophthora cinnamomi* and *P. citricola*. Phytopathology 75, 1042–1046.
Cohen, Y., Coffey, M.D., 1986. Systemic fungicides and the control of Oomycetes. Annu. Rev. Phytopathol. 24, 311–338.
Cohen, Y., Samoucha, Y., 1984. Cross-resistance to four systemic fungicides in metalaxyl-resistant strains of *Phytophthora infestans* and *Pseudoperonospora cubensis*. Plant Dis. 68, 137–139.
Elliott, M., Sumampong, G., Varga, A., Shamoun, S.F., James, D., Masri, S., Briere, S.C., Grünwald, N.J., 2009. PCR-RFLP markers identify three lineages of North American and European populations of *Phytophthora ramorum*. For. Pathol. 39, 266–278.
Elliott, M., Sumampong, G., Varga, A., Shamoun, S.F., James, D., Masri, S., Grünwald, N.J., 2011. Phenotypic differences among three clonal lineages of *Phytophthora ramorum*. Forest Pathol. 41, 7–14.
Erwin, D.C., Ribeiro, O.K., 1996. *Phytophthora* Diseases Worldwide. American Phytopathological Society Press, St. Paul, MN, USA, 562 pp.
Fenn, M.E., Coffey, M.D., 1984. Studies on the *in vitro* and *in vivo* antifungal activity of fosetyl-Al and phosphorous acid. Phytopathology 74, 606–611.
Ferguson, A.J., Jeffers, S.N., 1999. Detecting multiple species of *Phytophthora* in container mixes from ornamental crop nurseries. Plant Dis. 83, 1129–1136.
Ferrin, D.M., Kabashima, J.N., 1991. *In vitro* insensitivity to metalaxyl of isolates of *Phytophthora citricola* and *P. parasitica* from ornamental hosts in southern California. Plant Dis. 75, 1041–1044.
Garbelotto, M., Schmidt, D.J., Harnik, T.Y., 2007. Phosphite injections and bark application of phosphite + Pentrabark™ control sudden oak death in coast live oak. Arboric. Urban For. 33, 309–317.
Gisi, U., Chin, K.M., Knapova, G., Küng Färber, R., Mohr, U., Parisi, S., Sierotzki, H., Steinfeld, U., 2000. Recent developments in elucidating modes of resistance to phenylamide, DMI, and strobilurin fungicides. Crop Prot. 19, 863–872.
Goheen, E.M., Kanaskie, A., Parke, J., Roth, M., Osterbauer, N., Trippe, A., 2006. Applications of fungicides to protect four hosts from foliar infection by *Phytophthora ramorum* in Curry County, Oregon. In: Frankel, S.J., Shea, P.J., Haverty, M.I. (Eds.), Proceedings of the Sudden Oak Death Second Science Symposium: the State of Our Knowledge. US Department of Agriculture, Forest Service, Pacific Southwest Research Station, Albany, CA, pp. 513–514. General Technical Report PSW-GTR-196.
Goss, E.M., Larsen, M., Chastagner, G.A., Givens, D.R., Grünwald, N.J., 2009. Population genetic analysis infers migration pathways of *Phytophthora ramorum* in US nurseries. PLoS Pathog. 5 (9), e1000583. http://dx.doi.org/10.1371/journal.ppat.1000583.
Goss, E.M., Larsen, M., Vercauteren, A., Werres, S., Heungens, K., Grünwald, N.J., 2011. *Phytophthora ramorum* in Canada: evidence for migration within North America and from Europe. Phytopathology 101, 166–171.

Grünwald, N.J., Goss, E.M., Press, C.M., 2008. *Phytophthora ramorum*: a pathogen with remarkably wide host range causing sudden oak death on oaks and ramorum blight on woody ornamentals. Mol. Plant Pathol. 9, 729–740.

Grünwald, N.J., Garbelotto, M., Goss, E.M., Heungens, K., Prospero, S., 2012. Emergence of the sudden oak death pathogen *Phytophthora ramorum*. Trends Microbiol. 20, 131–138.

Guest, D.I., Pegg, K.C., Whiley, A.W., 1995. Control of *Phytophthora* diseases of tree crops using trunk-injected phosphonates. Hortic. Rev. 17, 299–330.

Heungens, K., De Dobbelaere, I., Maes, M., 2006. Fungicide control of *Phytophthora ramorum* on rhododendron. In: Frankel, S.J., Shea, P.J., Haverty, M.I. (Eds.), Proceedings of the Sudden Oak Death Second Science Symposium: the State of Our Knowledge. US Department of Agriculture, Forest Service, Pacific Southwest Research Station, Albany, CA, pp. 241–257. General Technical Report PSW-GTR-196.

Jackson, T.J., Burgess, T., Colquhoun, I., Hardy, G.E. St. J., 2000. Action of the fungicide phosphite on Eucalyptus marginata inoculated with *Phytophthora cinnamomi*. Plant Pathol. 49, 147–154.

Jeffers, S.N., 2003. Fungicides for managing *Phytophthora* species in nurseries. Sudden Oak Death Online Symposium. www.apsnet.org/online/SOD (website of the American Phytopathological Society).

Kliejunas, J.T., 2010. Sudden Oak Death and *Phytophthora ramorum*: a Summary of the Literature. US Department of Agriculture, Forest Service, Pacific Southwest Research Station. General Technical Report PSW-GTR-234, 181 pp.

Kristjansson, G.T., Miller, S.J., 2009. Pest risk assessment of *Phytophthora ramorum* Werres, de Cock & Man in't Veld Causal Agent of Ramorum Blight, Ramorum Bleeding Canker, Ramorum (shoot) Dieback and Sudden Oak Death. Canadian Food Inspection Agency. PRA 2000-39 Version 4.

Kuhajek, J.M., Jeffers, S.N., Slattery, M., Wedge, D.E., 2003. A rapid microbioassay for discovery of novel fungicides for *Phytophthora* spp. Phytopathology 93, 46–53.

Lamari, L., 2002. ASSESS Image Analysis Software for Plant Disease Quantification. The American Phytopathological Society Press, St. Paul, Minnesota, USA.

Liebhold, A.M., Brockerhoff, E.G., Garrett, L.J., Parke, J.L., Britton, K.O., 2012. Live plant imports: the major pathway for forest insect and pathogen invasions of the US. Front. Ecol. Environ. 135–143. http://dx.doi.org/10.1890/110198.

Linderman, R.G., Davis, E.A., 2008. Evaluation of chemical agents for the control of *Phytophthora ramorum* and other species of *Phytophthora* on nursery crops. Online Plant Health Progr. http://dx.doi.org/10.1094/PHP-2008-0211-01-RS.

Matheron, M.E., Porchas, M., 2000. Impact of azoxystrobin, dimethomorph, fluazinam, fosetyl-Al, and metalaxyl on growth, sporulation, and zoospore cyst germination of three *Phytophthora* spp. Plant Dis. 84, 454–458.

McCarren, K.L., McComb, J.A., Shearer, B.L., Hardy, G.E. St. J., 2009. *In vitro* influence of phosphite on chlamydospore production and viability of *Phytophthora cinnamomi*. For. Pathol. 39, 210–216.

Orlikowski, L.B., 2004. Chemical control of rhododendron twig blight caused by *Phytophthora ramorum*. J. Plant Prot. Res. 44, 41–46.

Perez, W., Lara, J., Forbes, G.A., 2009. Resistance to metalaxyl-M and cymoxanil in a dominant clonal lineage of *Phytophthora infestans* in Huanuco, Peru, an area of continuous potato production. Eur. J. Plant Pathol. 125, 87–95.

Pérez-Sierra, A., Álvarez, L.A., Vercauteren, A., Heungens, K., Abad-Campos, P., 2011. Genetic diversity, sensitivity to phenylamide fungicides, and aggressiveness of *Phytophthora ramorum* on *Camellia, Rhododendron*, and *Viburnum* plants in Spain. Plant Pathol. 60, 1069–1076.

Pest Management Regulatory Agency (PMRA) - Health Canada, 2013. Pesticides and pest management. http://www.hc-sc.gc.ca/cps-spc/pest/index-eng.php (accessed 09.08.14.).

Qi, R., Wang, T., Zhao, W., Li, P., Ding, J., Gao, Z., 2012. Activity of ten fungicides against *Phytophthora capsici* isolates resistant to metalaxyl. J. Phytopathol. 160, 717–722.

R Development Core Team, 2011. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria, ISBN 3-900051-07-0. URL: http://www.R-project.org/ (accessed 09.08.14.).

Revelle, W., 2013. Psych: procedures for Personality and Psychological research. Northwestern University, Evanston, Illinois, USA. http://CRAN.R-project.org/package=psych. Version = 1.3.2 (accessed 09.08.14.).

Riedel, M., Werres, S., Elliott, E., McKeever, K., Shamoun, S.F., 2012. Histopathological investigations of the infection process and propagule development of *Phytophthora ramorum* on rhododendron leaves. Forest Phytophthoras 2 (1). http://dx.doi.org/10.5399/osu/fp.2.1.3036 (accessed 09.08.14.).

Rizzo, D.M., Garbelotto, M., 2003. Sudden oak death: endangering California and Oregon forest ecosystems. Front. Ecol. Environ. 1, 197–204.

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

Shishkoff, N., 2005. The effect of systemic fungicides on detection by culturing of *Phytophthora ramorum*. Phytopathology 95, S96 (Abstract).

Stein, J.M., Kirk, W., 2002. Containment of existing potato late blight (*Phytophthora infestans*) foliar epidemics with fungicides. Crop Prot. 21, 575–582.

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

Turner, J., Jennings, P., McDonough, S., Liddell, D., Stonechouse, J., 2006. Chemical control of *Phytophthora ramorum* causing foliar disease in hardy nursery stock in the United Kingdom. In: Frankel, S.J., Shea, P.J., Haverty, M.I. (Eds.), Proceedings, Sudden Oak Death Second Science Symposium: the State of Our Knowledge. US Department of Agriculture, Forest Service, Pacific Southwest Research Station, Albany, CA, pp. 273–274. General Technical Report PSW-GTR-196.

Turner, J., Jennings, P., Werres, S., Wagner, S., 2008. Report response of isolates of *Phytophthora ramorum* exposed to fungicides commonly used to control *Phytophthora* diseases in nurseries. Deliverable Report 5. Forest Research, Central Sciences Laboratory, San Hutton, York, UK. EU sixth Framework Project, RAPRA.

Van Poucke, K., Franceschini, S., Webber, J.F., Vercauteren, A., Turner, J.A., Mccracken, A.R., Heungens, K., Brasier, C.M., 2012. Discovery of a fourth evolutionary lineage of *Phytophthora ramorum*: EU2. Fungal Biol. 116, 1178–1191.

Vercauteren, A., De Dobbelaere, I., Grünwald, N.J., 2010. Clonal expansion of the Belgian *Phytophthora ramorum* populations based on new microsatellite markers. Mol. Ecol. 19, 92–107.

Wagner, S., Kaminski, K., Werres, S., 2008. Evaluation of fungicides for control of *Phytophthora ramorum*. In: Frankel, S.J., Kliejunas, J.T., Palmieri, K.M. (Eds.), Proceedings of the Sudden Oak Death Third Science Symposium. US Department of Agriculure, Forest Service, Pacific Southwest Research Station, Albany, CA, pp. 481–482. General Technical Report PSW-GTR-214.

Werres, S., Marwitz, R., Man in't Veld, W.A., 2001. *Phytophthora ramorum sp. nov*: a new pathogen on *Rhododendron* and *Viburnum*. Mycol. Res. 105, 1155–1165.

Wilkinson, C.J., Shearer, B.L., Jackson, T.J., Hardy, G.E. St. J., 2001. Variation in sensitivity of Western Australian isolates of *Phytopthora cinnamomi* to phosphite *in vitro*. Plant Pathol. 50, 83–89.