In Vitro Testing of Biological Control Agents on A1 and A2 Isolates of *Phytophthora ramorum*[1]

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Abstract

Biological control products were tested *in vitro* with six isolates of *Phytophthora ramorum*. These isolates were geographically diverse and were selected based on their pathogenicity to detached Rhododendron leaves. In addition to five commercially available biocontrol products, nine species of *Trichoderma* were tested. The *in vitro* tests included dual culture methods and detached *Rhododendron* leaf assays. Best results were obtained with the actinomycete *Streptomyces lydicus* (Actinovate®) in both culture and detached leaf tests. There were differences among *P. ramorum* isolates in their sensitivity to the biocontrol agents tested.

Key words: *Phytophthora ramorum*, *Trichoderma* spp., *Streptomyces*, biological control.

Introduction

Phytophthora ramorum, the Oomycete causing sudden oak death, is a problem in wildlands and nurseries (Garbelotto 2004). Biological control methods are being sought as an alternative to chemical fungicide use in nurseries and landscape plantings. Several organisms have been demonstrated to be effective against Oomycetes, such as species of the fungus *Trichoderma*, the bacterium *Bacillus*, and the actinomycete *Streptomyces*. Commercially available products containing these biological control agents exist but have not been tested with *P. ramorum*.

The objective of this study was to evaluate fungal and bacterial antagonists to *P. ramorum in vitro* and on detached leaves of *Rhododendron*. In addition, variability in response to biocontrol agents among isolates of *P. ramorum* belonging to different mating types and from North American and European populations was examined.

Methods

Based on preliminary screening of pathogenicity to *Rhododendron* and growth rates at various temperatures, six isolates of *P. ramorum* were selected for screening of biocontrol agents (BCAs) (table 1).

To test the efficacy of biocontrol products in controlling *P. ramorum*, five commercially available biocontrol products were tested *in vitro* (table 2). In addition, several isolates of *Trichoderma, Penicillium*, and bacteria were tested. Antibiotic production was estimated based on the size of the zone of inhibition (Rajkumar and

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others 2005). For products containing *Trichoderma*, mycoparasitic activity was assessed (Goldfarb and others 1989).

Detached leaves of *Rhododendron* were treated with BCAs at manufacturers recommended concentrations in 200 ml sterile distilled (sd) H_2O and incubated 24 hours in a plastic box containing moist sterile vermiculite in the dark at 20°C. The following day leaves were wounded with forceps next to midrib about and a 7mm plug of *P. ramorum* inoculum placed mycelium side down over the wounded area on the abaxial (under) side. Leaves were sprayed with sdH2O and incubated at 20°C in the dark for 14 days. At the end of the incubation period, percent lesion area was measured using a flatbed scanner and the program ASSESS (Lamari 2002). Percent lesion area was compared to controls not treated with the BCAs.

Table 1—Isolates of *Phytophthora ramorum* selected for the biocontrol study

Isolate	Strain Number	Pathogenicity[a]	MT	Origin	Location
5067	Pr 106	6.51	A2	Forest	CA
5073	RHCC-23	31.09	A2	Nursery	CA
5074	RHCC-4	34.28	A2	Nursery	CA
5039	03-74-D12-A	43.59	A1	Nursery	OR
5084	CSL2266, BBA9/95	32.20	A1	Nursery	EU
5086	CSL2268	31.71	A1	Nursery	EU

[a]Percent lesion area on detached *Rhododendron* leaves after 14 days.

Table 2—Biocontrol products

Name	Organism	Company
Serenade	*Bacillus subtilis* QST 713	Agraquest
Plant Helper	*Trichoderma atroviride* CHS 861	Ampac
Actinovate	*Streptomyces lydicus* WYEC 108	Natural Industries Inc
Companion	*Bacillus subtilis* GB03, other *B. subtilis, B. lichenformis, B. megaterium*	Growth Products
Soilgard	*Gliocladium virens* strain GL-21	Certis

Results and Discussion

Preliminary results indicate that preparations containing *Bacillus subtilis* are very effective in controlling *P. ramorum in vitro* (fig. 1), but best results were obtained with Actinovate® (*Streptomyces lydicus* WYEC 108) on leaves. There was great variability among *P. ramorum* isolates in their behavior with biocontrol agents, both *in vitro* and on detached leaves.

Bacterial biocontrol agents are more effective against *P. ramorum* on leaves than *Trichoderma* (fig. 2). *Trichoderma* may be more effective in controlling the soil phase, or perhaps better formulations of *Trichoderma* are needed for foliar application. Plant Helper (*T. atroviride*) performed very well in *in vitro* tests (table 3), but poorly on detached leaves.

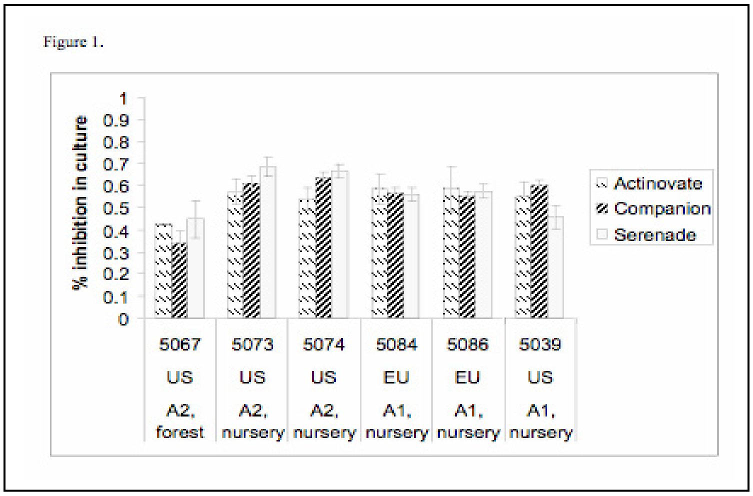

Figure 1—*In vitro* tests of commercial biocontrol agents with six isolates of *P. ramorum*. Percent inhibition of mycelial growth relative to a control plate containing no biocontrol agent is shown.

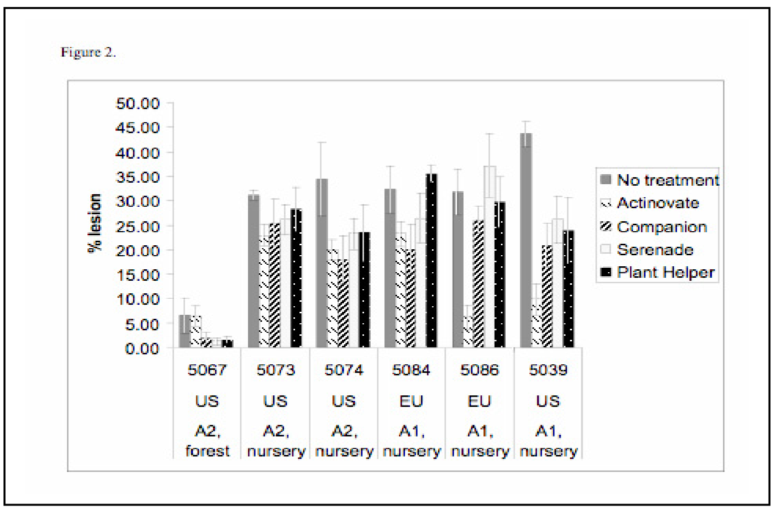

Figure 2—Results of *Rhododendron* leaf tests with four commercial biocontrol agents and an untreated control. Percent lesion area for each treatment is shown. Information about these biocontrol agents can be found in table 2.

Table 3—Percent inhibition of six *P. ramorum* isolates by several *Trichoderma* species

Isolate	Species	A2 5067	A2 5074	A2 5073	A1 5084	A1 5086	A1 5039
PH	*atroviride*	1.00	1.00	1.00	1.00	1.00	1.00
5005	*hamatum*	0.06	0.20	0.26	0.00	0.09	0.00
5006	*hamatum*	0.12	0.61	0.30	0.96	0.96	0.96
5092	*hamatum*	1.00	0.09	0.10	0.25	0.25	0.23
5097	*harzianum*		0.27	0.28		0.22	0.23
5022	*koningii*	0.95	0.57	0.45	1.00	1.00	1.00
5031	*koningii*	1.00	1.00	1.00	1.00	1.00	1.00
5101	*koningii*	0.45	0.07	0.10	0.29	0.29	0.30
5002	*polysporum*	1.00	1.00	1.00	1.00	1.00	1.00
5034	*polysporum*	0.95	0.57	0.37	0.51	0.68	0.89
5093	*pseudokoningii*		0.04	0.00	0.11	0.00	0.02
5094	*saturnisporium*	0.95	0.00	0.02	0.12	0.19	0.00
5095	*virens*	0.00	0.12	0.16	0.00	0.00	0.07
5098	*virens*	0.41	0.06	0.17	0.21	0.26	0.25
5102	*virens*	0.99	0.55	0.57	0.53	0.96	0.48
5020	*viride*	1.00	1.00	1.00	1.00	1.00	1.00
5023	*viride*	1.00	0.91	1.00	1.00	1.00	1.00
5037	*viride*	1.00	1.00	1.00	1.00	1.00	1.00

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