



Sudden Oak Death (*Phytophthora ramorum*): Research up-date, risk analysis and mitigation approach for Canada

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***Phytophthora*- the plant destroyer**

Potato blight – *Phytophthora infestans*

Irish Famine 1845





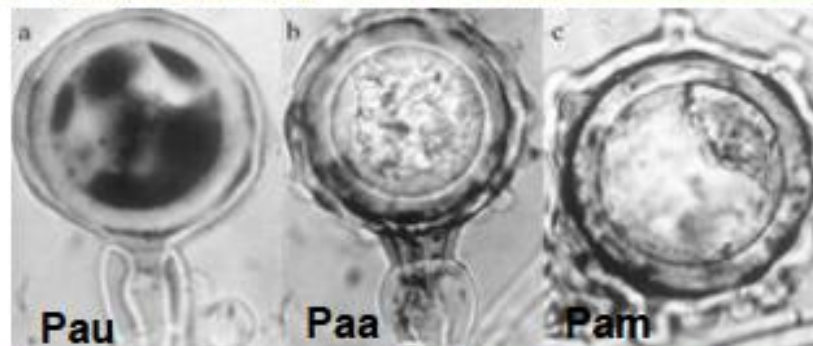
Emerging *Phytophthora* spp. worldwide



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Phytophthora pinifolia on radiata pine in Chile

Octubre 2004

Young plantation (damage 2004-2006)



Bioforest S.A./División Protección Fitosanitaria



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Photo from R. Ahumada et al.

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Adult plantation (Foliage damaged 2006)

Photo from R. Ahumada et al.



P. cinnamomi on Fraser fir (Christmas tree) in North Carolina, USA



- Symptoms of chlorosis, wilting, necrosis and mortality of Fraser fir due to *Phytophthora* root rot.

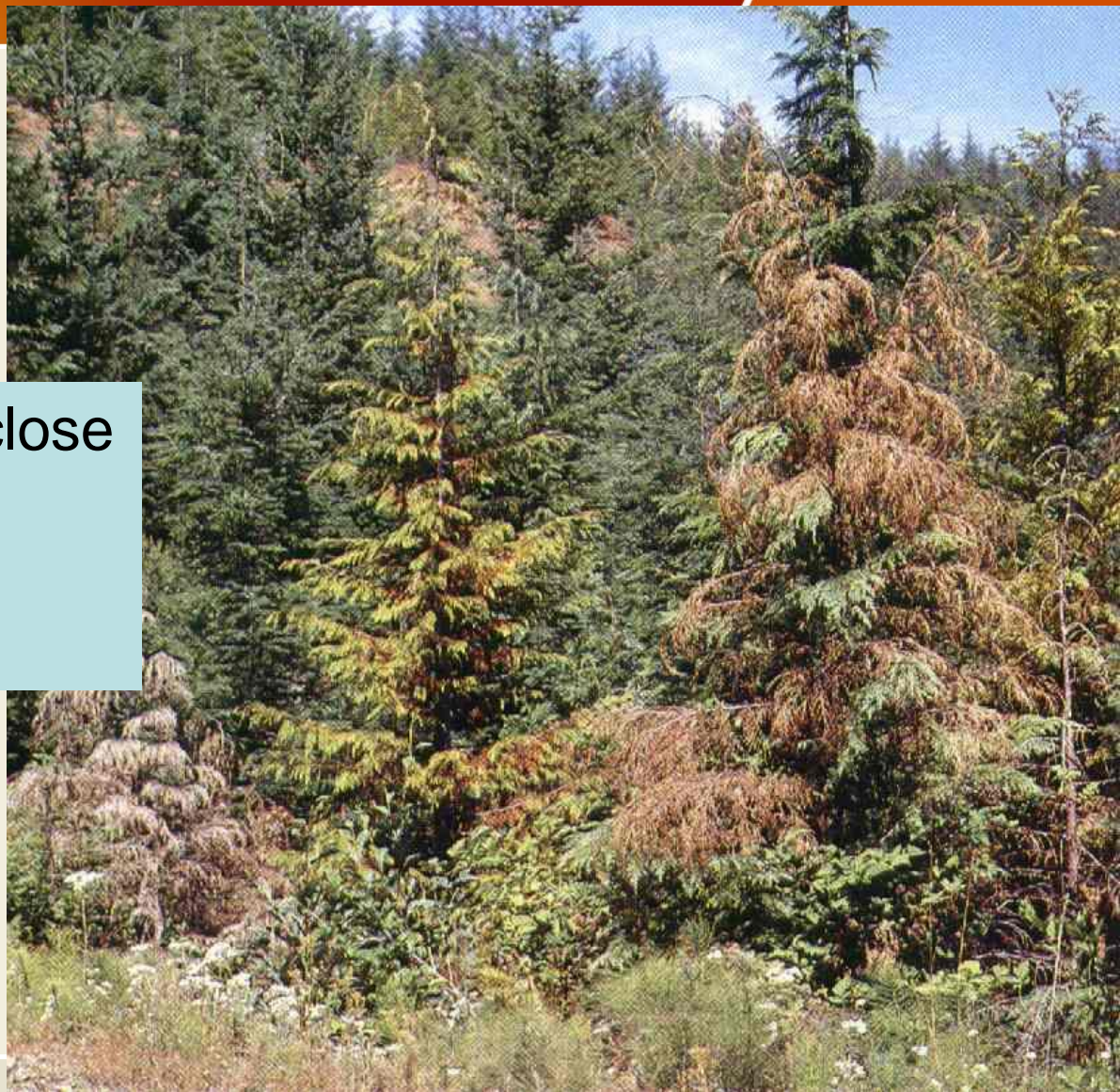




Phytophthora lateralis

P. lateralis

P. lateralis (genetically close to *P. ramorum*) on Port Orford cedar in Oregon, USA.





SOD VS. SLD

Japanese Larch (*Larix kaempferi*): 600,000 trees

(California- SOD)



(England- SLD)



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What is Sudden Oak Death (SOD)?

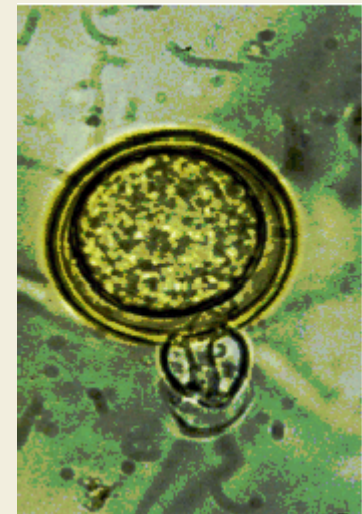
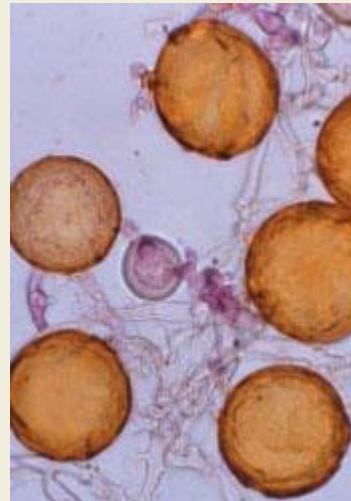
- A quarantine organism with serious regulatory consequences
- Caused by *Phytophthora ramorum*
- In coastal CA forests & southwest OR, nurseries in North America and Europe





Phytophthora spore stages

- *Phytophthora* thrives in wet conditions
- *P. ramorum* infections initiated by sporangia and zoospores
- Chlamydospores can persist in soil and leaf litter
- Sexual oospores are resistant to damage





Costs of SOD to BC Nursery Industry

- Value \$500 million with \$170 million in export sales to US
- Cost for eradication is estimated to be \$8.5 million
- South coastal BC risk is considered to be medium (CFIA 2011)





Sudden Oak Death (SOD)

- First seen in early 1990s
- Two types of symptoms:
 - Canker
 - Foliar blight
- Host range: more than 120 plant hosts
- Most of the outbreak in central coastal CA & Southwest Oregon

Photos by Joseph O'Brien, USDA Forest Service, United States

Website: www.forestryimages.org



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SOD Hosts in Canada

- Maples
- Oaks
- Douglas-fir
- Understory vegetation (e.g. Salal, Kalmia, Vaccinium)
- Horticultural plants —
 - Rhododendrons
 - Camellias



Vine maple



Arbutus



Salal



Douglas-fir





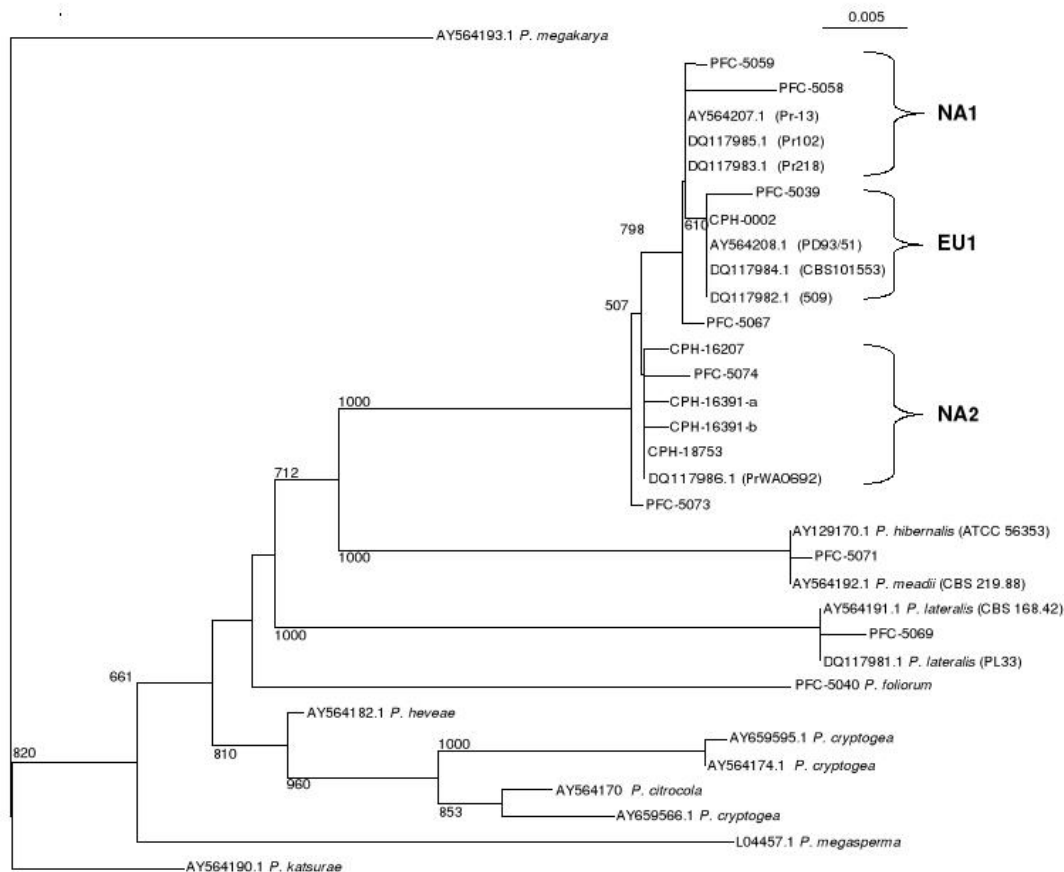
Research Objectives:

- To identify populations of *P. ramorum* and closely related species using PCR-RFLP DNA markers
- To screen several fungicides with different modes of action for effectiveness on three life stages of *P. ramorum*
- To evaluate bacterial and fungal antagonists to *P. ramorum in vitro* and on detached leaves. Also, to evaluate the efficacy of Chontrol®- *C. purpureum* for control of tanoak resprouts- field trials in southwest Oregon.
- To assess susceptibility of six eastern Canadian forest tree species to *P. ramorum* infection





Separation of 3 distinct *P. ramorum* lineages



- It is possible to identify which lineage an isolate of *P.r.* belongs to using PCR-RFLP of the *Cox1* gene
- First using *Apo1* to separate *P.r.* from other species and EU1 from North American populations
- And then *Ava1* to distinguish between NA1 and NA2 lineages

Combining the results of the two PCR-RFLP we can construct a tree that separates the 3 lineages of *P. ramorum*





Effects of Fungicides on *P. ramorum* -*In vitro* tests

Name	Active ingredient	Recommended dose, ppm	EC50, mycelium, 1 week	EC50, mycelium, 4 weeks	EC50, chlamydospores	EC50, zoospores
Acrobat	Dimethomorph	25-50	0.14 (Y)	0.41 (Y)	0.24 (Y)	9 (Y)
Aliette	Fosetyl-Al	79-495	1567 (N)	1944 (N)	355 (Y)	> 1000 (N)
Daconil	Chlorothalonil	101-511	9.55 (Y)	Variable	222 (Y)	54 (Y)
Kocide 2000	Copper hydroxide	135	35 (Y)	117 (Y)	34 (Y)	82 (Y)
Manzate	Mancozeb	135-372	34 (Y)	67 (Y)	3 (Y)	4 (Y)
Previcur N	Propamocarb	129-155	> 10,000 (N)	> 10,000 (N)	161 (N)	37 (Y)
Reason	Fenamidone	27-108	>1000 (N)	>1000 (N)	0.41 (Y)	70 (Y)
Truban	Etridiazole	15-28	1.5 (Y)	>1000 (N)	28 (N)	3.34 (Y)
Subdue Maxx	Mefanoxam	0.43-4.4	0.028 (Y)	0.027 (Y)	0.007 (Y)	0.083 (Y)

- EC50 values are given for percent inhibition of mycelial growth after one week and four weeks, chlamydospore production after four weeks, and zoospore germination after 48 hours.
- Fungicides are considered to be effective if their EC₅₀ value is less than the recommended dosage





Summary – Fungicide Results

- There were differences between *P. ramorum* isolates and lineages in their response to treatment with chemical fungicides
- Chlamydospore production increased then decreased at higher concentrations for some fungicides
- The contact fungicides Manzate and Kocide both worked well on all spore stages but they are broad-spectrum and kill beneficial fungi and bacteria
- The systemic fungicides Acrobat and Subdue Maxx controlled all spore stages at low concentrations and are oomycete specific, but resistance was seen to develop in some isolates
- Subdue Maxx most effective on all 3 life stages but problem with resistance on EU lineage reported





Effect of Biocontrol on *P. ramorum* -Percent inhibition of *P. ramorum* mycelial growth relative to untreated control

P. ramorum population

Biocontrol Product	All	NA1	NA2	EU1
Untreated	0.00	0.00	0.00	0.00
Companion	0.39	0.65	0.33	0.38
Serenade	0.37	0.64	0.28	0.38
Actinovate	0.29	0.30	0.31	0.31
Plant Helper	1.00	1.00	1.00	1.00
SoilGard	0.31	0.24	0.27	0.31

- Both *Bacillus subtilis* products (Companion and Serenade) inhibited NA1 better than NA2 and EU1
- Actinovate *Streptomyces lydicus* performed the least well of the 3 bacterial BCAs *in vitro*
- *T. atroviride* (Plant Helper) caused 100% inhibition of all lineages
- *T. virens* (Soil Gard) was only about 30% effective.

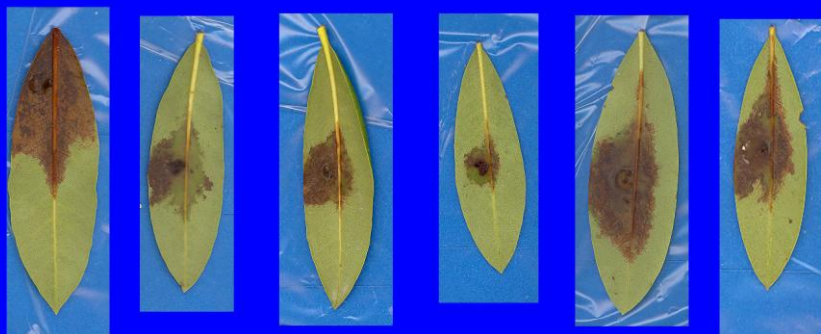




In vitro leaf test

Rhododendron

Biocontrol agents for *Phytophthora ramorum* on Rhododendron foliage



Untreated

Companion

Bacillus subtilis GB03,
other *B. subtilis*, *B.*
lichenformis, *B.*
megaterium

Serenade

Bacillus subtilis
QST 713

Actinovate

Streptomyces
lydicus WYEC
108

Soilgard

Gliocladium
virens strain
GL-21

Plant
Helper

Trichoderma
atroviride CHS
861

P. ramorum NA2 genotype PFC 5074

Camellia

Biocontrol agents for *Phytophthora ramorum* on Camellia foliage



Untreated

Companion

Bacillus subtilis GB03,
other *B. subtilis*, *B.*
lichenformis, *B.*
megaterium

Serenade

Bacillus subtilis
QST 713

Actinovate

Streptomyces
lydicus WYEC
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Soilgard

Gliocladium
virens strain
GL-21

Plant
Helper

Trichoderma
atroviride CHS
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P. ramorum NA2 genotype PFC 5074



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Differences in percent lesion area among biocontrol treatments for each *P. ramorum* genotype on detached rhododendron and camellia leaves.

<i>P. ramorum</i> population	Rhododendron				Camellia			
	All	NA1	NA2	EU1	All	NA1	NA2	EU1
Untreated	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Companion	0.40	0.42	0.50	0.49	0.00	0.00	0.00	0.47
Serenade	0.45	0.76	0.54	0.54	0.43	0.29	0.52	0.59
Actinovate	0.67	0.40	0.78	0.75	0.00	0.00	0.21	0.38
Plant Helper	0.31	0.23	0.42	0.49	0.28	0.08	0.41	0.66
SoilGard	0.26	0.46	0.20	0.39	0.23	0.15	0.51	0.42

- Biological control agents tended to be most effective against the NA2 and EU1 populations of *P. ramorum*.
- Overall, best results were obtained from Serenade and Plant Helper on Camellia.
- All treatments reduced *P. ramorum* lesion size on Rhododendron with Actinovate providing the most control. However, there was no control on Camellia.





Biological control of tanoak resprouts using *Chondrostereum purpureum* “Chontrol®”



Commercial Partner: MycoLogic Inc., Uvic



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Field trials in Southwest Oregon



Treated -

Chontrol®- *C.
purpureum*



Control- no *C. purpureum* Chontrol®



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Tree susceptibility

Acer saccharum (sugar maple)

Betula alleghaniensis (yellow birch)

Fraxinus americana (white ash)

Quercus rubra (red oak)

Abies balsamea (balsam fir)

Larix laricina (tamarack)

1.1 Foliage

1.2 Stems

P. ramorum lineage NA1





1.1 INOCULATIONS - FOLIAGE

Susceptibility and sporulation

1.1.1 Detached
leaves/needles

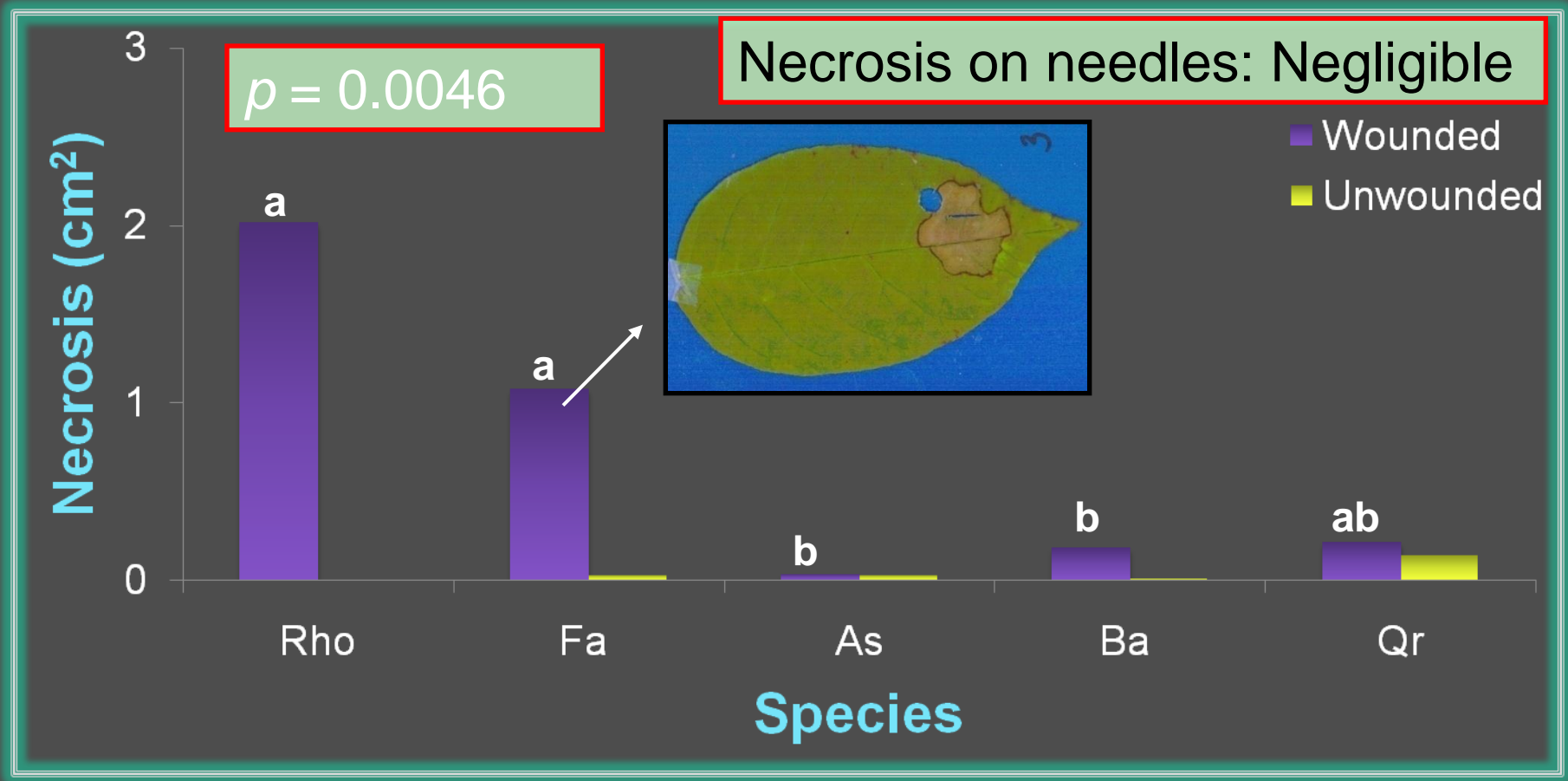
1.1.2 Plant dip

Rhododendron 'Nova Zembla' (control)



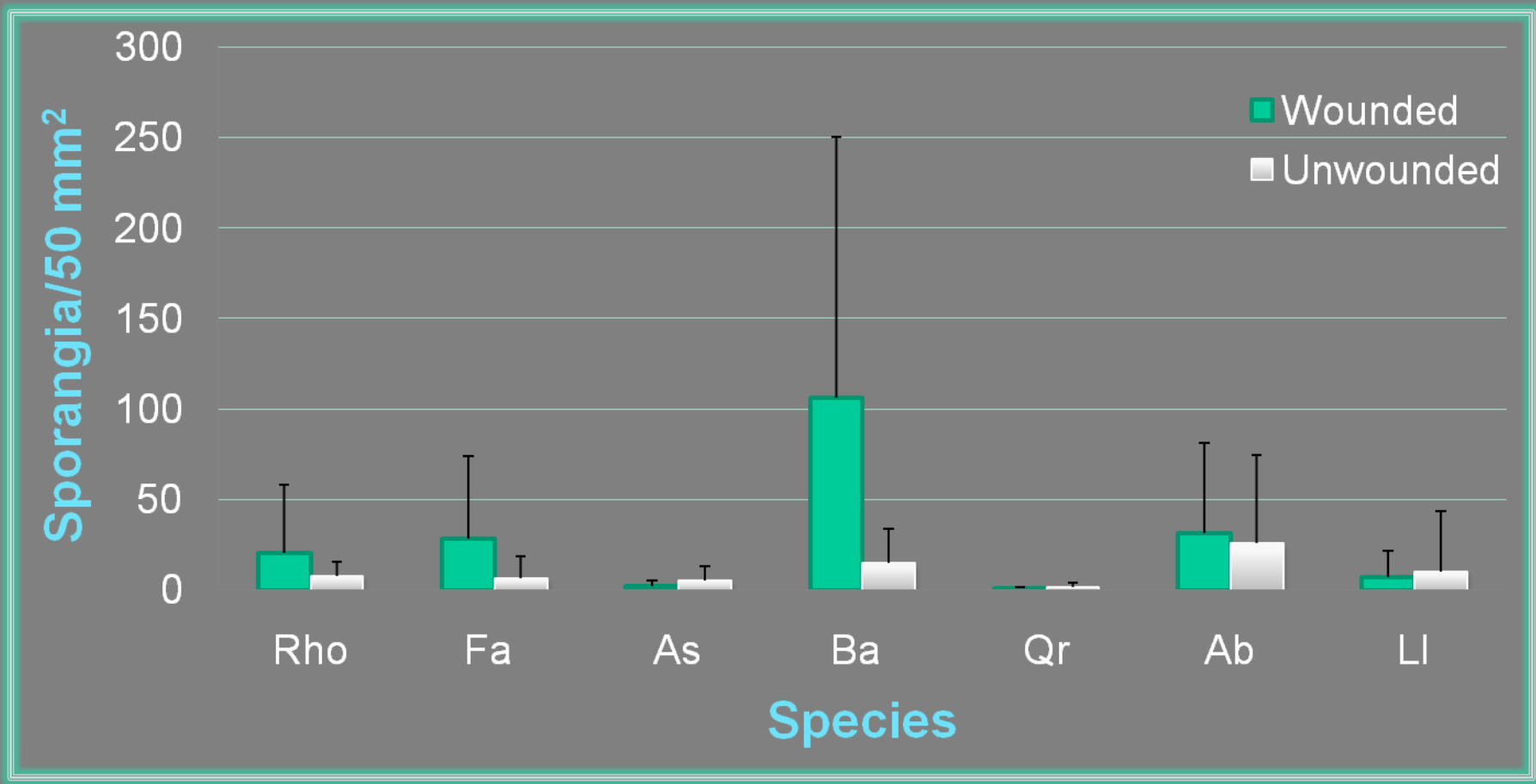
FOLIAGE

Plant-dip assays - Necrosis



FOLIAGE

Plant-dip assays - Sporulation





1.2 INOCULATIONS - STEMS



- Yellow birch
- Sugar maple
- White ash
- Red oak
- Balsam fir
- Tamarack



Results

Necrosis	Species
> 5 cm ²	<i>Larix laricina</i> <i>Abies balsamea</i>
2 – 5 cm ²	<i>Quercus rubra</i>
1 – 2 cm ²	<i>Fraxinus americana</i>
< 1 cm ²	<i>Betula alleghaniensis</i> <i>Acer saccharum</i>





Red Oak



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Balsam fir



Tamarack



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Mitigation measures to prevent the introduction of SOD- *Phytophthora ramorum* into BC & Canada

- Preventing the introduction and spread of *P. ramorum* is the key to minimize its impact on the nursery and environment.
- Commercial nurseries are advised to adopt the recommended Best Management Practices (BMPs) and the *P. ramorum* Nursery Certification Program (running by CNLA) to safeguard the industry.





Cont. Mitigation measures

- Plant species belonging to genus *Rhododendron*, *Camellia*, *Viburnum*, *Pieris*, *Kalmia* and *Magnolia* (Filthy 6)!! are considered highly susceptible to *P. ramorum* and nurseries are advised to take extra precaution when handling these plants. Keep these plants in a separate area or surround blocks with non-host plants.
- Scout for visible symptoms, particularly during spring, early summer and fall when the pathogen is active. If suspected, immediately notify CFIA or CFS.





Cont. Mitigation measures

- **Fungicides:** Dimethomorph (Acrobat 50 WP), fosetyl-AL (Aliette) and metalaxyl-M (Subdue MAXX) are registered for preventative use in nurseries and landscape plantings. These fungicides will not eliminate existing infection.
- **Avoid overhead irrigation.** Irrigate in a manner to avoid prolonged leaf wetness of 12 hours or more.





Cont. Mitigation measures

- Irrigation water from any source other than well or municipal water supplies should be monitored and tested to confirm that it is free from *P. ramorum*.
- An import policy D-01-01 titled, "Phytosanitary Requirements to Prevent the Entry of *Phytophthora ramorum*" (<http://www.inspection.gc.ca/english/plaveg/p/roctect/dir/d-01-01e.shtml>) has been implemented to prevent the introduction of *P. ramorum* into Canada (CFIA – revised September 16, 2010; 17th revision).





Summary

- PCR-RFLP DNA markers identify three lineages of North American and European *Phytophthora ramorum* populations
- Several fungicides & commercial biocontrol products have shown promise *in vitro* & *in vivo* against SOD-*Phytophthora ramorum*
- Chontrol®- *Chondrostereum purpureum* treatment as a biocontrol of tanoak resprouts is showing promise & would be indispensable alternative to chemical herbicides





Cont.- Summary

- Susceptibility of Six eastern Canadian tree species to infection by *P. ramorum*: A) Foliage: White Ash, Yellow Birch and Balsam Fir could propagate the SOD- *P. ramorum* ; B) Stems (risk of mortality): Red Oak, Balsam fir and Tamarack
- Preventing the introduction & spread of SOD- *P. ramorum* is the key to minimize its impact on Canadian nurseries & environment





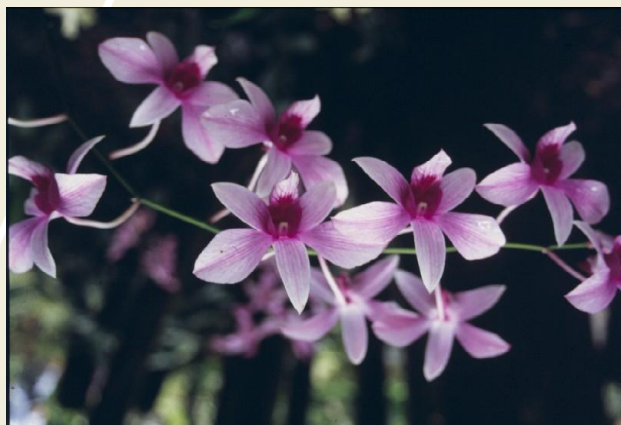
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Thank you/ Merci
Any questions?



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