A Technique for Determining Inoculum Threshold for the Spread of *Phytophthora ramorum* in Irrigation Water and Examining Fungicide Resistance and Pathogenicity Among Clonal Lineages in *Phytophthora ramorum*

*OR*

# What is the risk of *P. ramorum* spread from nurseries via waterways?

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# Examining Fungicide Resistance and Pathogenicity Among Clonal Lineages in *Phytophthora ramorum*

Nurseries and other locations where *P. ramorum* samples were obtained and number of isolates from each clonal lineage used in this study

| Nursery #* | County | Year | NA1 | NA2 | EU1 | EU1/NA2 |
|---|---|---|---|---|---|---|
| 20 | Cowlitz | 2007 | 4 | | | |
| 38 | Snohomish | 2007 | 2 | | | |
| 35 | King | 2007, 2008 | 16 | | 2 | |
| 37 | King | 2007 | | 4 | | |
| CA | Santa Clara | 2006 | 10 | | | |
| 44 | Clark | 2008 | 5 | | 1 | |
| 19 | Pierce | 2007, 2008 | 4 | | | |
| 23 | Pierce | 2006, 2010 | 3 | 5 | | |
| 45 | Pierce | 2009 | | 2 | | 4 |
| OR | Clackamas | 2003 | 1 | | | |
| 41 | Thurston | 2008, 2009 | | | 9 | |
| 40 | King | 2008 | | | 2 | |
| 43 | Snohomish | 2009 | | | 6 | |
| 21 | Snohomish | 2007 | 1 | | | |
| S | King | 2006 | 1 | | | |

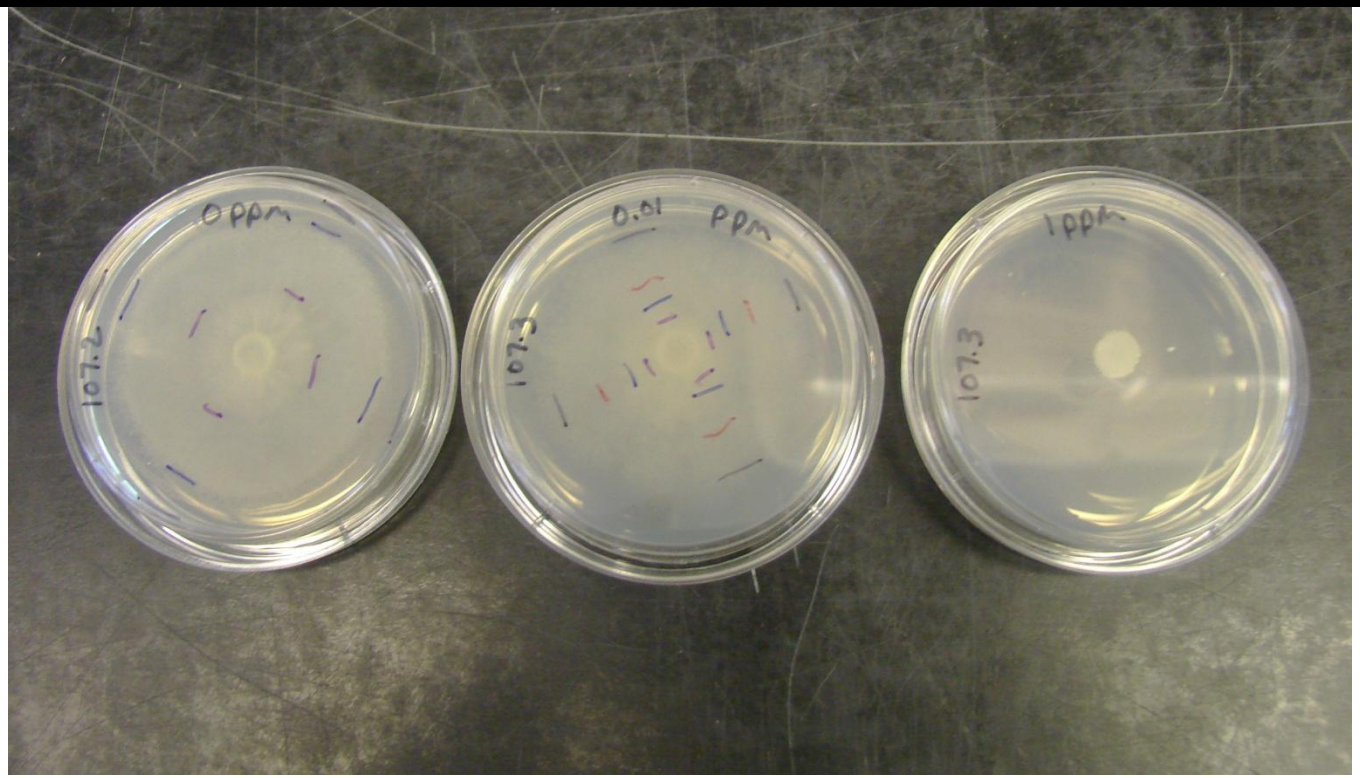
*S = stream bait, CA = California, OR = Oregon

# EU1/NA2

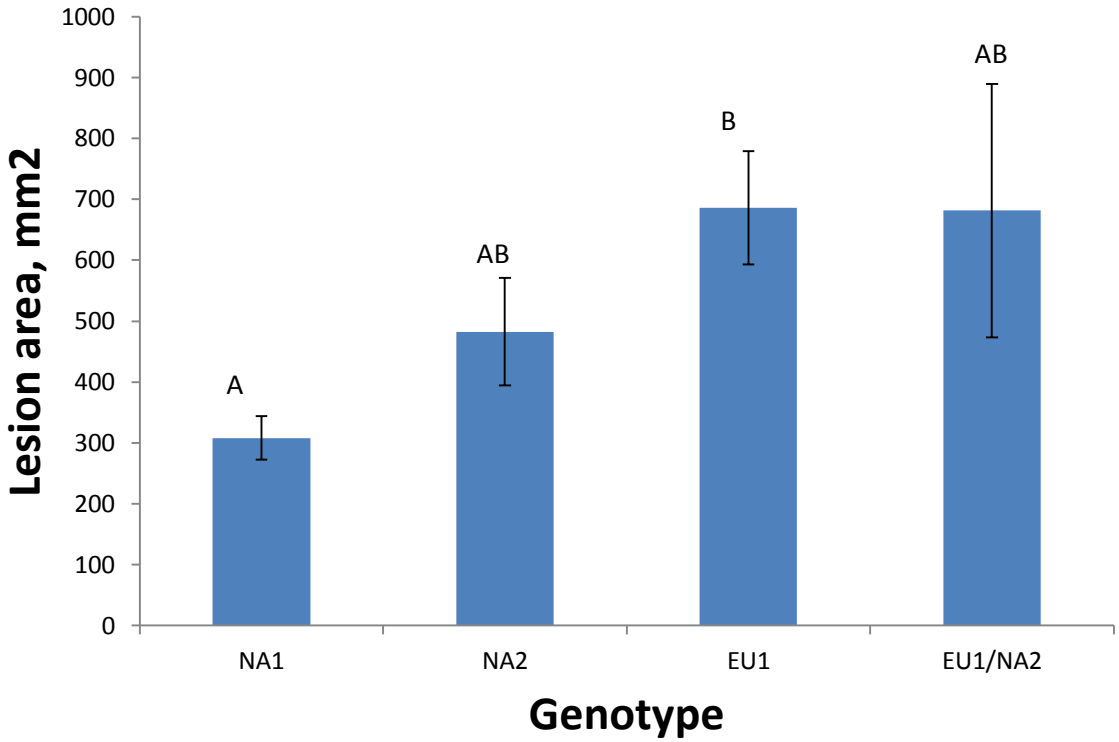
- Acquired from 3 Rhododendron plants at nursery 45
- 8 isolates from leaf and stem lesions
- Not hybridizing or sexually reproducing – a heterokaryon?
- Single-zoospore isolates were all NA2 (n=40)

# Fungicide screening

- 85 *P. ramorum* isolates, most from WA nurseries
- % inhibition by mefenoxam at 0.01 and 1 ppm a.i.
- Standard curve on 19 isolates 0 – 100 ppm a.i.



# Pathogenicity




Lesion area produced by three clonal lineages and one comingled sample of *Phytophthora ramorum* after ten days on detached Rhododendron 'Nova Zembla' leaves. Bars with different letters are significantly different at p = 0.05 (Kruskal-Wallis test with Dunn's multiple comparisons).

# NA1

| isolate | EC50 ppm | avg lesion area $mm^2$ | WSU # | Host | Cultivar | Nursery # |
|---|---|---|---|---|---|---|
| NA1 6 | 0.022 | 292 | 106-0016 | Douglas-fir (*Pseudotsugae menziesii)* | | CA |
| NA1 8 | 0.007 | 74 | 106-0019 | Mistletoe *(Phoradendron serotinum* subsp.*macrophyllum)* | | CA |
| NA1 39 | 0.011 | 22 | 107-0072 | *Rhododendron* | Pentanthera | 35 |
| NA1 40 | 0.003 | 44 | 107-0073 | *Rhododendron* | Pentanthera | 35 |
| NA1 47 | 0.004 | 56 | 107-0095 | *Rhododendron* | | TF35 |
| NA1 52 | 0.005 | 64 | 107-0100b | *Rhododendron* | | TF35 |

Some NA1 isolates were low in pathogenicity.

# EU1

| isolate | EC50 ppm | avg lesion area $mm^2$ | WSU # | Host | Cultivar | Nursery # |
|---|---|---|---|---|---|---|
| EU1 23 | 0.004 | 463 | 107-0037 | *Rhododendron* | Unique | OR |
| EU1 65 | 0.004 | 805 | 108-0028 | *Viburnum tinus* | Spring bouquet | 40 |
| EU1 66 | 1.695 | 876 | 109-0012 | soil bait | | TF41 |
| EU1 88 | 0.794 | 856 | 108-0039 | *Rhododendron* | Purpureum elegans | TF41 |
| EU1 93 | 0.003 | 1087 | 109-0058 | *Rhododendron* | Dexter's Pink | 43 |
| EU1 99 | 2.761 | 946 | 109-0093 | *Rhododendron* | | 41 |

EU1 isolates from nursery 41 were tolerant of the fungicide.

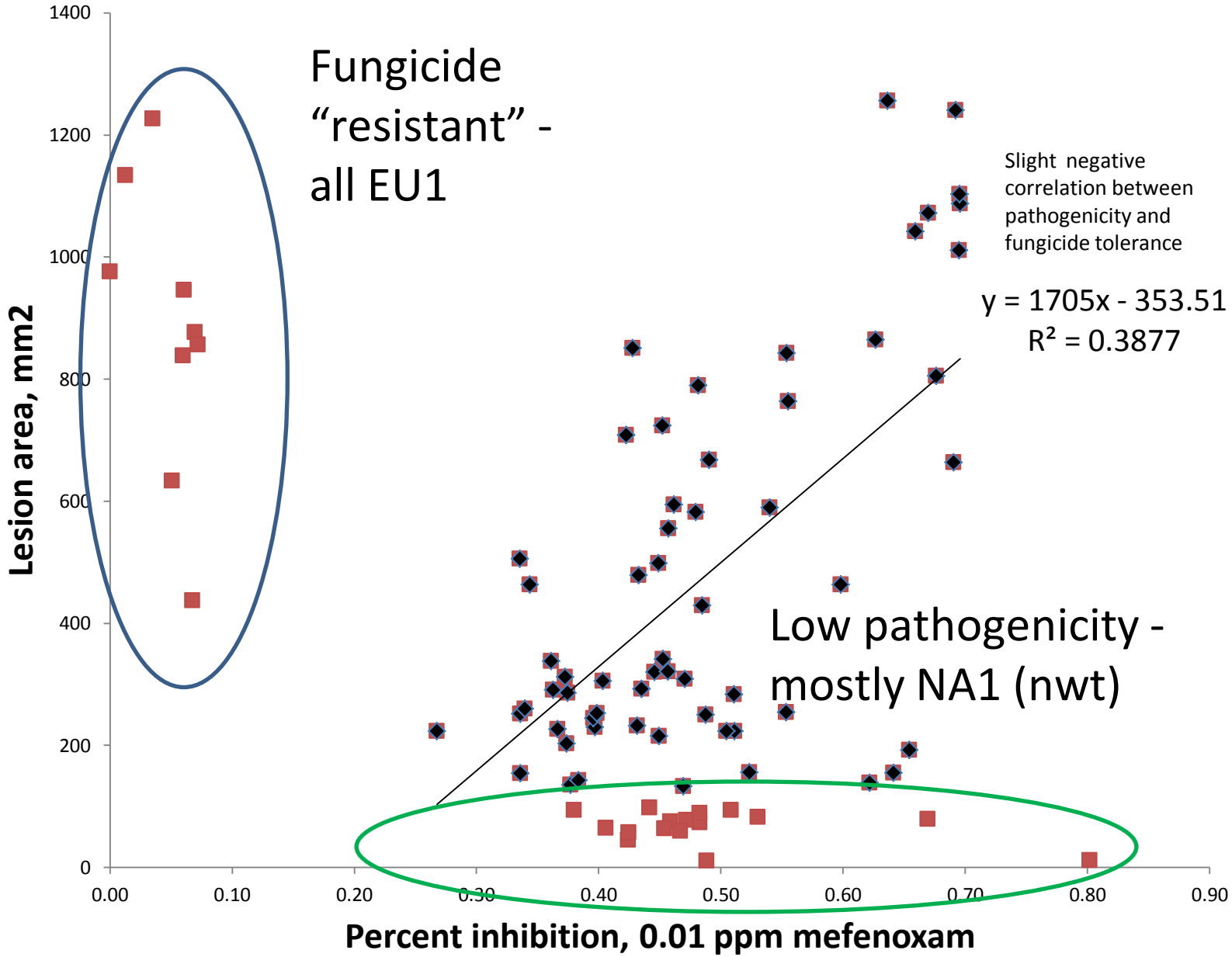
EC50 > 10 ppm is resistant.

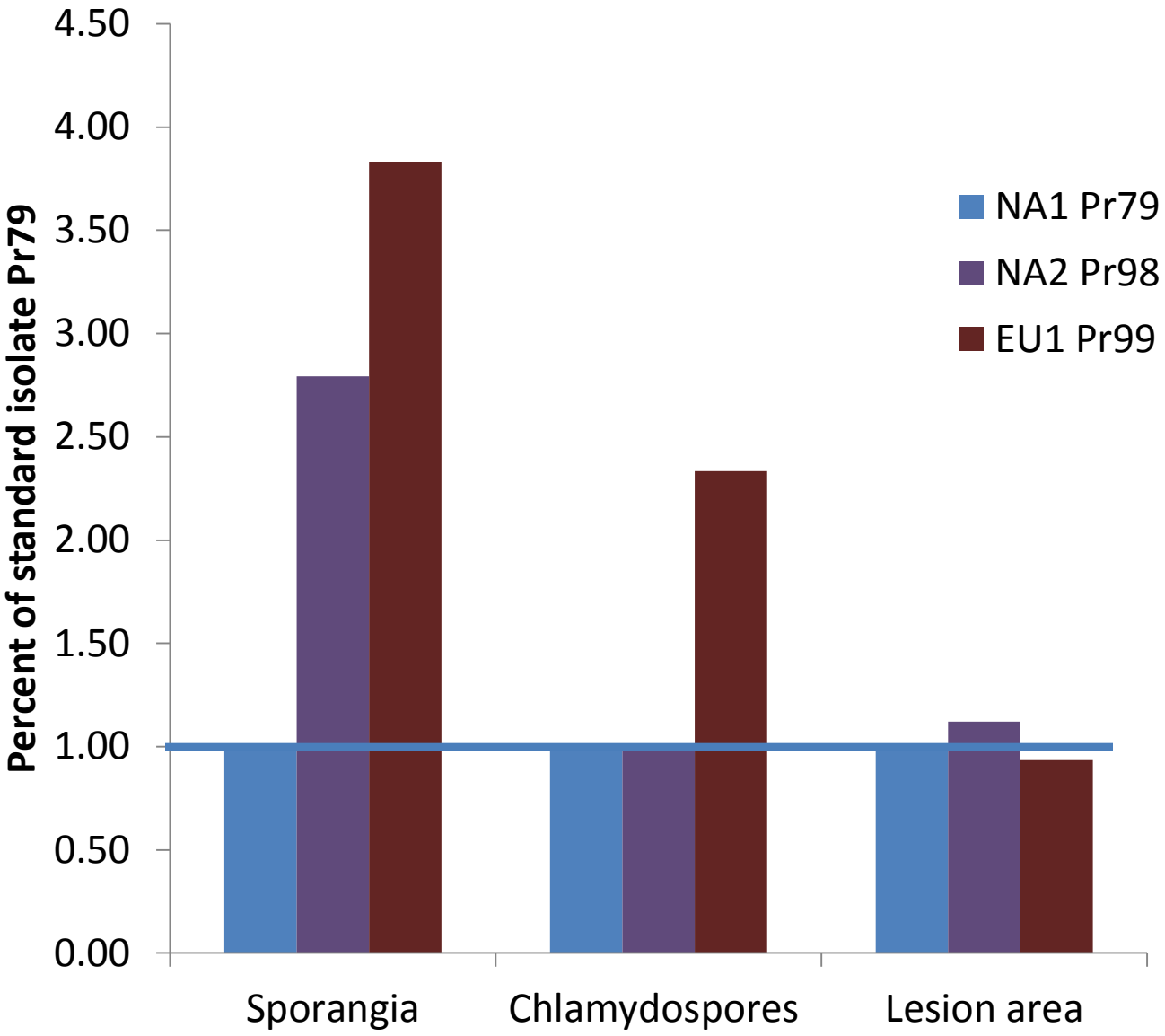
# NA2

| isolate | EC50 ppm | avg lesion area $mm^2$ | WSU # | Host | Cultivar | Nursery # |
|---|---|---|---|---|---|---|
| NA2 17 | 0.002 | 338 | 107-0011 | *Kalmia latifolia* | Raspberry Glow | 37 |
| NA2 37 | 0.008 | 251 | 107-0021 | soil bait | | 37 |
| NA2 98 | 0.005 | 308 | 109-0074 | salal (*Gaultheria shallon)* | | 45 |
| NA2 103 | 0.002 | 1042 | 109-0098 | *Rhododendron* | Paul Bosley | 45 |
| NA2 105 | 0.006 | 851 | 110-0022 | *Rhododendron* | | 23 |

# EU1/NA2

| isolate | EC50 ppm | avg lesion area $mm^2$ | WSU # | Host | Cultivar | Nursery # |
|---|---|---|---|---|---|---|
| EU1/NA2 100 | 0.005 | 135 | 109-0095 | *Rhododendron* | Paul Bosley | 45 |
| EU1/NA2 101 | 0.005 | 763 | 109-0096 | *Rhododendron* | Paul Bosley | 45 |

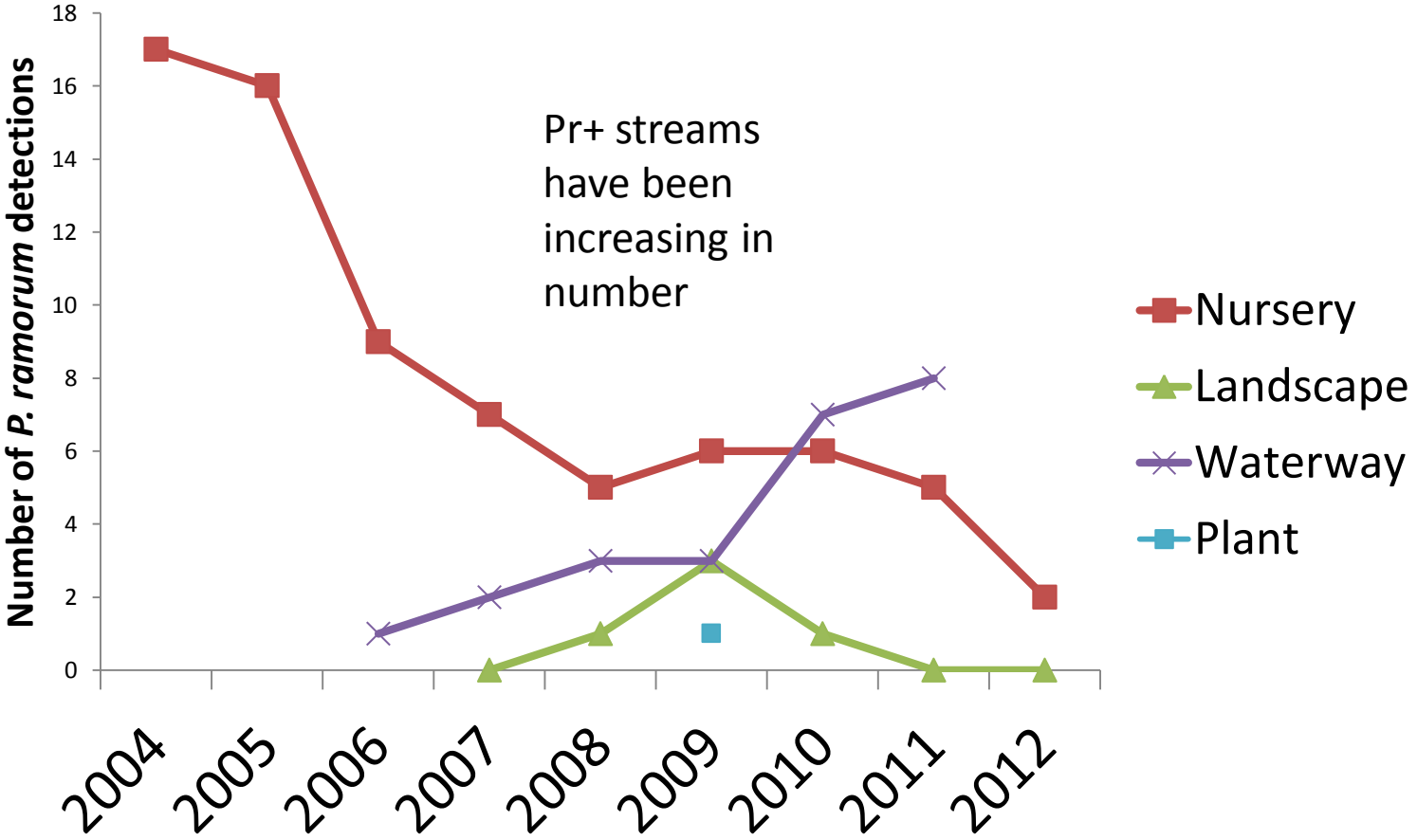

Fungicide "resistant" - all EU1
Slight negative correlation between pathogenicity and fungicide tolerance
y = 1705x - 353.51
R² = 0.3877
Low pathogenicity - mostly NA1 (nwt)
Lesion area, mm2
Percent inhibition, 0.01 ppm mefenoxam
0
200
400
600
800
1000
1200
1400
0.00
0.10
0.20
0.30
0.40
0.50
0.60
0.70
0.80
0.90


4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Percent of standard isolate Pr79
NA1 Pr79
NA2 Pr98
EU1 Pr99
Sporangia
Chlamydospores
Lesion area

# Conclusion:

All *P. ramorum* is not created equal.

# *P. ramorum* in Washington State



# Transfer of Pr from water to vegetation

2009
On salal outside nursery #45

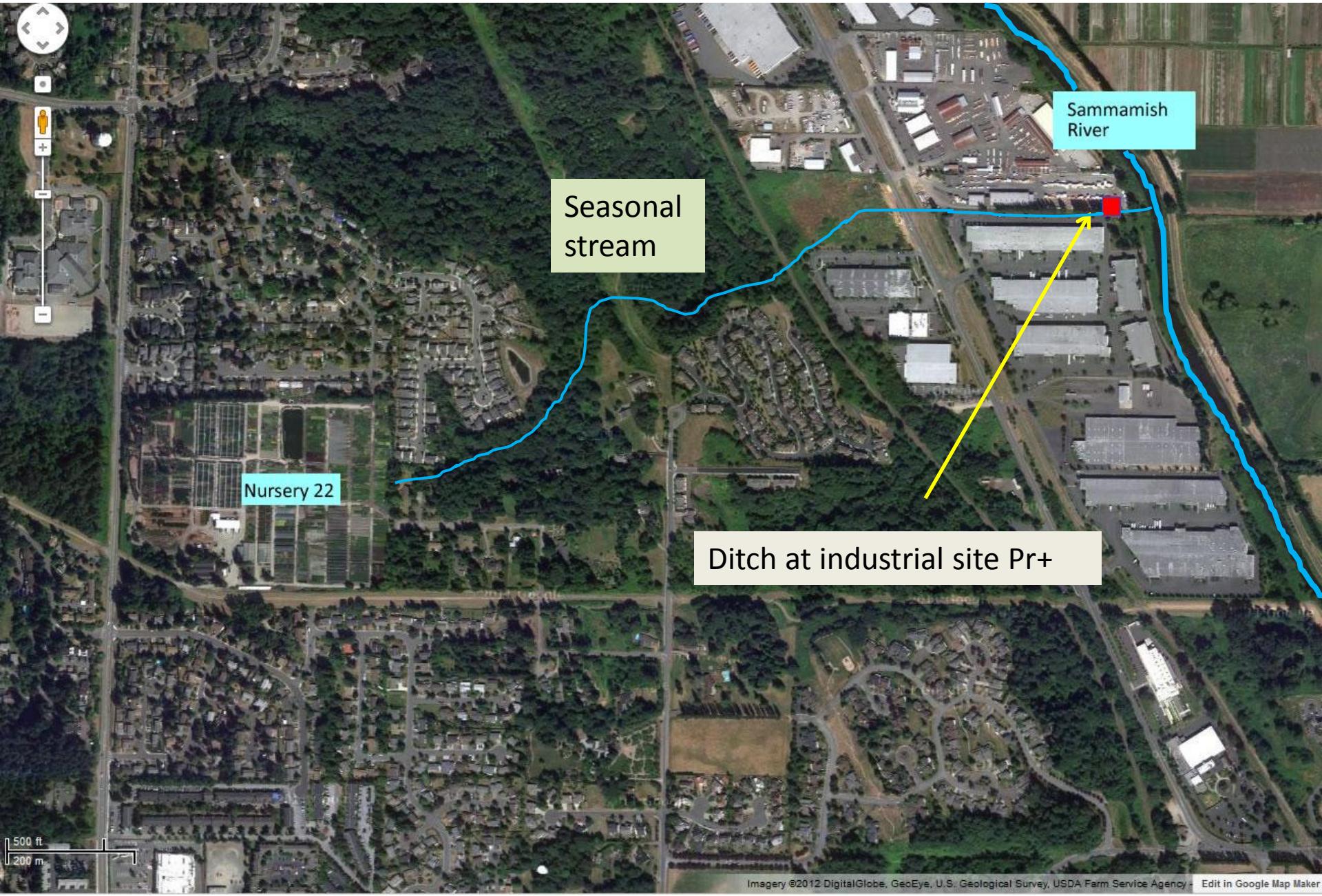
NA2 lineage (isolate # Pr98)

# A Technique for Determining Inoculum Threshold for the Spread of *Phytophthora ramorum* in Irrigation Water

# Some nurseries and landscapers use stream water for irrigation

WA Dept. of Ecology Records (May 2009) indicate that 46 entities have rights to use water from the Sammamish River in King County, WA to irrigate almost 2,800 acres of land.

# The “Sammamish Shower”


Sammamish River
Seasonal stream
Nursery 22
Ditch at industrial site Pr+
500 ft
200 m
Imagery ©2012 DigitalGlobe, GeoEye, U.S. Geological Survey, USDA Farm Service Agency
Edit in Google Map Maker

# Results of pilot study

## Lab testing

- Plants overhead irrigated with 10L water containing 1 x $10^4$ zoospores/ml
- Plant foliage had very low levels of infection – 2%
- Roots and soils – 90% infection

## Field testing

- No Pr detected on plants or soils
- All stream baits negative for Pr

Oomycetes isolated from leaf baits from three locations in June 2011. AS = “alder site”, a known *P. ramorum* positive site on the Sammamish River, IS = industrial site ditch, upstream from shower, also known to be positive for *P. ramorum*, SS = water from shower that had been applied to plants. Totals for all three sampling periods are given. No *P. ramorum* was detected at any of the sites.

| **Oomycete species** | **AS** | **IS** | **SS** | **all sites** |
|---|---|---|---|---|
| *Pythium ‘Group F’* | 4 | 1 | 9 | 14 |
| *Pythium undulatum* | 3 | 0 | 2 | 5 |
| *Phytopythium* spp. | 1 | 6 | 0 | 7 |
| *Phytophthora* taxon 'salixsoil' | 7 | 2 | 0 | 9 |
| *Phytophthora gonapodyides* | 1 | 1 | 0 | 2 |
| *Phytophthora* taxon 'pgchlamydo' | 0 | 0 | 2 | 2 |
| *Halophytophthora* sp. | 0 | 1 | 0 | 1 |

# Further study planned

Inoculum

- Quantify inoculum levels using filtration/culturing, BOB (Bait in a Bottle), and qPCR
- Testing in lab and 3 field sites
- Sample foliage and soil/roots for *P. ramorum*

*P. ramorum* fitness

- Sporulation potential
- Temperature growth rates
- Behavior of fungicide “resistant” isolates on fungicide treated Rhododendrons

# Acknowledgements

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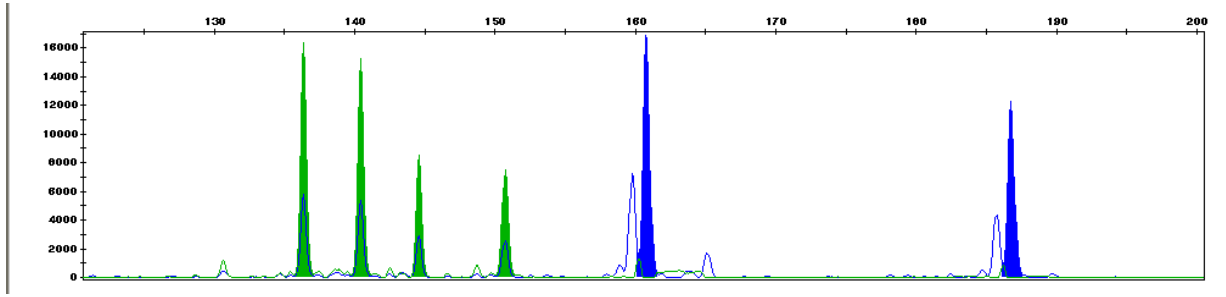
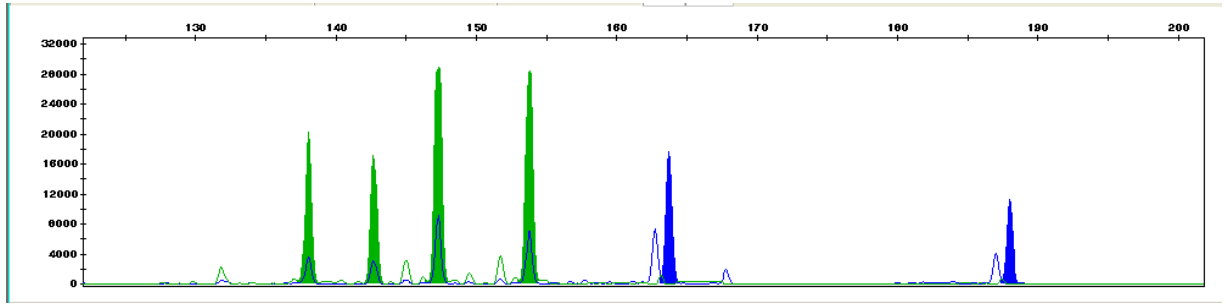
Kathy Riley
Mark Kurkov
Lucy Rollins
Giselle Baptiste

Visit our website:

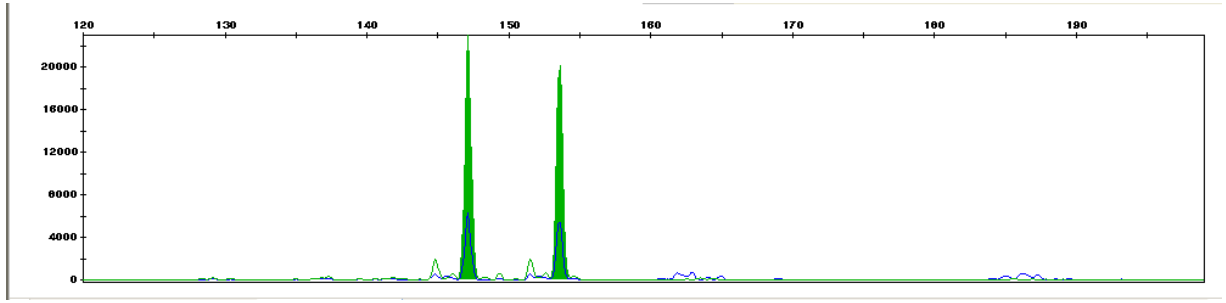
http://www.puyallup.wsu.edu/ppo/sod/



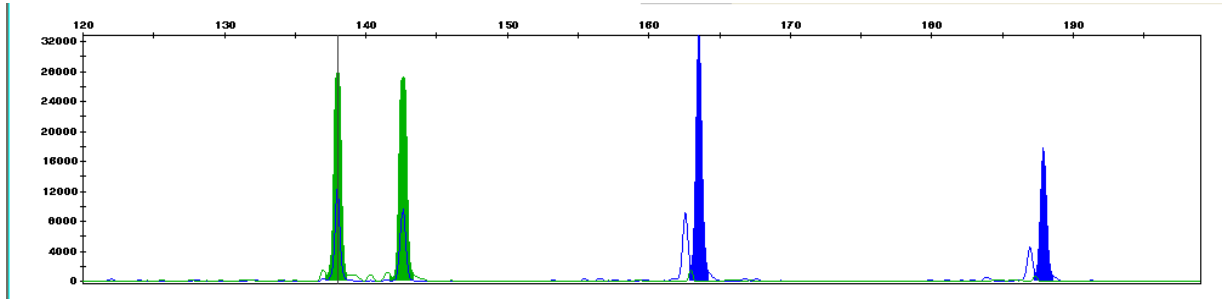

EU1/NA2




EU1/NA2




NA2




EU1

PrMS39

PrMS45