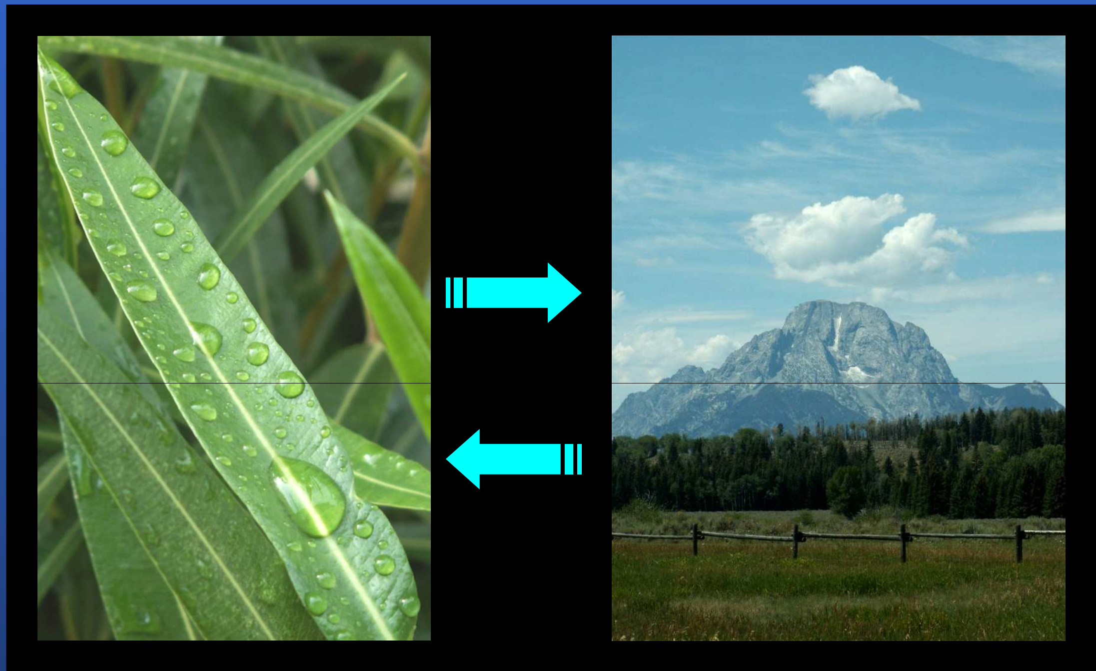
Ecology of Pseudomonas syringae : role of the water cycle

Cindy E. Morris, Catherine Glaux, Caroline Guilbaud

Pseudomonas syringae : Partner and actor in the natural cycle of water



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Pseudomonas syringae, an archetype among plant pathogenic bacteria

- Ubiquitous epiphyte
- Genome 6 Mbp
- Cool weather opportunist
- Multiple means of dissemination including airborne
- Ice nucleation active

Plant Pathology 100 - Final Exam:

Which habitats are reservoirs of *Pseudomonas syringae*?

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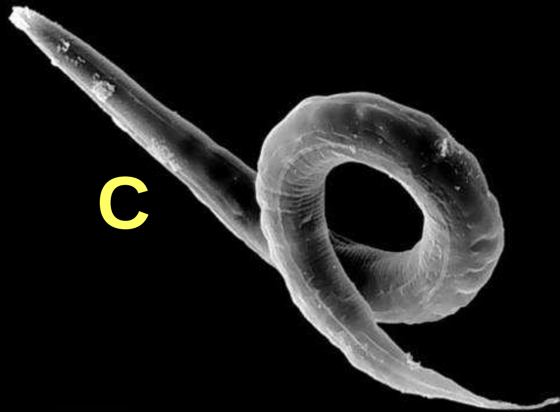
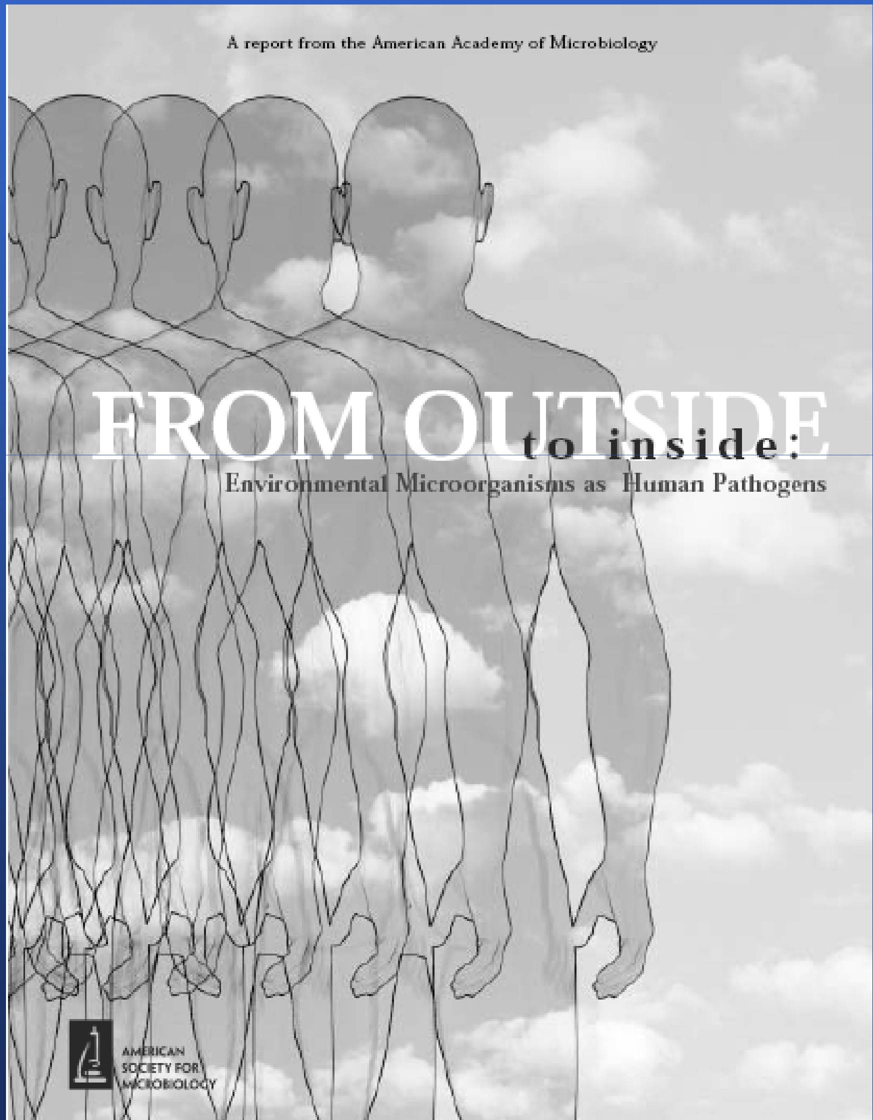


From outside to inside: Environmental microorganisms as human pathogens.



American Academy of Microbiology 2004

Cangelosi, Freitag & Buckley, 18 p.

http://www.asm.org/ASM/files/ccLibraryFiles/FILENAME/000000001402/EnvironMicroBW.pdf

Critical point from report:

Acquisition / evolution of factors involved in pathogenicity to humans and in resistance to medically-important antibiotics can occur in environments that:

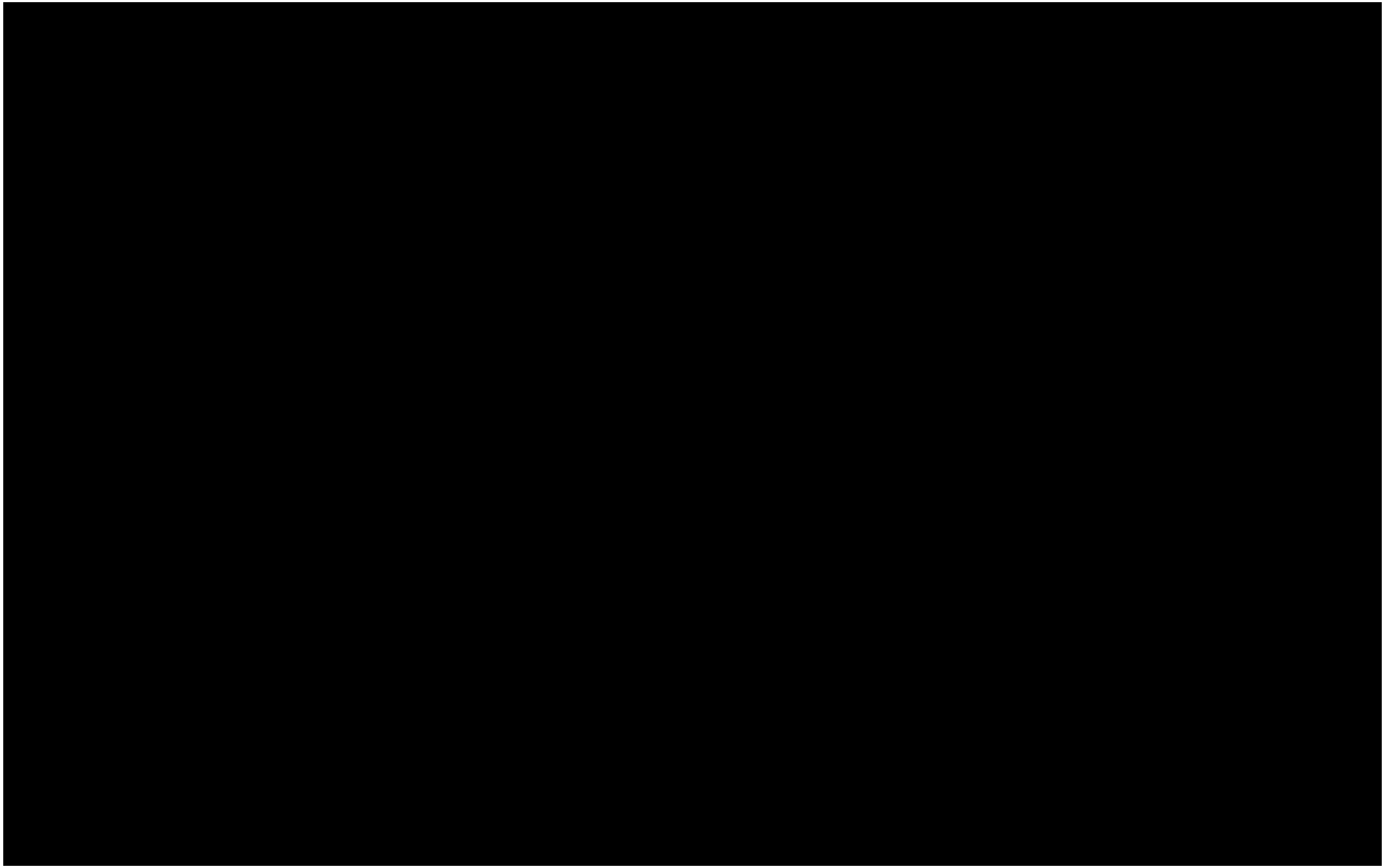
- do not involve interactions with human hosts
- do not involve contact with these antibiotics

In Plant Pathology, can we address analogous hypotheses concerning the role of non agricultural habitats in diversification of pathogens and in the evolution of their pathogenicity?

Have we adequately elucidated the non agricultural (and non plant) habitats of plant pathogens? Are we overly agro-centric?

A bit of intuition:

H_o: *P. syringae* is ubiquitous outside of agricultural contexts



A bit of intuition:

H_o: *P. syringae* is ubiquitous outside of agricultural contexts

Survey of *Pseudomonas syringae* in wild places

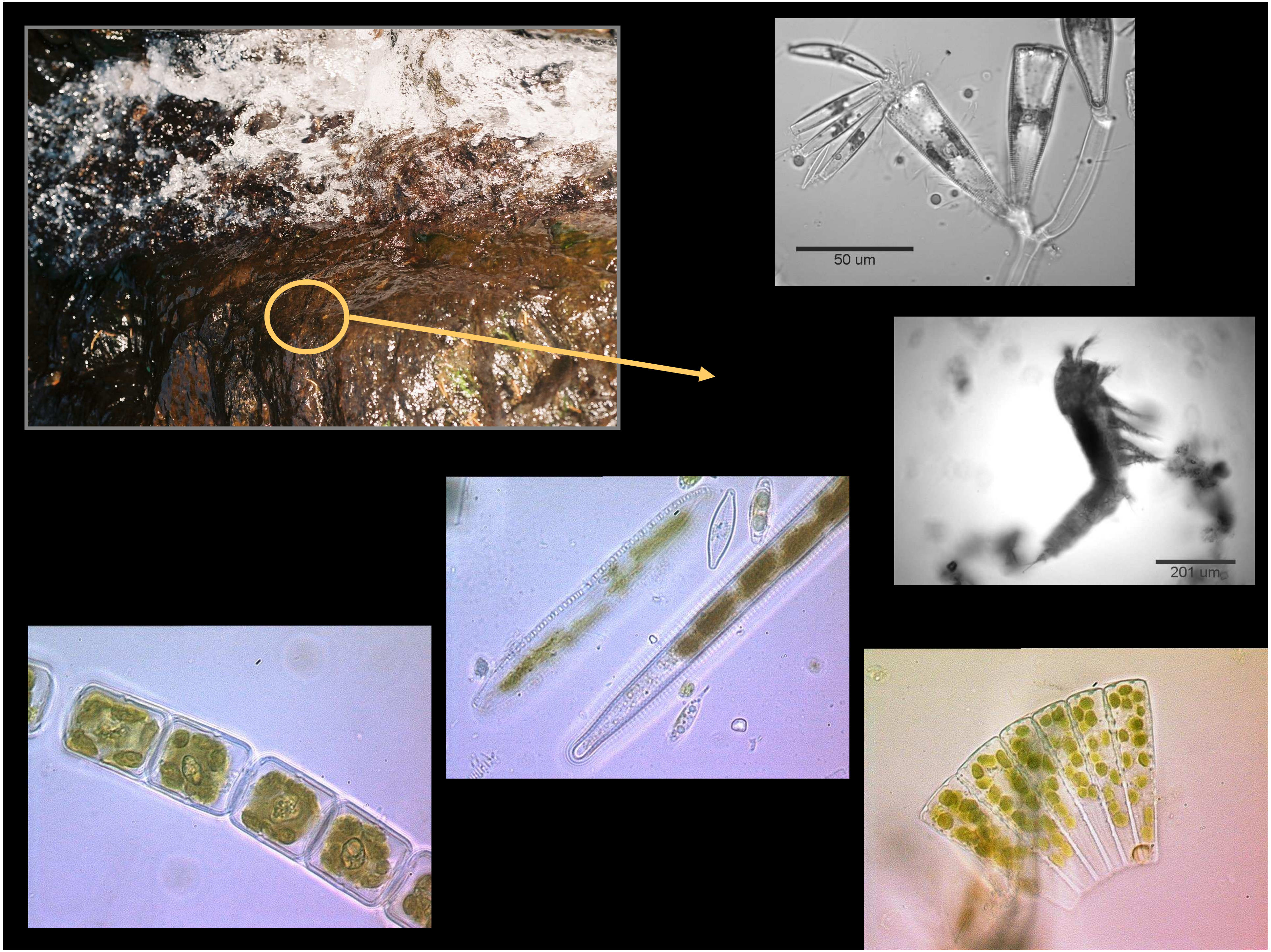
Quantitative isolation from non-agricultural sites

Selective medium

Phenotypic characterization

Confirmation of identity by molecular typing – 4 housekeeping genes: *cts, gyrB, gapA, rpoD*

Epilithon
(biofilms on rocks)


50 um
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Wild plants, for example :

***Primula* spp.**

Hutchinsia alpina

Silene acaulis

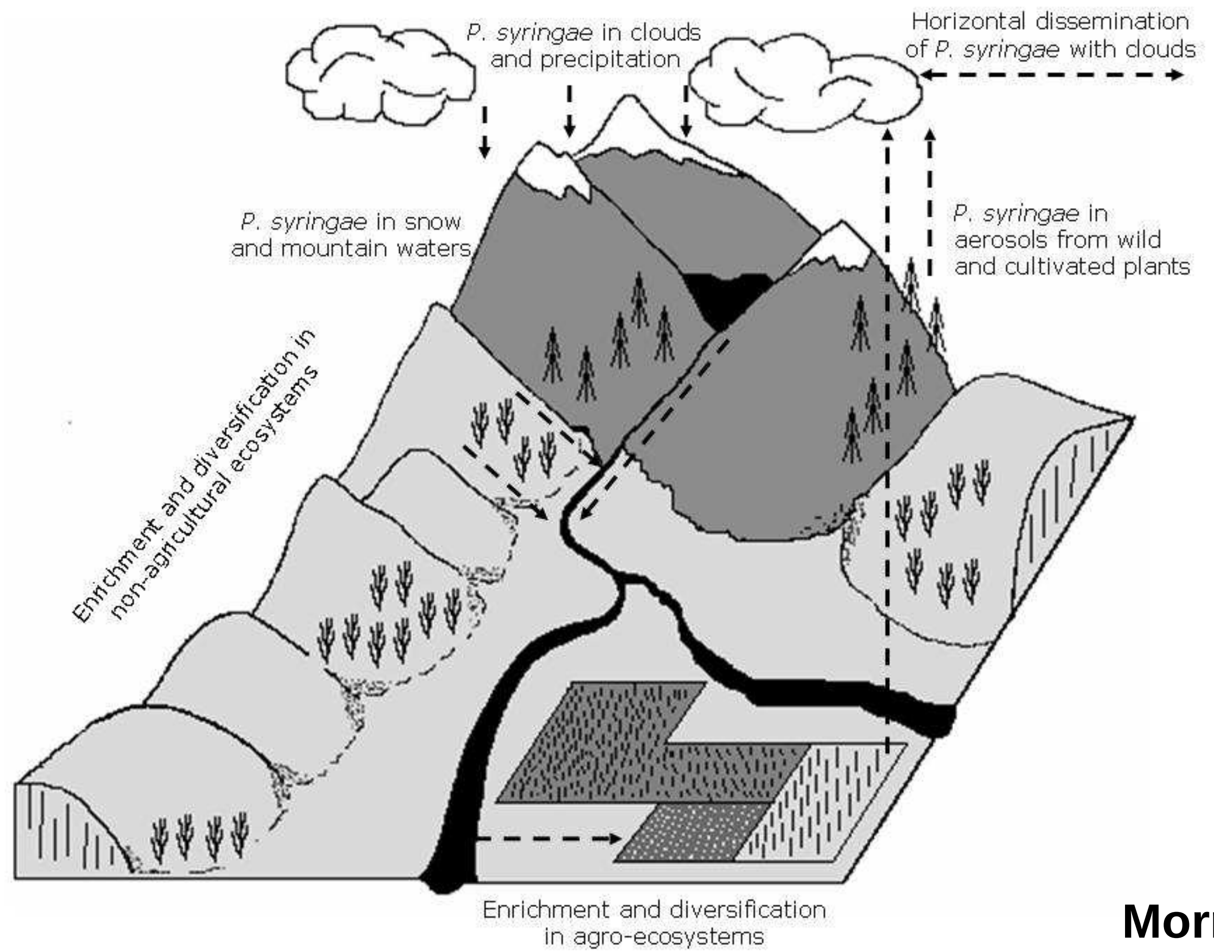
Fresh and firn snow


2800 m, Otzal Valley, Austria

Survey of *P. syringae*

Substrate	Nt	n+	Known regions	Pop. density
Crops			Worldwide, temperate zones	10^2 - 10^8 cfu/ leaf(g)
Wild plants Primulaceae other	 21 5	 14 4	FR, Italy, USA	500 - 10^5 cfu/g
Aerosols			USA	↑ 30 cfu/m^2/sec
Cloud water (Amato el al 2007)	5	3	FR, USA	ca. 10^4 cfu/L
Rain	4	2	FR	100 - 10^4 cfu/L
Snow	14	6	FR, Austria	100 - 10^5 cfu/L meltwater
Mountain lakes and streams	8	5	FR, USA, NZ	100 - 10^4 cfu/L
Epilithic biofilms	16	7	FR, USA	50 - 10^4 cfu/g
Irrigation water			FR	12 - 70 cfu/L

Pseudomonas syringae : widespread in diverse substrates along the water cycle

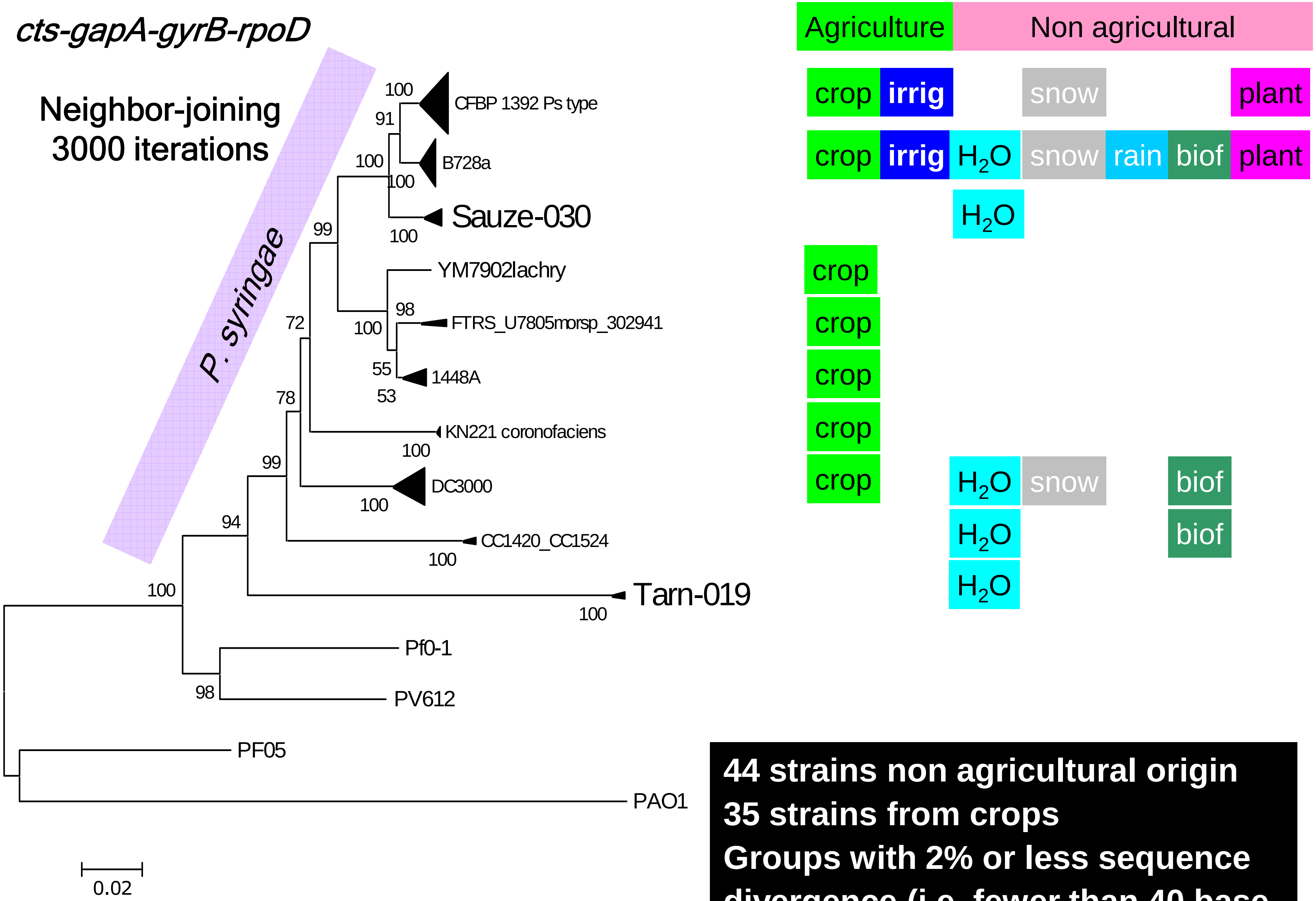


Morris et al 2008

Pseudomonas syringae in non agricultural habitats: are they similar to strains from agricultural ?

Genotype: *MLST of housekeeping genes*

Phenotype: *host range, toxins, ice nucleation activity*



proportion differences per 1915 bases

44 strains non agricultural origin
35 strains from crops
Groups with 2% or less sequence divergence (i.e. fewer than 40 base differences).

Pseudomonas syringae in non agricultural habitats: are they similar to strains from agricultural ?

Genomic groups of *P. syringae* fall into 3 ecotypes:

- Only in crops (few genomic groups)
- Only in water (few genomic groups)
- Widespread in many or all substrates (most groups)

Evaluation of host range

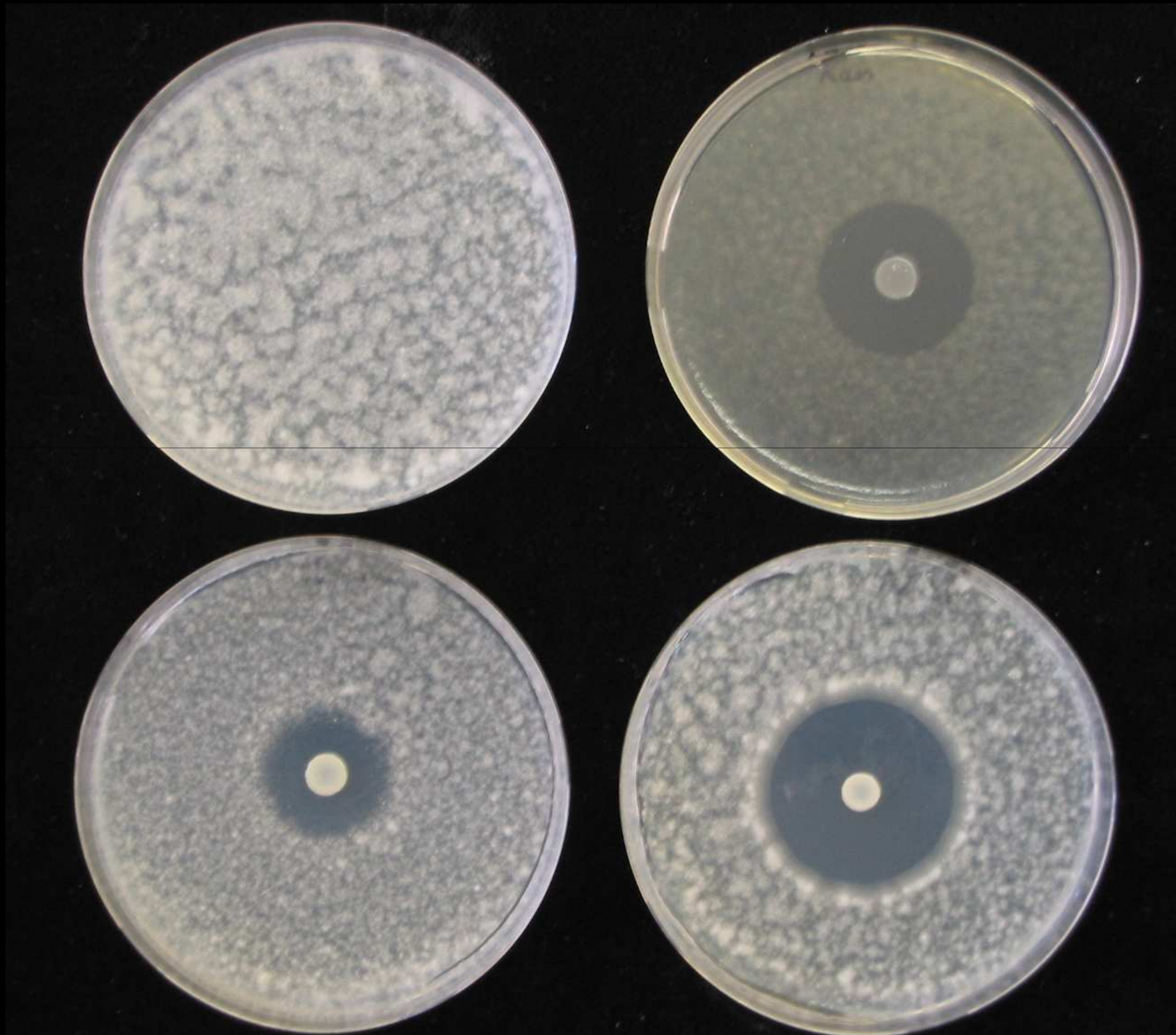
lettuce

cantaloupe

sugar beet

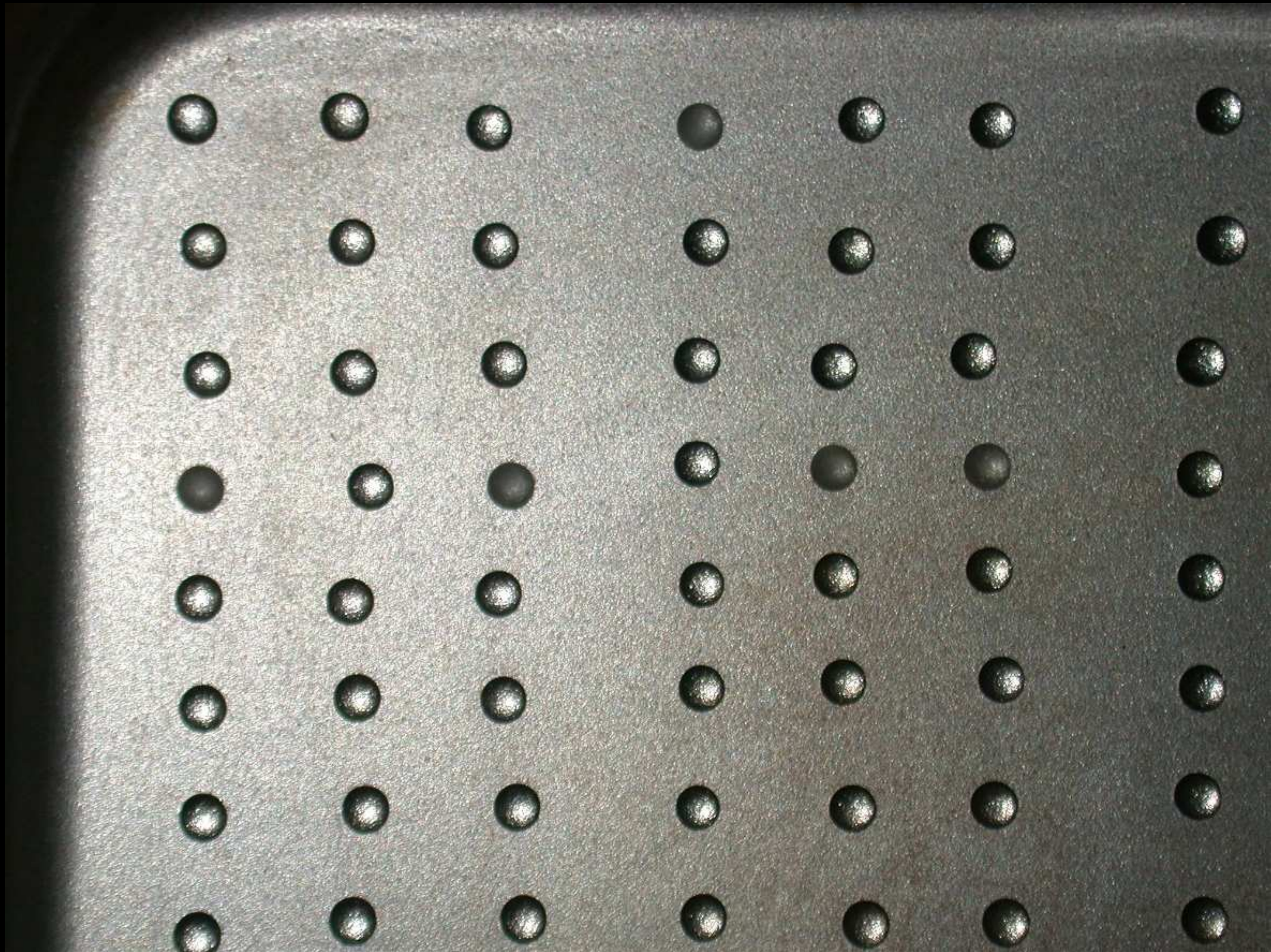
P. syringae / bacterial blight of cantaloupe

Bioassay for syringomycin-like toxin



Assay for ice nucleation activity

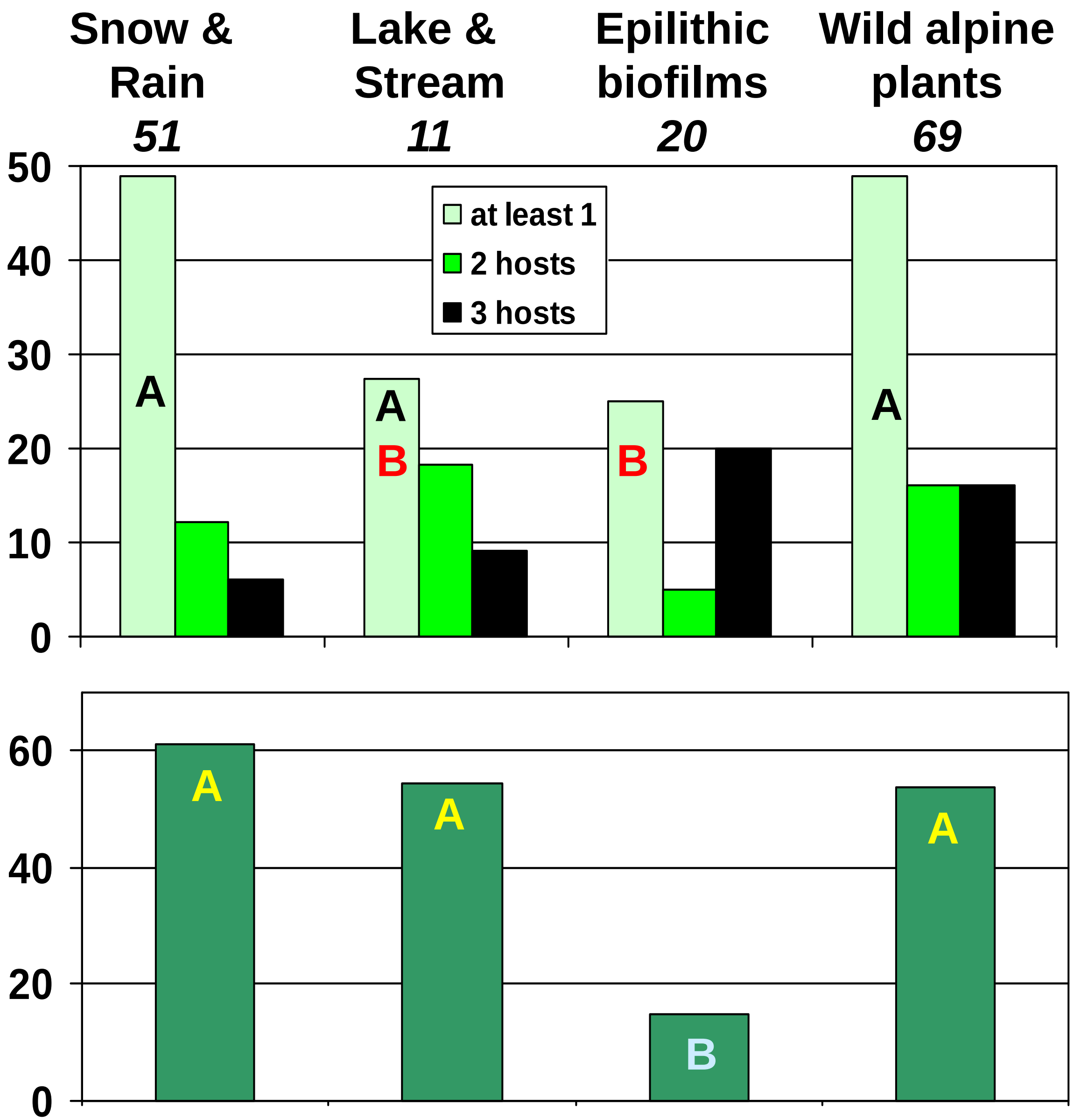
-2°C to -8°C / 10^4 and 10^6 cells



Traits of *Pseudomonas syringae* in wild places

Host range
(% of strains)

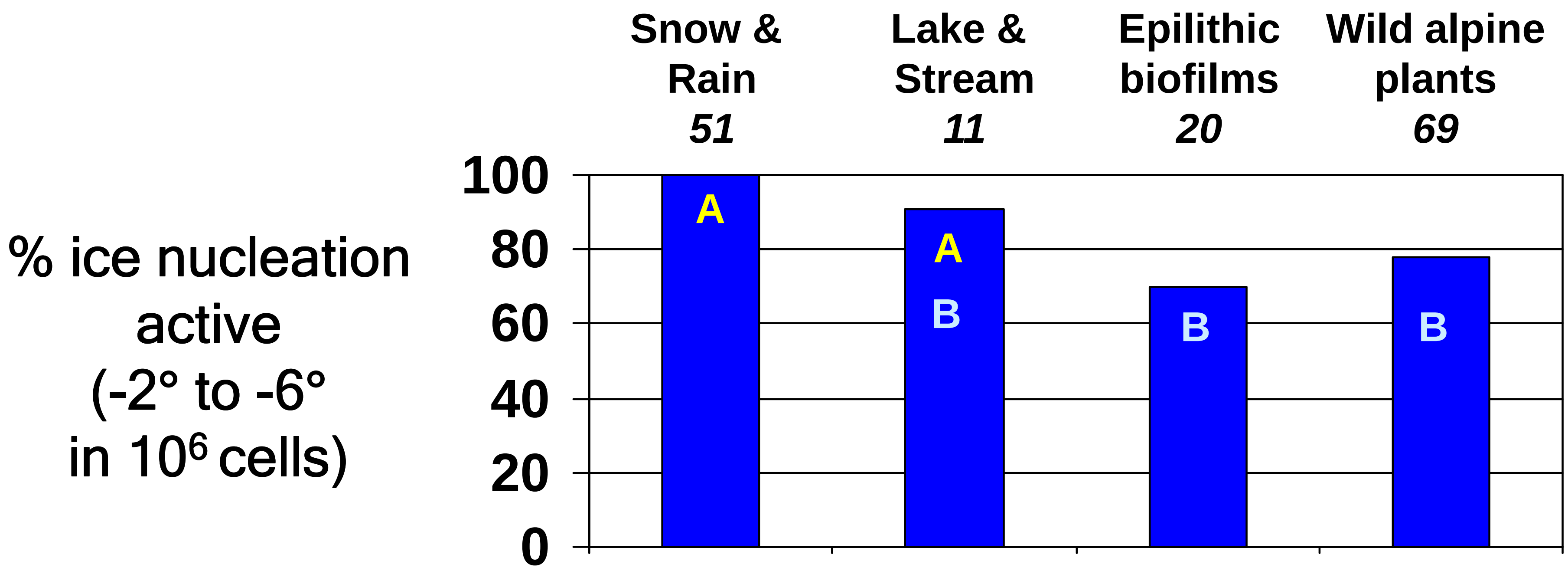
% strains producing
syringomycin-like
toxin



pair-wise χ^2, $p \leq 0.05$

Traits of *Pseudomonas syringae* in wild places

All strains of *P. syringae* from precipitation are INA
at warm temperatures – unlike other substrates



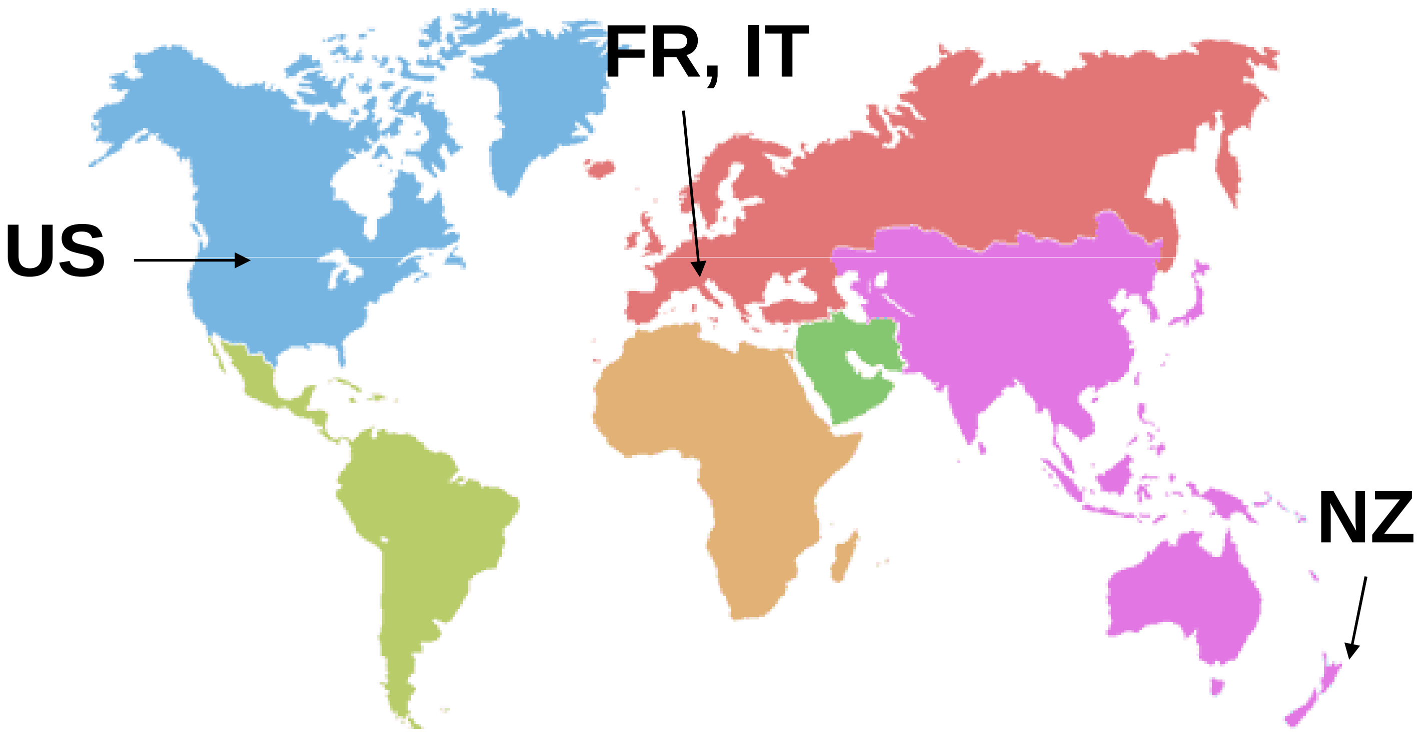
pair-wise χ^2, $p \leq 0.05$

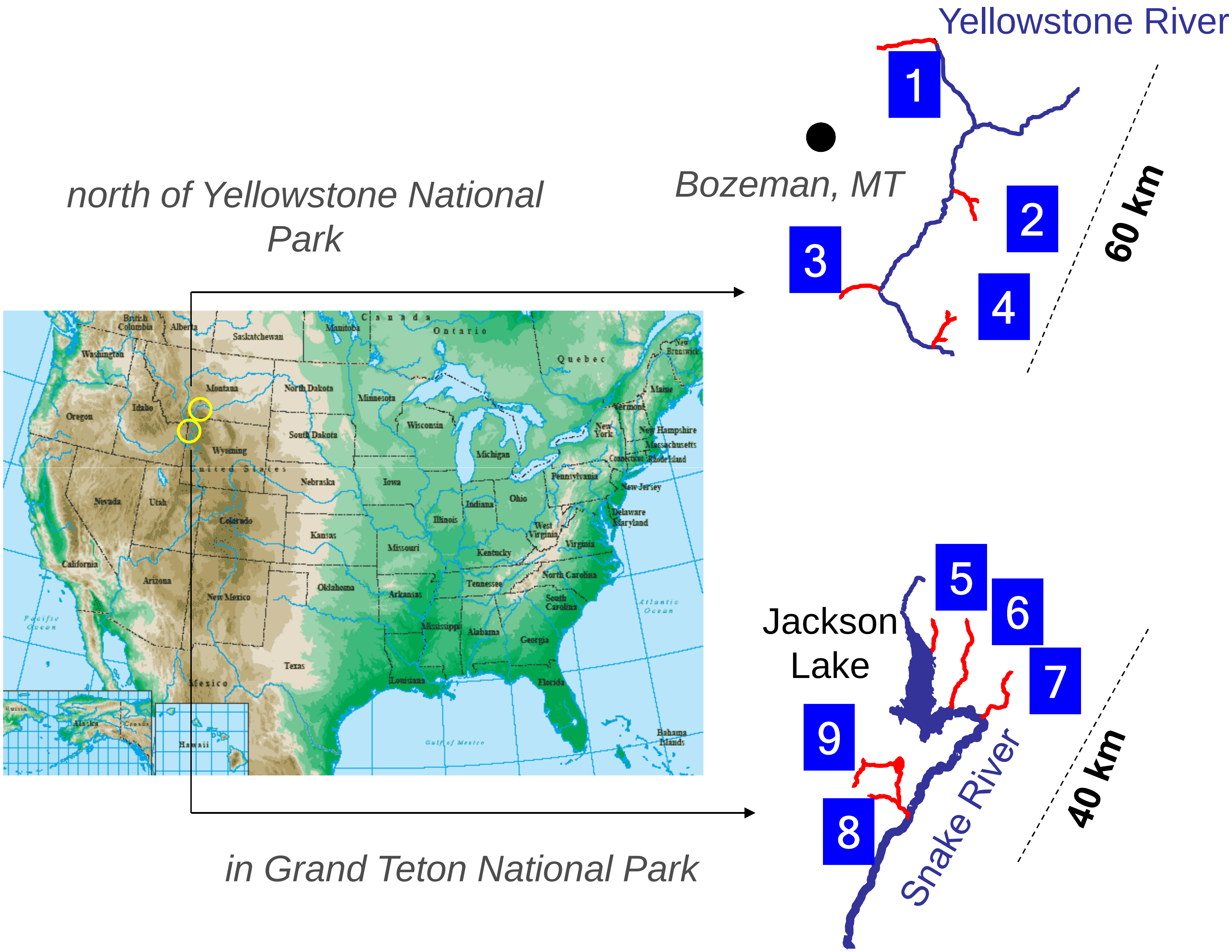
Implications of non agricultural habitats of a plant pathogen for epidemiology, ecology and evolution

Some preliminary questions:

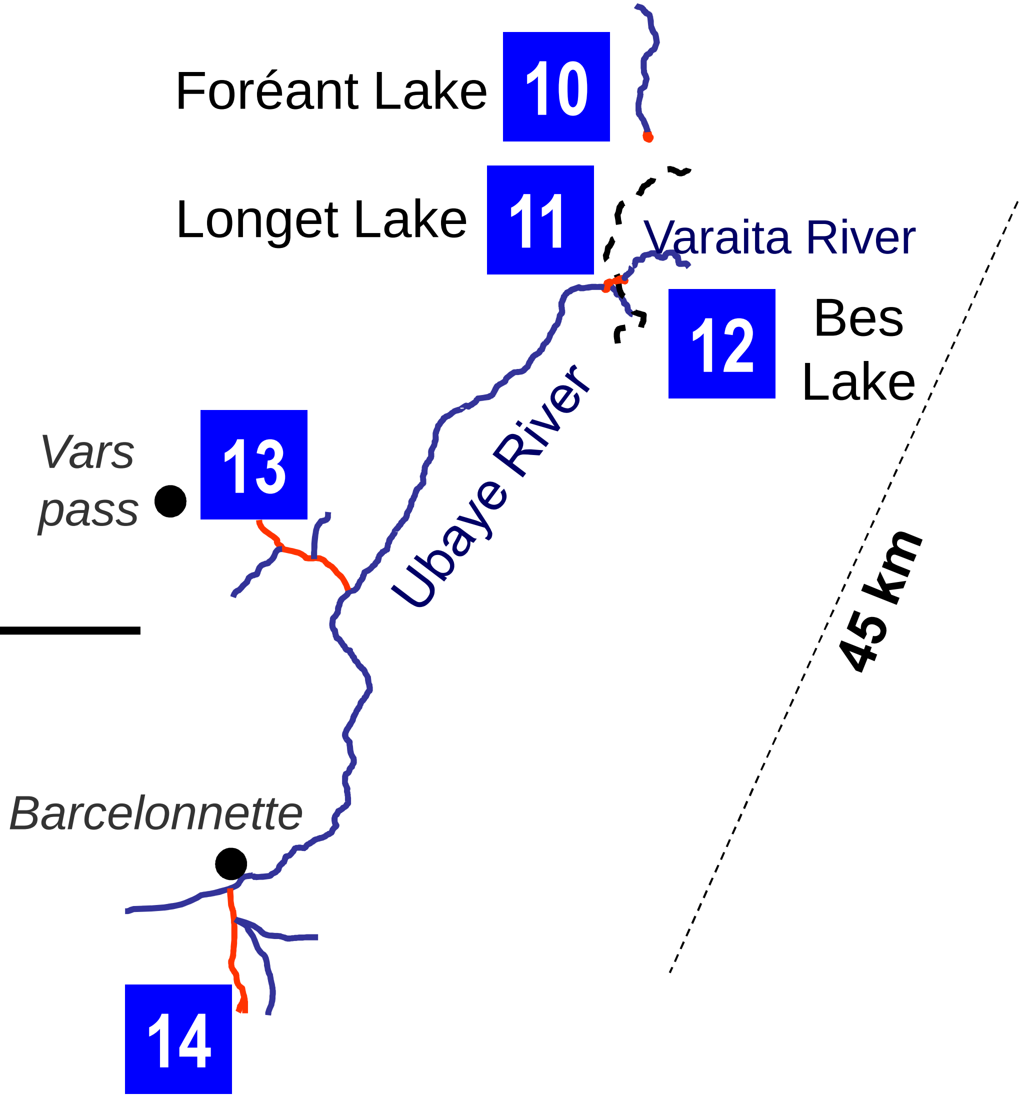
- Can we evaluate the biogeography of a pathogen independent from cropping patterns and transport of plant materials?
- Do plant pathogens have other key roles in the environment?

Biogeography of *Pseudomonas syringae* in source waters of rivers



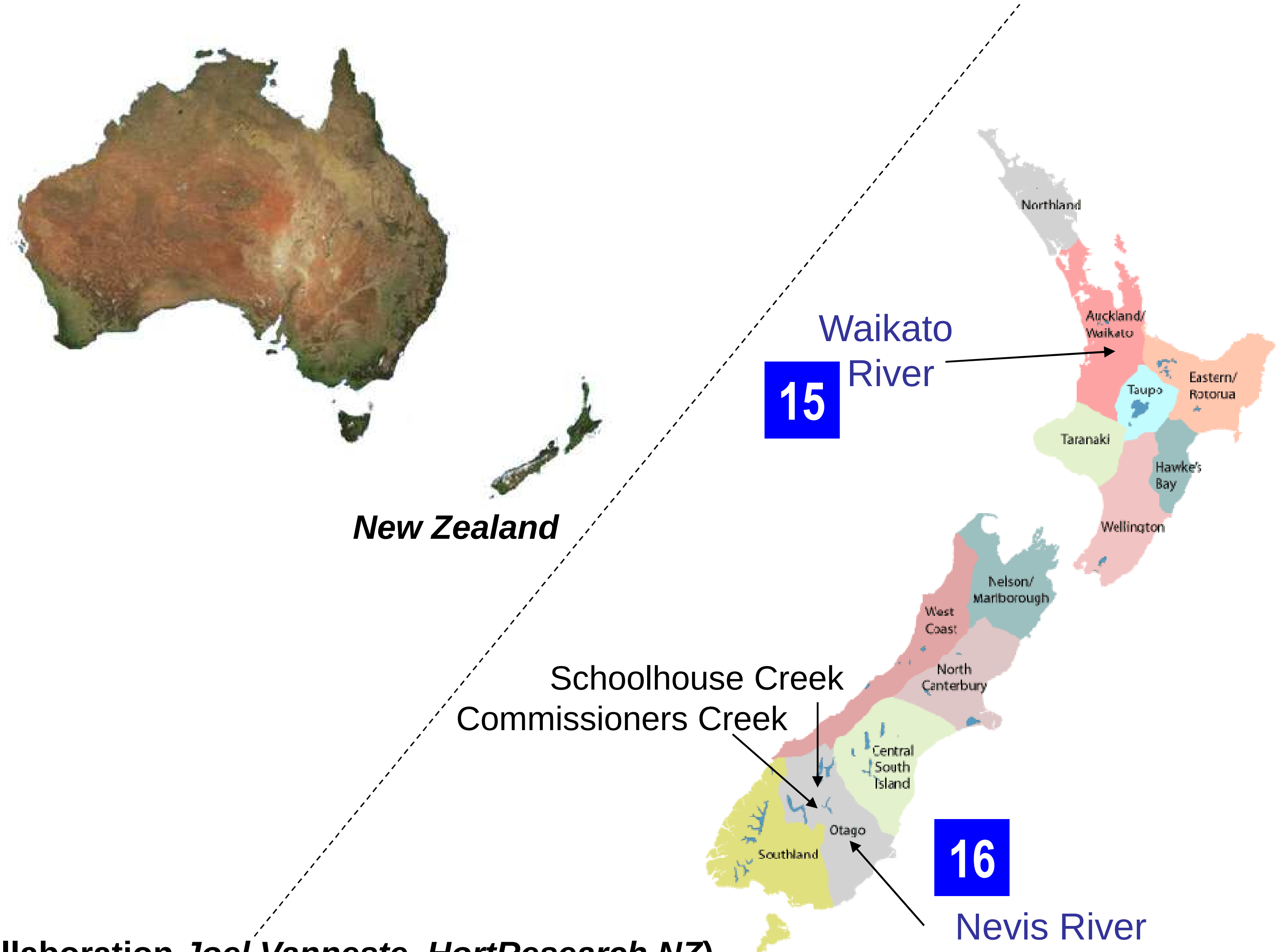


north of Yellowstone National Park
in Grand Teton National Park
Yellowstone River
Bozeman, MT
60 km
1
2
3
4
Jackson Lake
Snake River
40 km
5
6
7
8
9



21 **source of Tarn River**

22 **source of Viaur River**



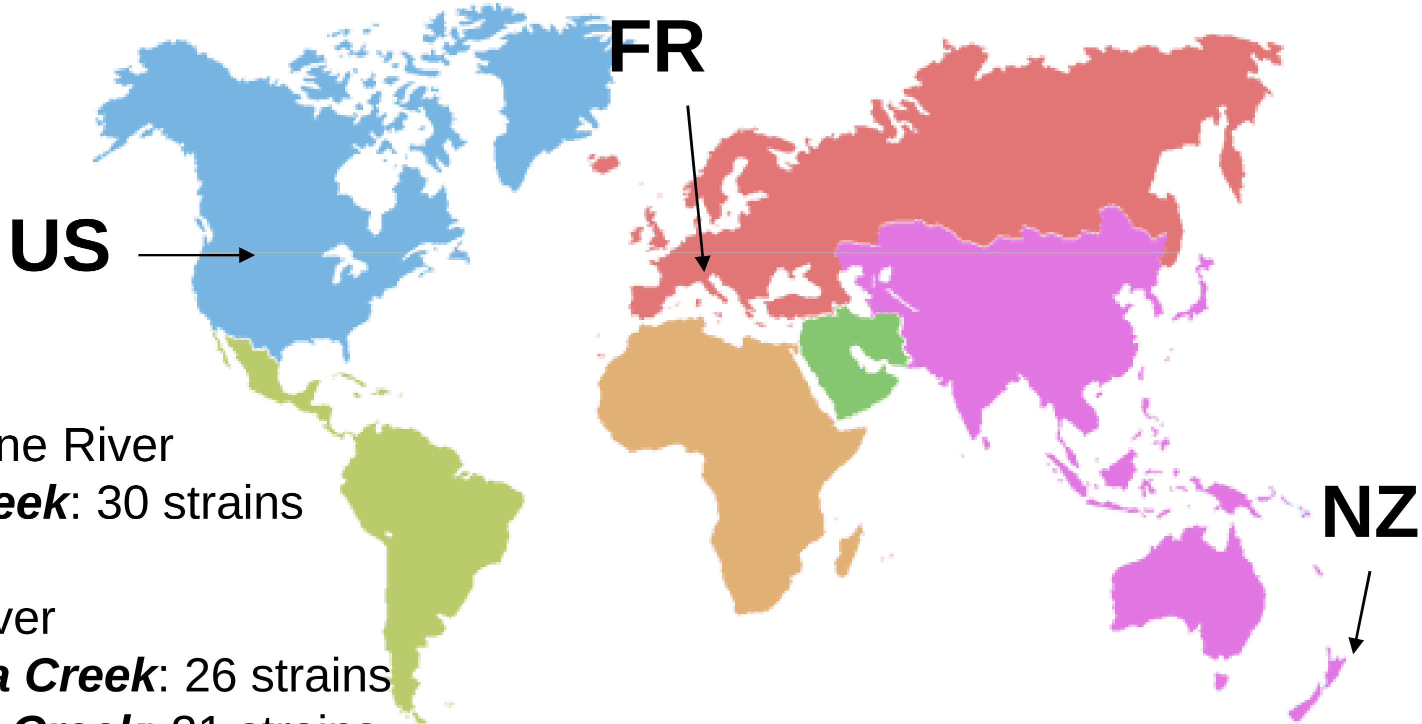
(collaboration *Joel Vanneste, HortResearch NZ*)

		log cfu / L	
Site	date	*P. syringae*	total bacteria
1	Aug 2007	3.92	6.93
2	Aug 2007	2.75	5.84
3	Aug 2007	3.04	6.27
4	Aug 2007	2.62	6.25
5	Aug 2007	2.86	7.48
6	Aug 2007	2.69	6.82
7	Aug 2007	2.86	8.10
8	Aug 2007	(<1.00)	6.48
9	Aug 2007	2.84	6.32
15	Dec 2006	2.49	4.95

		log cfu / L	
Site	date	*P. syringae*	total bacteria
10	Jun 2006	4.41	*nd*
11	Jun 2006	2.69	*nd*
12	Jun 2006	3.75	*nd*
13	Jul 2006	2.77	6.32
13	Apr 2007	1.52	6.04
13	May 2007	(<1.00)	5.28
14a	Apr 2007	4.32	7.25
14a	May 2007	2.16	5.55
14b	Apr 2007	3.38	7.30
14b	May 2007	1.56	5.62
21	Apr 2007	2.65	7.24

Strain collection for biogeographical analysis

Ubaye: ca. 400 strains
Sauze: ca 120 strains
Tarn: 24 strains
Viaur: 30 strains

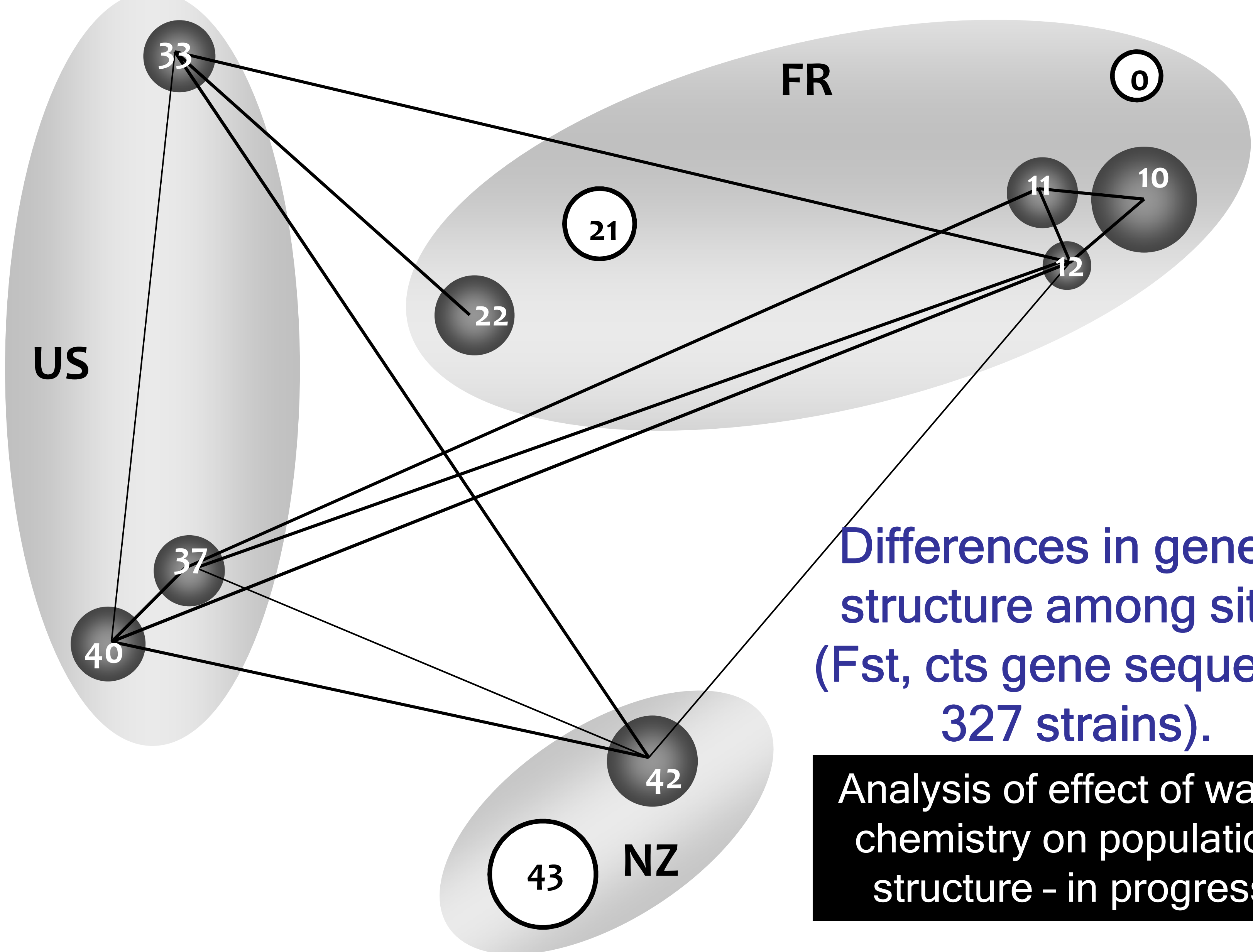


Yellowstone River
Pine Creek: 30 strains

Snake River
Arizona Creek: 26 strains
Pilgrim Creek: 21 strains
Cascade Creek: 29 strains

Schoolhouse Creek: 75 strains
Commissioners Creek**:** 30 strains

Preliminary results - Population structure vs. geographic location: any dissemination patterns?



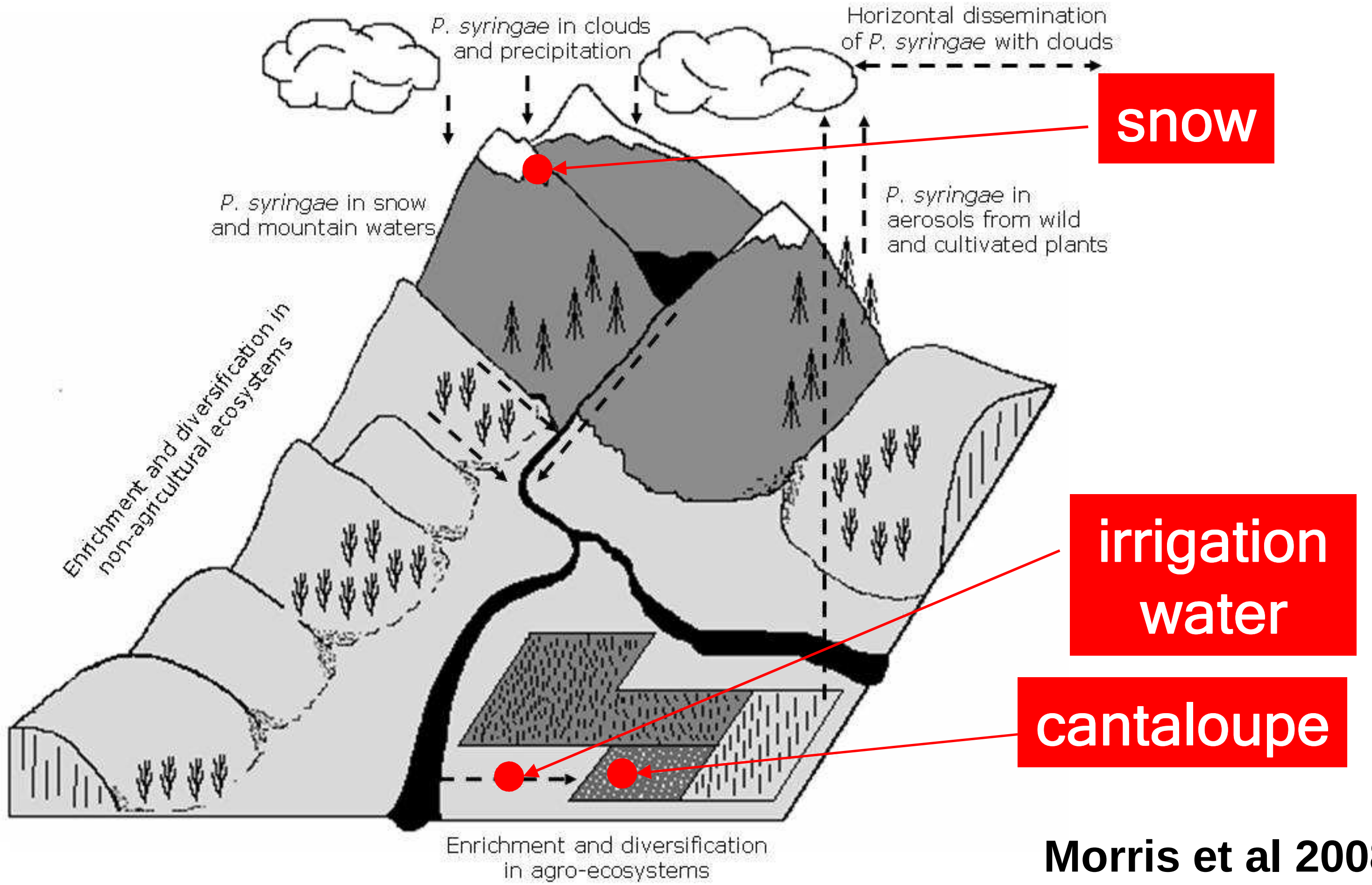
Differences in genetic structure among sites (Fst, cts gene sequence 327 strains).

Analysis of effect of water chemistry on population structure - in progress

How is *P. syringae* related to the water cycle : simple passenger or active partner?

How is *P. syringae* related to the water cycle : simple passenger or active partner?

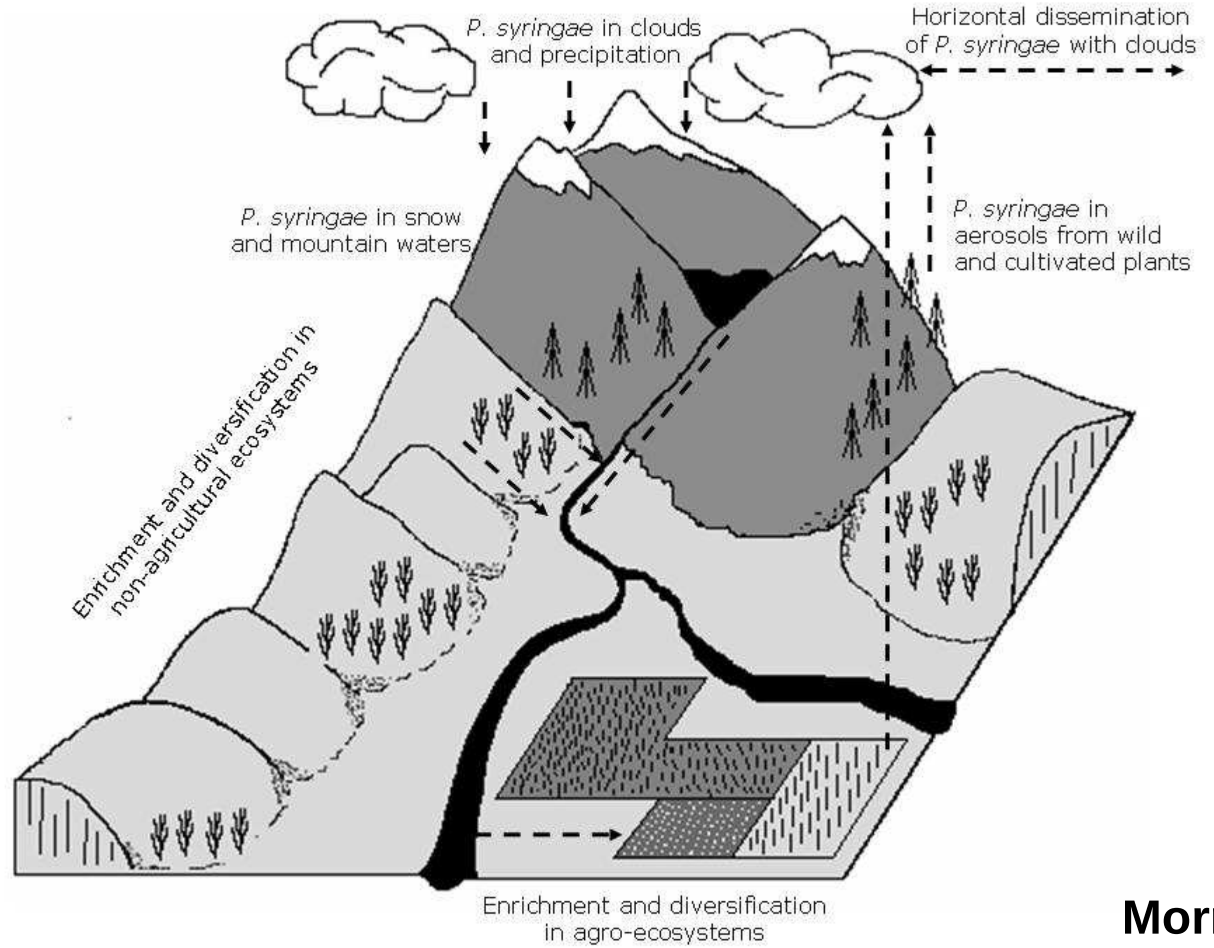
It 'rides' the water cycle: clones are present in different substrates (identical for 10 housekeeping gene sequences, Vinatzer et al).



Morris et al 2008

How is *P. syringae* related to the water cycle : simple passenger or active partner?

Does it play a role in the water cycle: induction of precipitation via INA ?

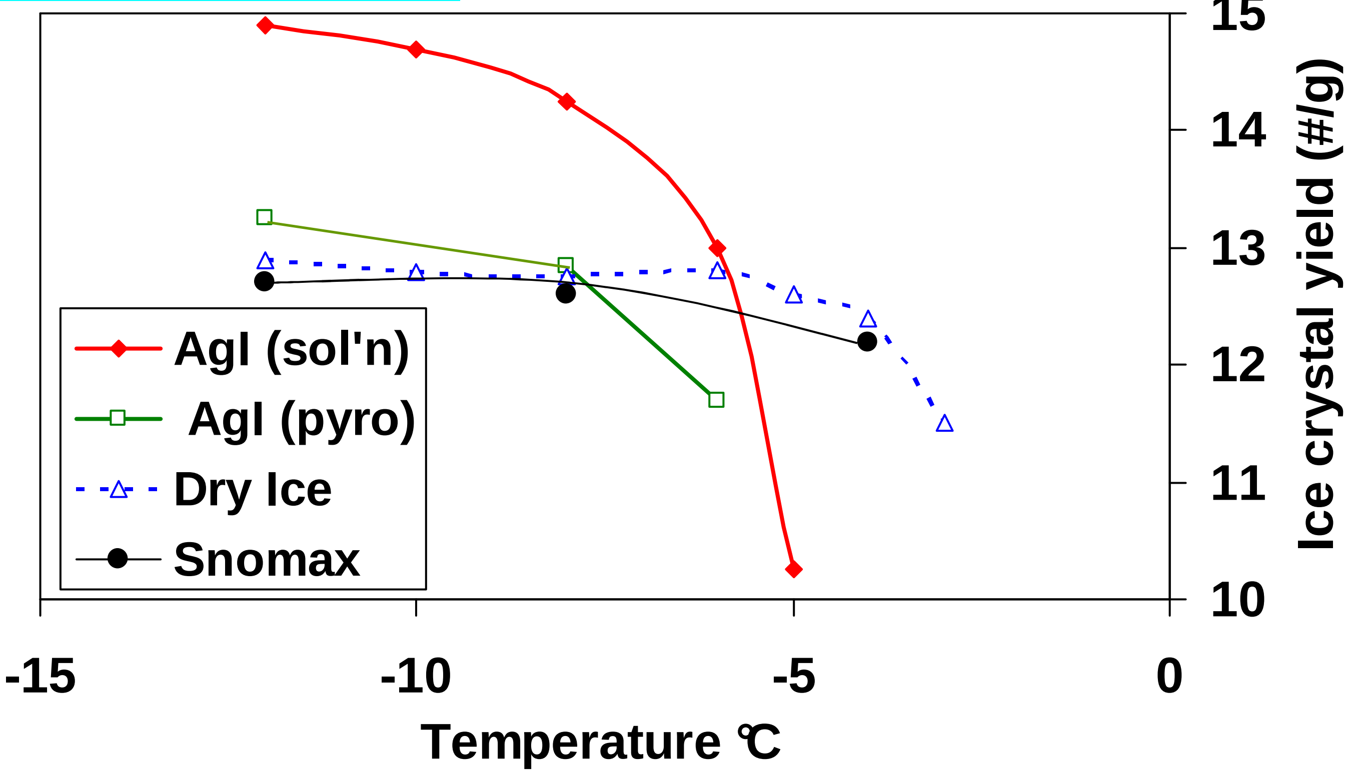


Morris et al 2008

Indirect evidence concerning the role of *P. syringae* in precipitation

Ward, P.J., and P.J. DeMott. 1989. Preliminary experimental evaluation of Snomax snow inducer, *Pseudomonas syringae*, as an artificial ice nucleus for weather modification. *J. Weather Modif.* 21:9-13.

Chamber experiments



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Cloud seeding experiments in the field:

« In one case, visible glaciation effects were apparent as warm as the -5℃ level…. No ice crystal concentration data were collected »

Indirect evidence concerning the role of *P. syringae* in precipitation

Möhler, O., et al. 2008. Heterogeneous ice nucleation activity of bacteria: new laboratory experiments at simulated cloud conditions. *Biogeosciences* 5:1425-1435.

AIDA experiments

Institute for Meteorology and Climate Research, Karlsruhe, Germany

Strains of
P. syringae, *P. viridiflava*, *E. herbicola*

- No significant ice activity above −7C.
- They are ice-active @ −7 and −11C
- INA fraction = 10^{-4}.

To demonstrate that *P. syringae* plays a role in precipitation

Interdisciplinary research: meteorology, atmosphere physics, microbiology, modeling

Ideal field sites: orographic clouds, sites with important changes in land use patterns, 'controlled' sources of *P. syringae*

Historical records: the archives of ice cores

Tools for quantifying biological ice nuclei and *P. syringae* ice nuclei

« Microbiological Meteorology »

Implications of non agricultural habitats of a plant pathogen for epidemiology, ecology and evolution

Conceptual:

To what type(s) of niches did the ancestor of *P. syringae* adapt?

Is *P. syringae* an aquatic bacterium ?

What environmental factors drive the evolution of ‘modern’ *P. syringae* ?

Are there environmental factors that are equal or stronger selective pressures than crop genotype for emergence of pathotypes? (co-evolution)

Implications of non agricultural habitats of a plant pathogen for epidemiology, ecology and evolution

Epidemiology and disease control:

Does *P. syringae* play beneficial roles in the environment? If so, how do we balance disease control strategies with protection of these other roles?

If *P. syringae* is in old ice, does this give new meaning to "latency" in epidemiological cycles (as the ice melts) ?

Is ice nucleation activity a means of assuring deposition of bacteria (and other light particles with which it is associated)?

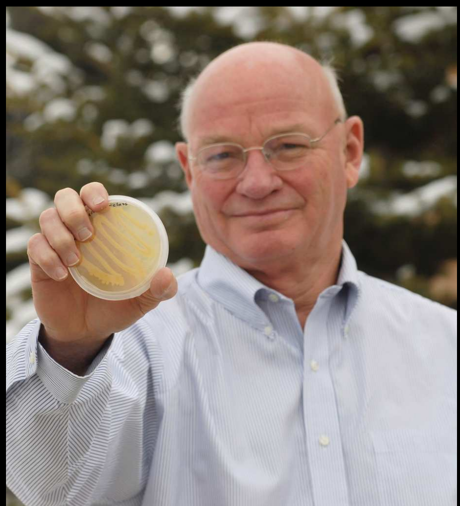
In progress

Modeling the flow of *P. syringae* from snowpack to surface waters in the geological context of the southern Alps and its consequence on disease epidemiology.

Doctoral thesis, Caroline Monteil

Association of rust spores with ice nucleation active *P. syringae*: impact on dissemination and survival.

Collaboration with ICARDA (Syria) and
Montana State University



Dave Sands, MSU

Boris Vinatzer, VA Tech

Joel Vanneste, Plant & Food Res., NZ