Perspectives on the roles of ice nucleation in the microbial world

Is there a role for ice nucleation activity in bacterial dissemination?

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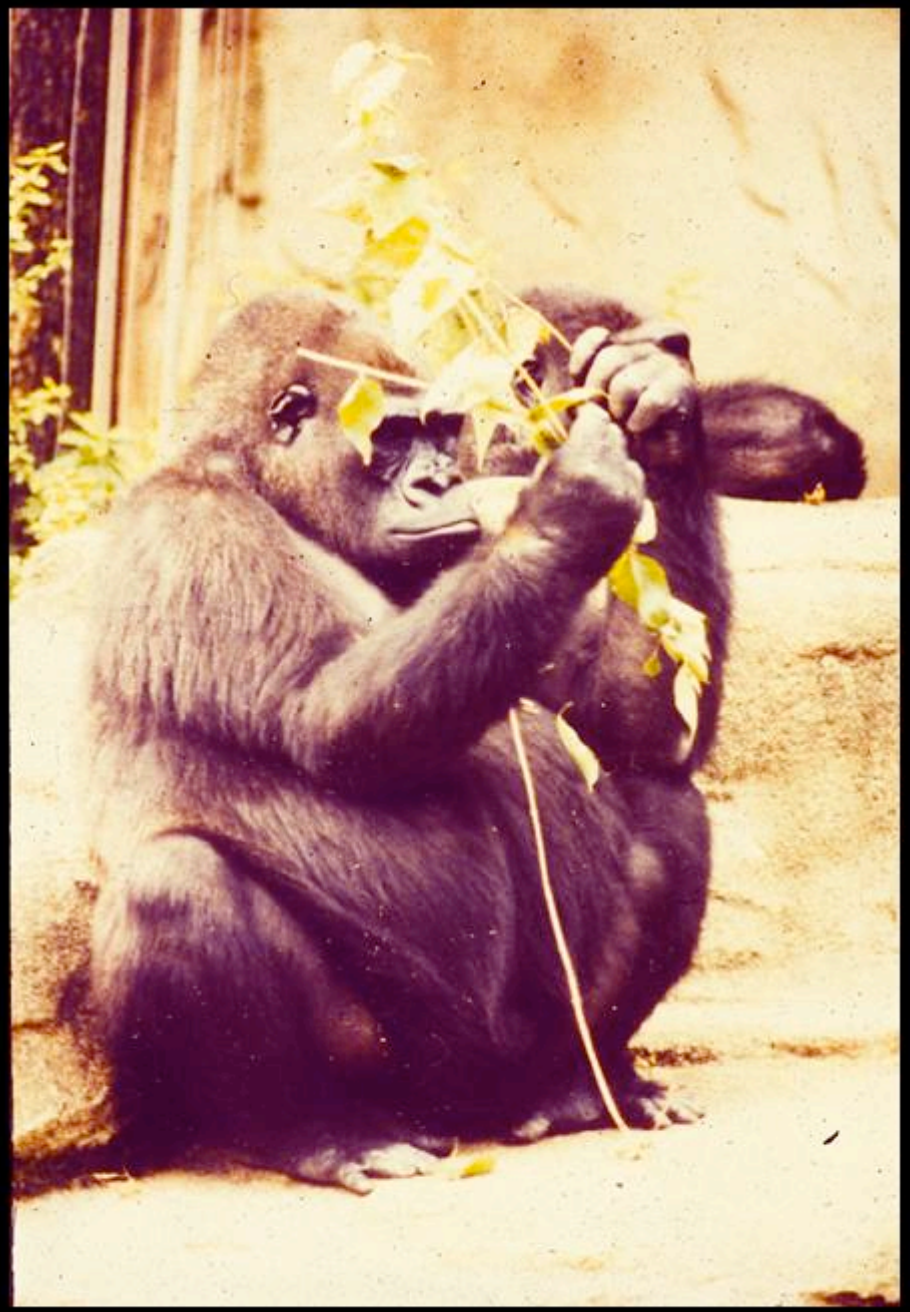


Plant Pathology
The oldest profession

INA plant pathogenic bacteria: different lifestyles

Xanthomonas campestris

all strains are host specific

e.g.: *X. campestris* pv. *translucens*

Pseudomonas syringae

host specific and broad host range strains

e.g.: *P. syringae* pv. *syringae*

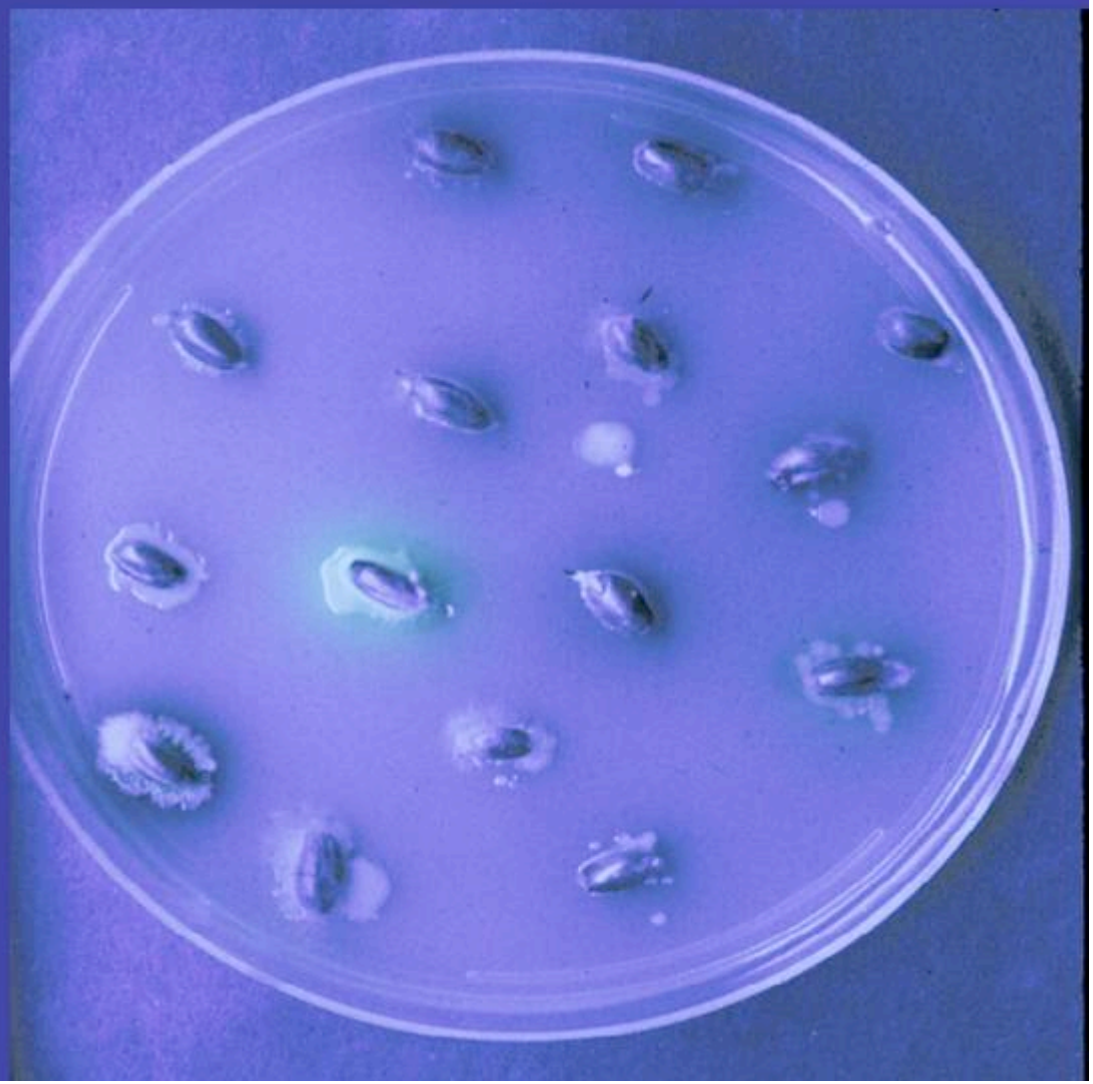
P. syringae pv. *tomato*

P. syringae pv. a
Leaf Blight on Barley

X. campestris pv. *transluscens*
Black chaff of wheat

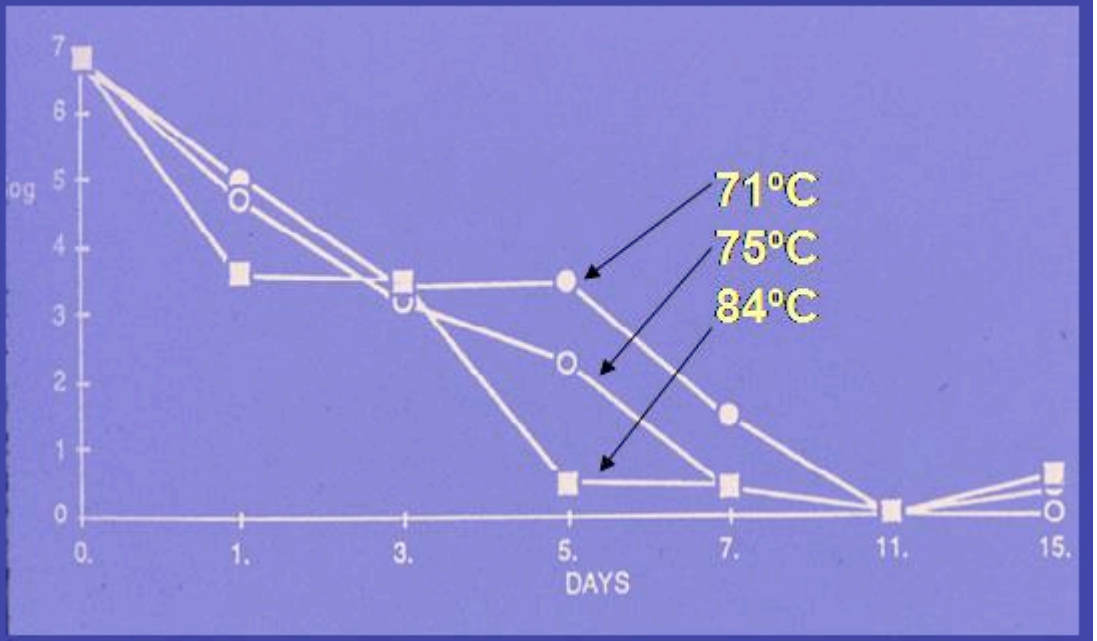
X. campestris pv. *transluscens* and *P. syringae* pv. asas are seed borne

Checking wheat seed for infestation by *P. syringae* on BCBRVB selective medium



In general, seed treatment is thought to be an effective means to control seed-borne diseases

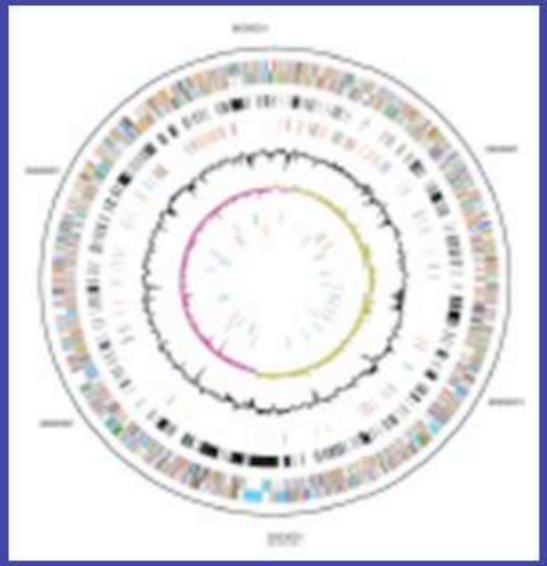
Chemical treatment (Bronopol) for *P. syringae*



Heat treatment for *X. campestris*

SEED TREATMENT -- BRONOPOL

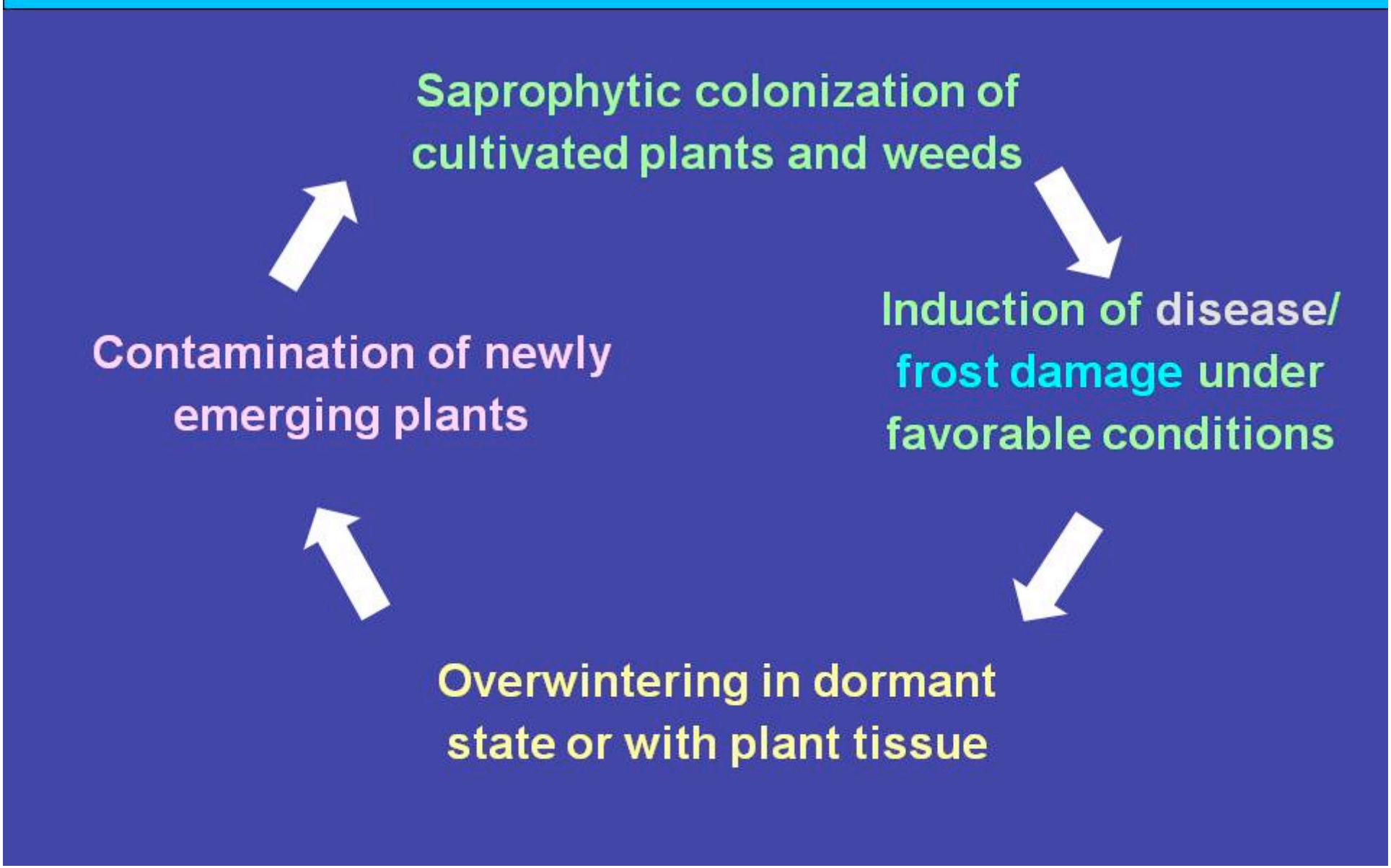
Pseudomonas syringae, an archetype among plant pathogenic bacteria



Genome
6 Mbp

Protein-coding genes
5000

An agro-centric perspective dominates our understanding of the life history of *P. syringae*



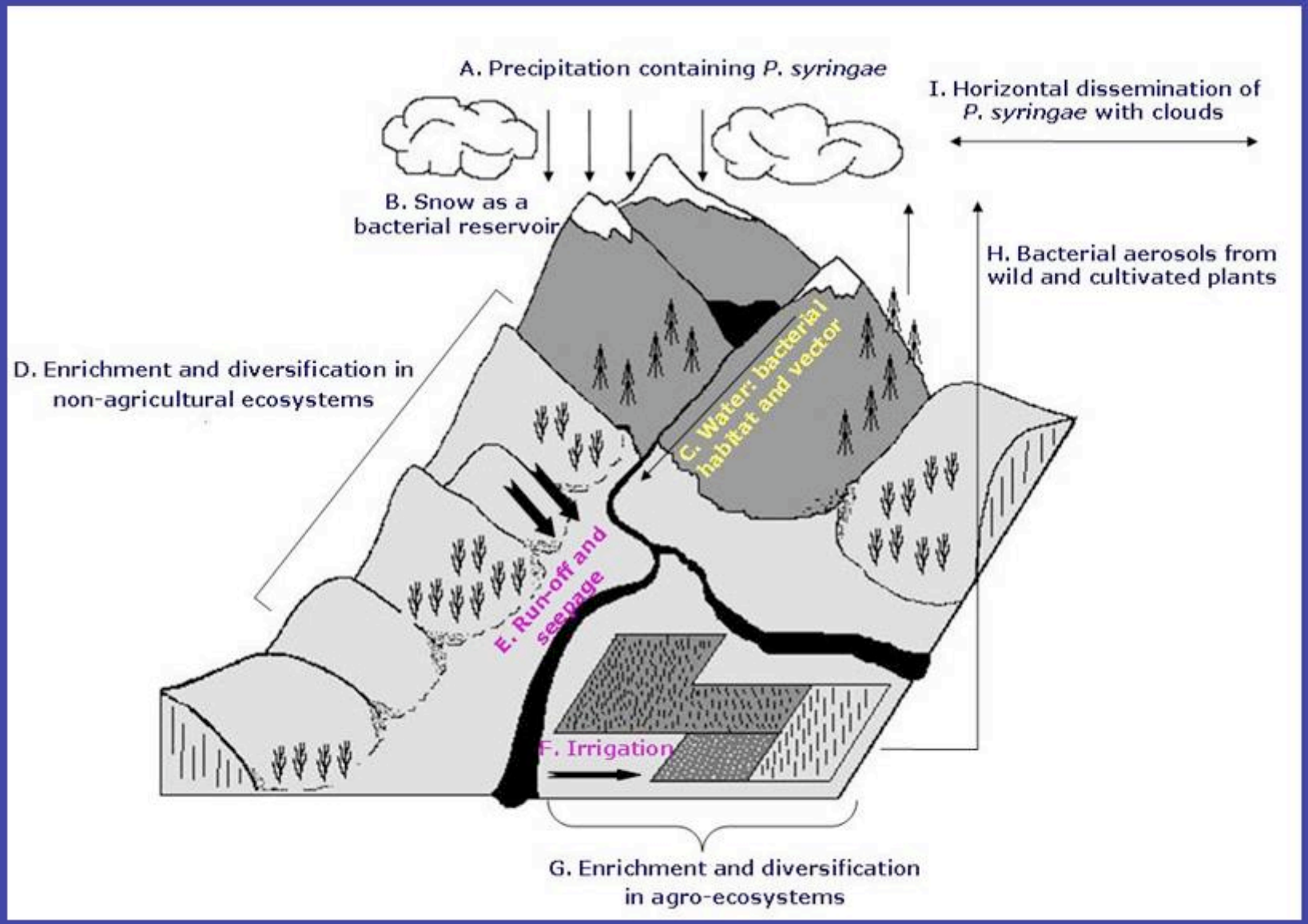
Data illustrating the abundance of *P. syringae* in numerous non-agricultural reservoirs

Substrate	# samples	# positive	Country and site		*Ps* (HR+) CFU / g or / L
Snow, fresh	9	3	Fr	1200 m	$150 - 10^5$
Snow, firn	5	3	Fr, Au	2600 - 3000 m	$100 - 5000$
Lakes and streamheads	7	4	Fr, It	2100 - 2600 m	$100 - 10^4$
Epilithic biofilms	16	7	Fr, USA	roadside; alpine > 2000 m	$50 - 10^4$
Rain	4	1	Fr	poolside backyard	10^4

Data illustrating the abundance of *P. syringae* in numerous non-agricultural reservoirs

Substrate	#samples	#positive	Country and site		*Ps* (HR+) CFU / g or / L
Primulaceae	21	14	Fr, It, USA	forest; roadside; alpine $\geq$ 2000 m; border of national parks	$\leq 10^4$
other wild flowers (4 spp)	5	4	Fr, It	forests; alpine $\geq$ 2000 m	10^5
Cloud water (Amato et al)	?	2	Fr	1450 m	ca. 10^4

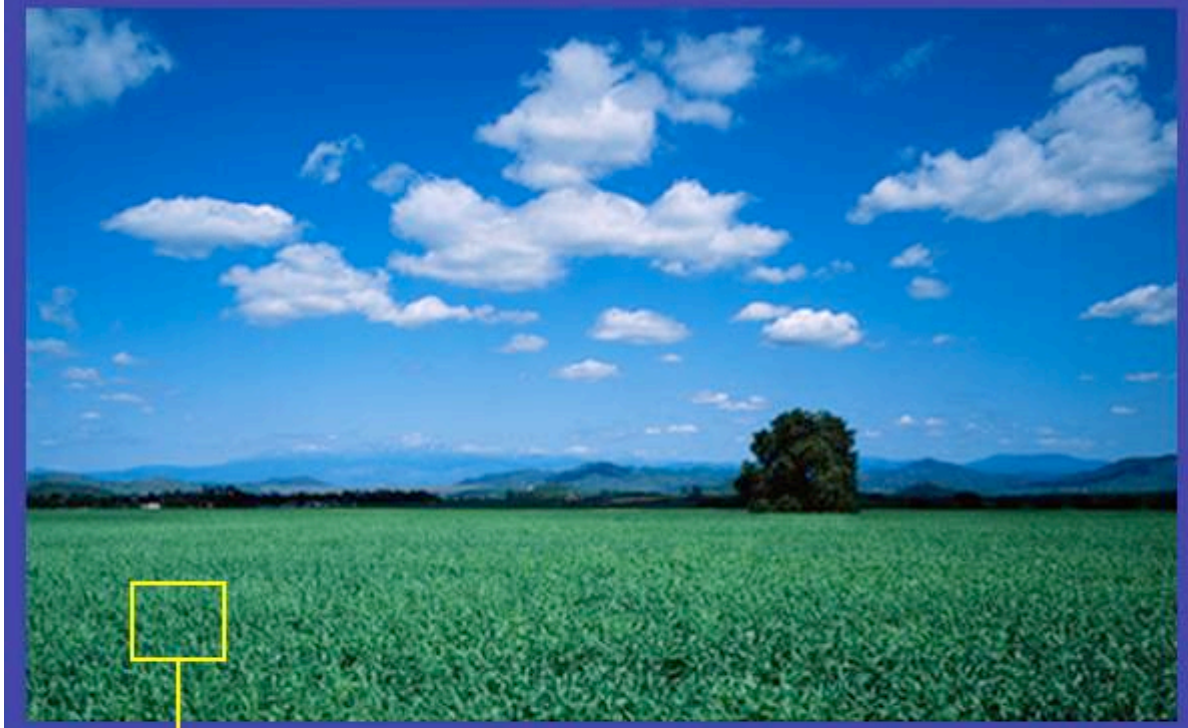
A comprehensive view of the habitats and flux of *P. syringae* in the environment is emerging.



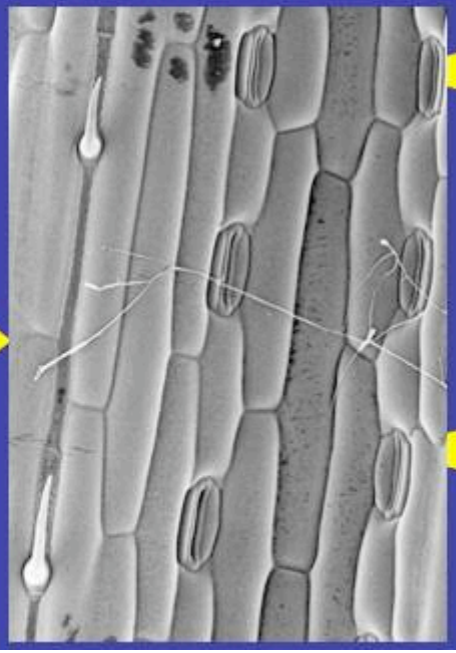
Properties of *P. syringae* from different habitats

source	# strains tested	% INA ≥ -6°C	% virulent on ≥1 plant spp.	% virulent on 3 plant spp.	% producing syringomycin
Primula spp.	61	75	54	18	52
Snow	49	100*	49	12	61
River epilithon	20	70	25	5	15*

* Significantly different ($p < 0.05$) from other sources based on Fisher's exact test



200 X



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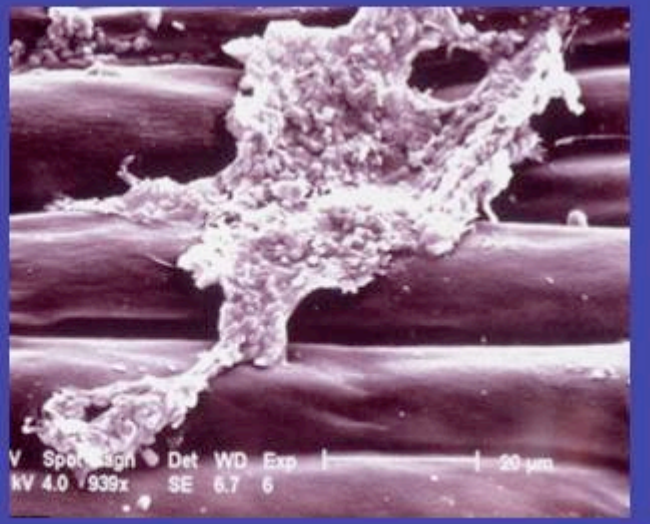
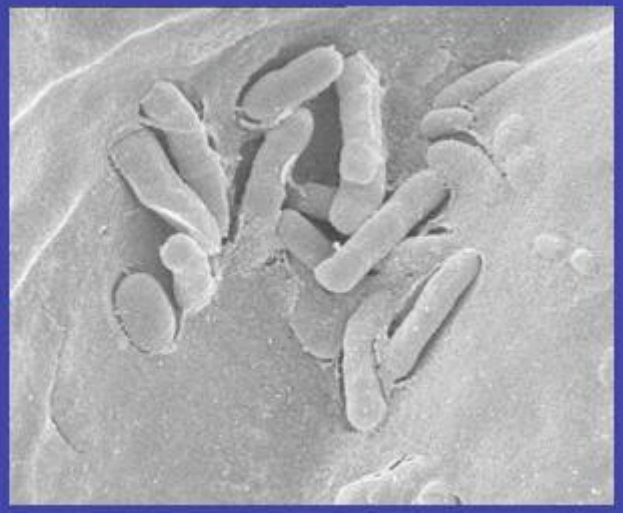


20 X



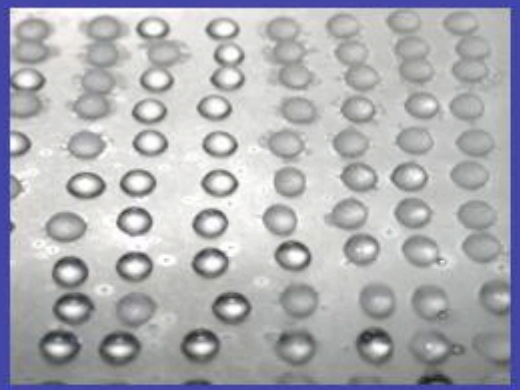
Bacteria on plants

- Common on leaves and other aerial parts of healthy plants
- Large diversity of types (species) co-inhabit leaves
- Numbers on leaves:

  $10^4 – 10^8$ bacteria / cm^2

Ice nucleation-active bacteria:
common leaf surface bacteria

- **Ability to nucleate ice embryos is <u>rare</u> among micro-organisms**
- **But, several species of widespread plant bacteria are INA:**



Pseudomonas syringae
Erwinia herbicola
Xanthomonas campestris

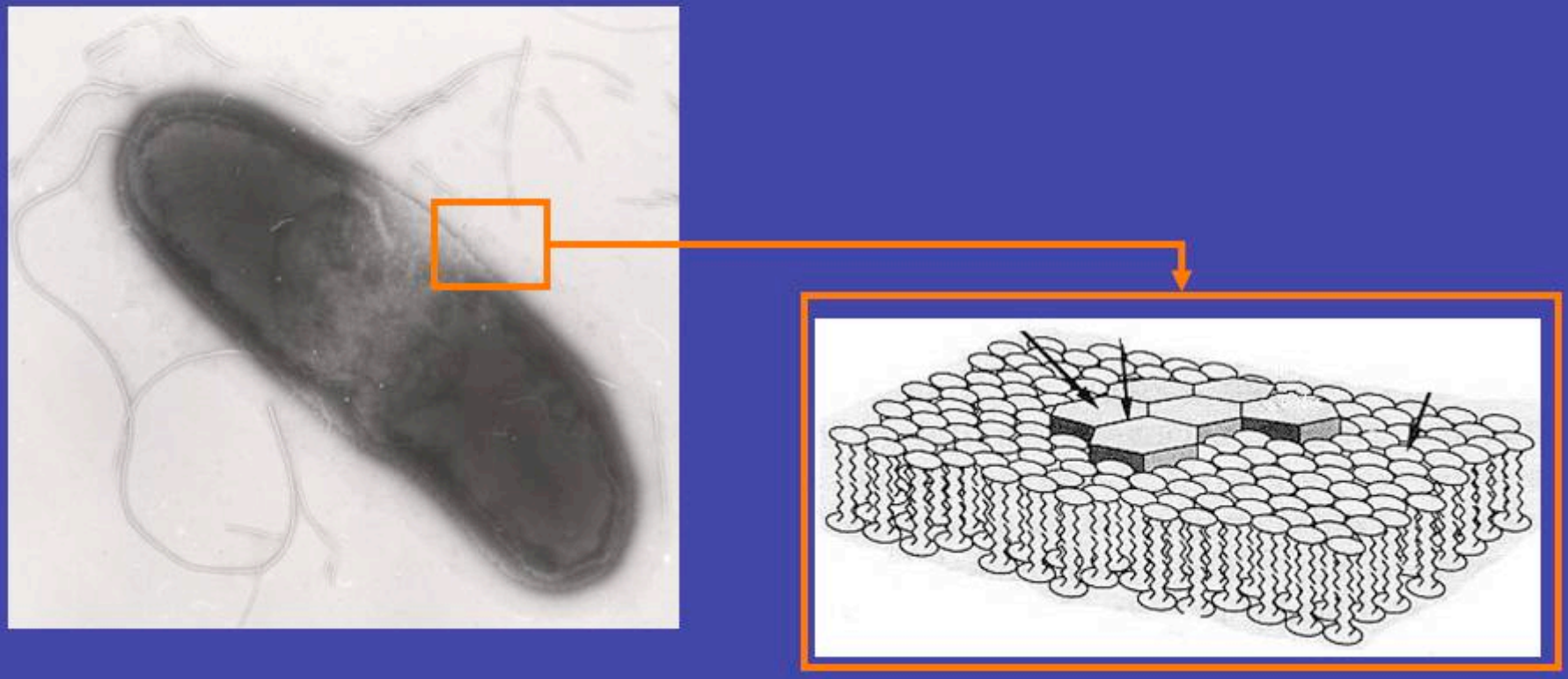
Bacterial ice nuclei: the most active naturally-occurring ice nuclei

Warmest temperature of activity of some ice nucleation-active materials

silver iodide	-8°C
pollen	-5°C
forest aerosols (tree oils + iodine)	-4°C
atmospheric dust	-8°C
cristalline metaldehyde	-0.4°C
Pseudomonas syringae	-2°C

Bacterial ice nuclei: biology

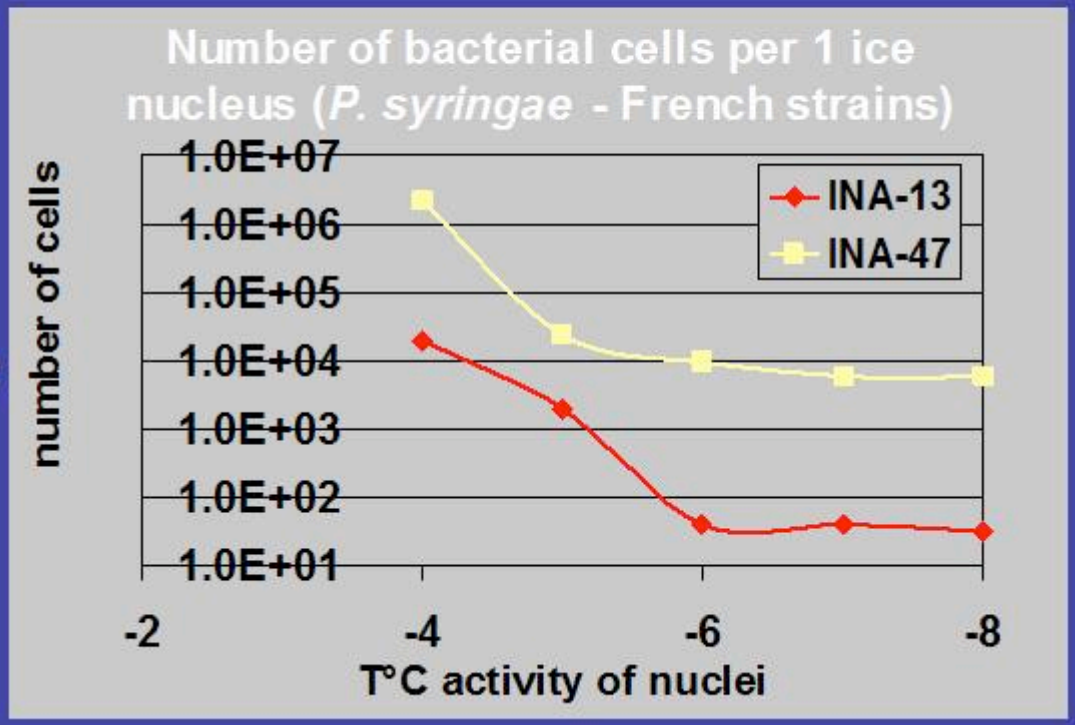
- The ice nuclei of bacteria are proteins in the outer membrane of the cell that interact with water.



Bacterial ice nuclei: biology

- **Bacterial INA is enhanced by environmental conditions (cool temperatures and low nutrients lead to warmer T° of activity).**

- **Not all cells in a population of bacteria have the same T° of activity.**



Bacterial ice nuclei: biology

- **Different plants harbor different amounts of INA bacteria.**

Different plant species

Bacteria / g of leaf (from Lindow et al, 1978).

Amaranth	1.3×10^3
White mustard	2.5×10^5
Soy bean	2.0×10^2
Snap bean	1.6×10^5
Potato	7.9×10^2
Zinnia	1.6×10^6
Quackgrass	1.2×10^4
Kentucky bluegrass	1.6×10^4
Boxelder	6.3×10^6
Red oak	7.9×10^1
Quaking aspen	1.6×10^3

Different varieties within a plant species

Bacteria / g of barley leaf (from Georgakopoulos and Sands, 1992)

CULTIVAR:	
AR9	1.3×10^2
AR3	1.5×10^4
Ershabet	5.2×10^4
AR13	3.4×10^5

Bacterial ice nuclei: biology

- **INA bacteria are naturally disseminated in the air.**

- **Net upward flux (dry conditions)**

  ca. 10^5 bacteria /m^2/h (Lindemann et al, 1982)

- **Dry deposition ca. 2 x 10^3 bacteria /m^2/h**

  (Lindemann & Upper, 1985)

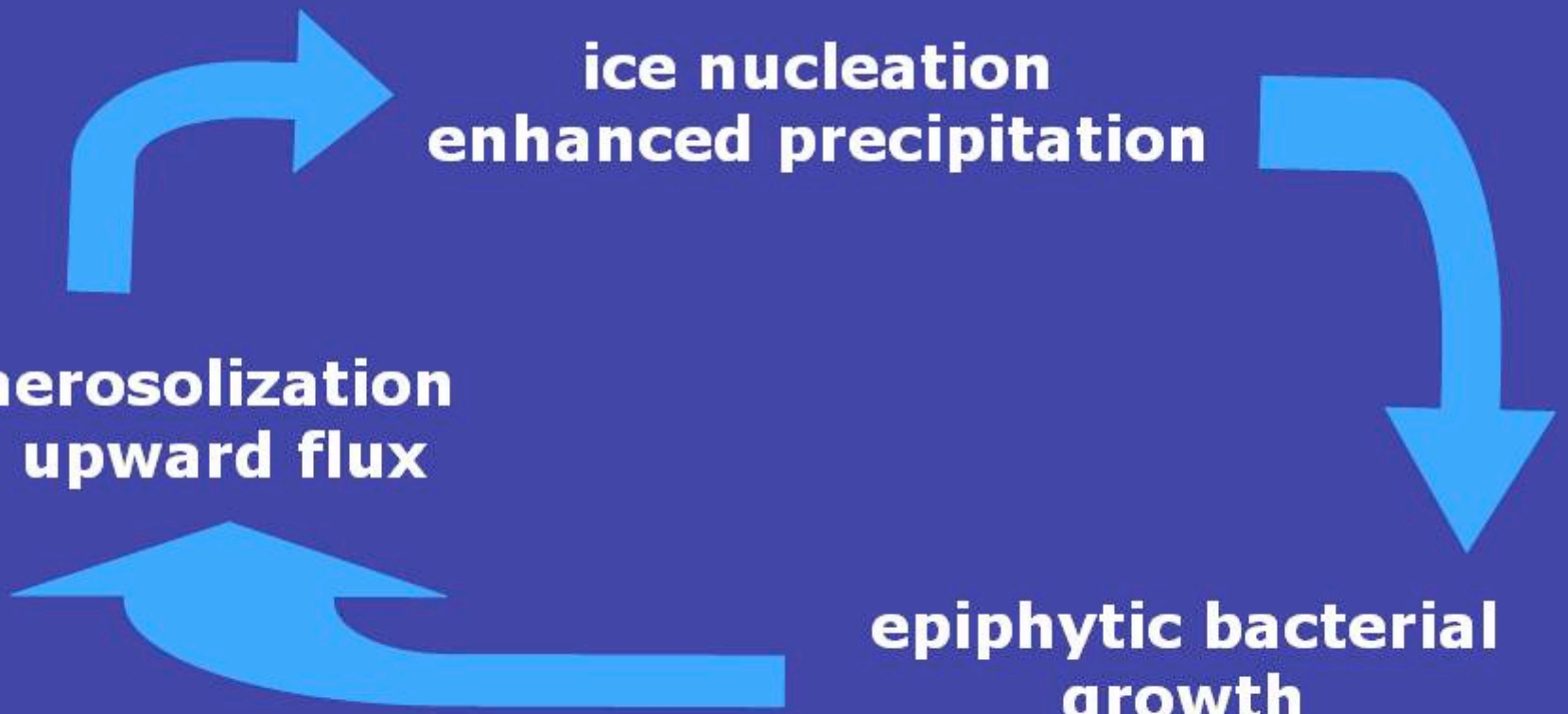
- **Diurnal cycles**

- **Detected in rain clouds**

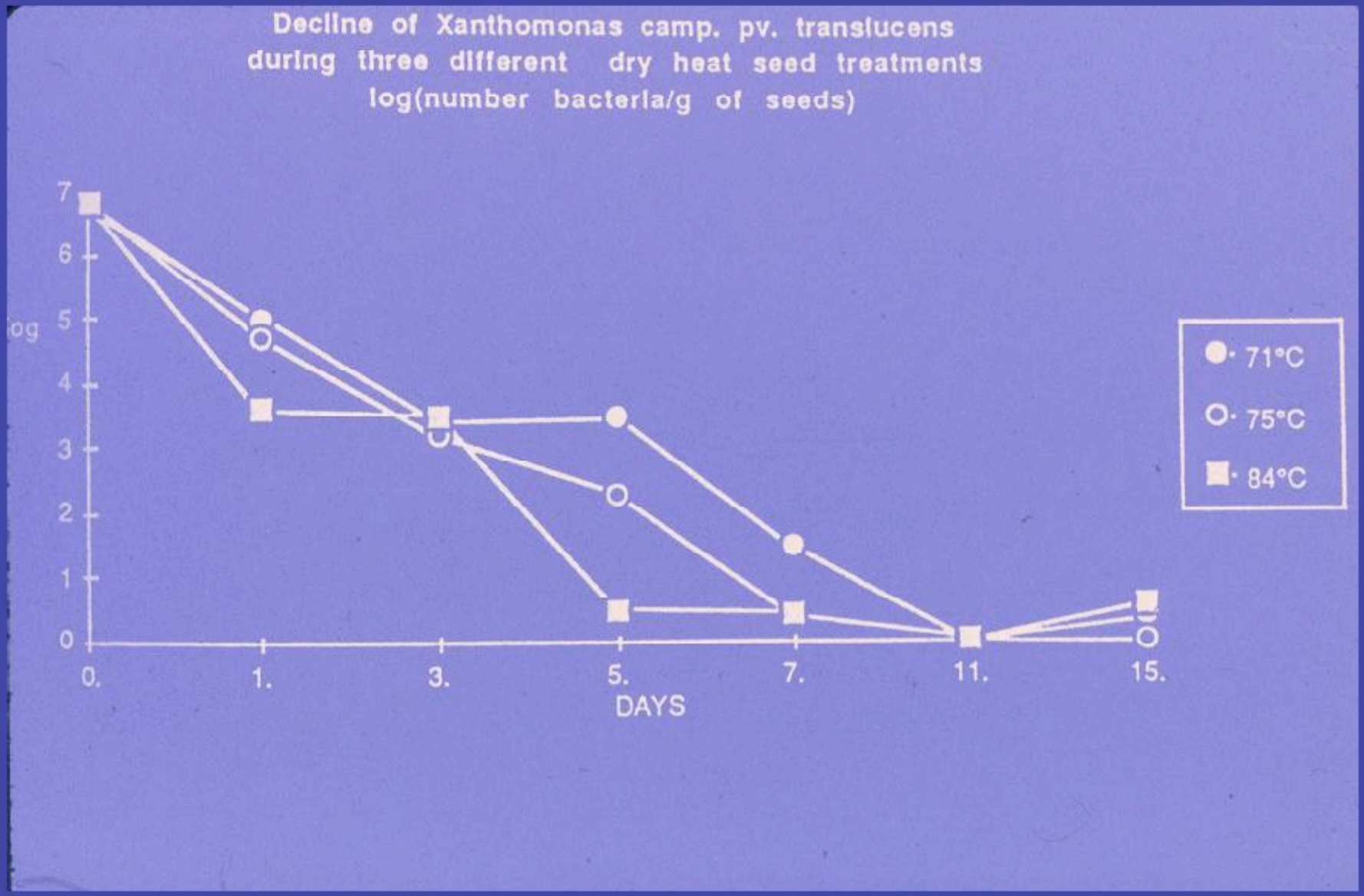
  (Jayaweera & Flanagan, 1982; Sands et al, 1982)

Bioprecipitation concept

Sands et al, 1982, 1985



Dry heat effect on Xanthomonas seed infestation



Germination Reduced by only ca 5%, Infestation by 6 logs

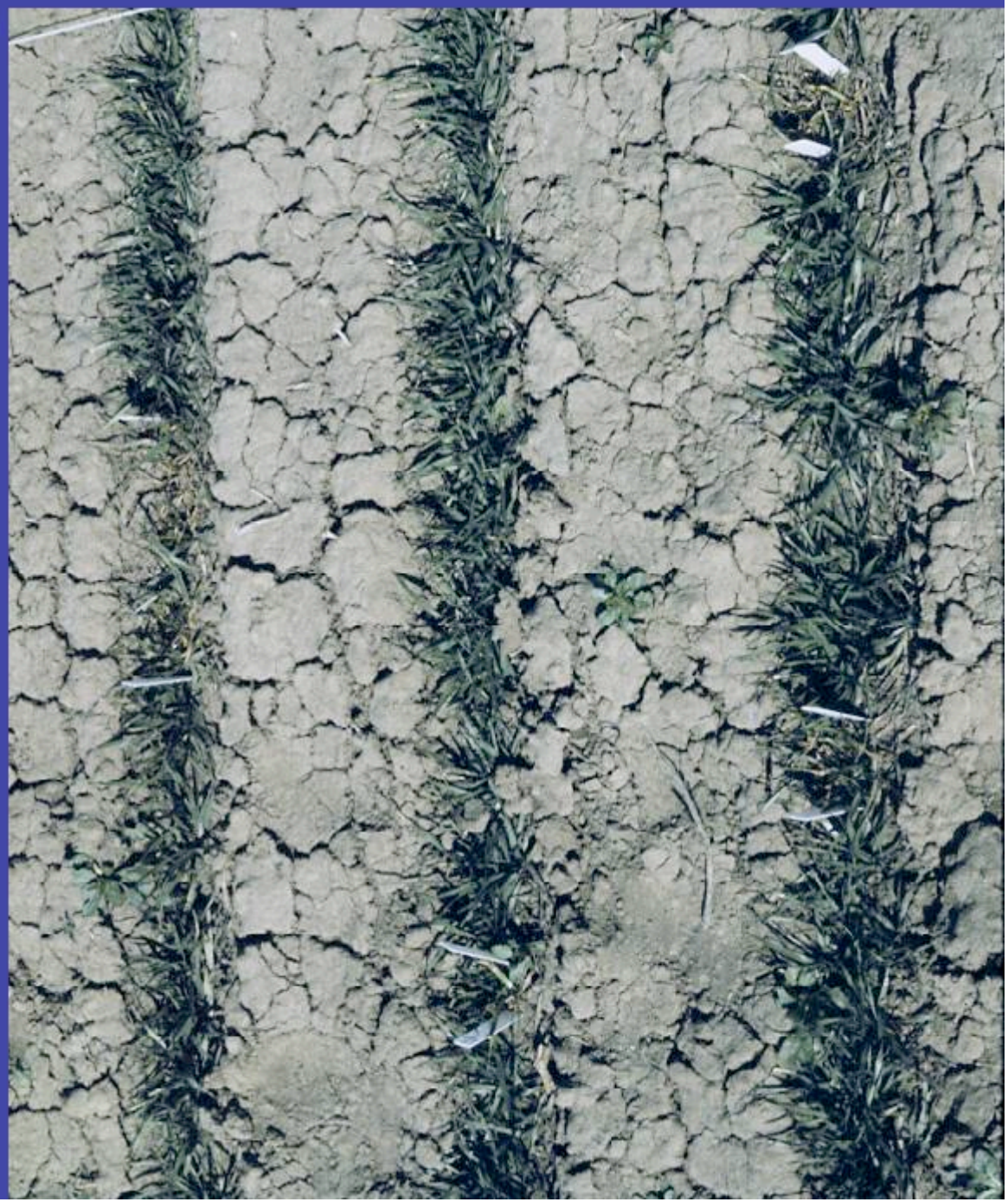
Frost damage, barley, Osorno, Chile, Associated—Pseudomonas syringae

SEED TREATMENT -- BRONOPOL

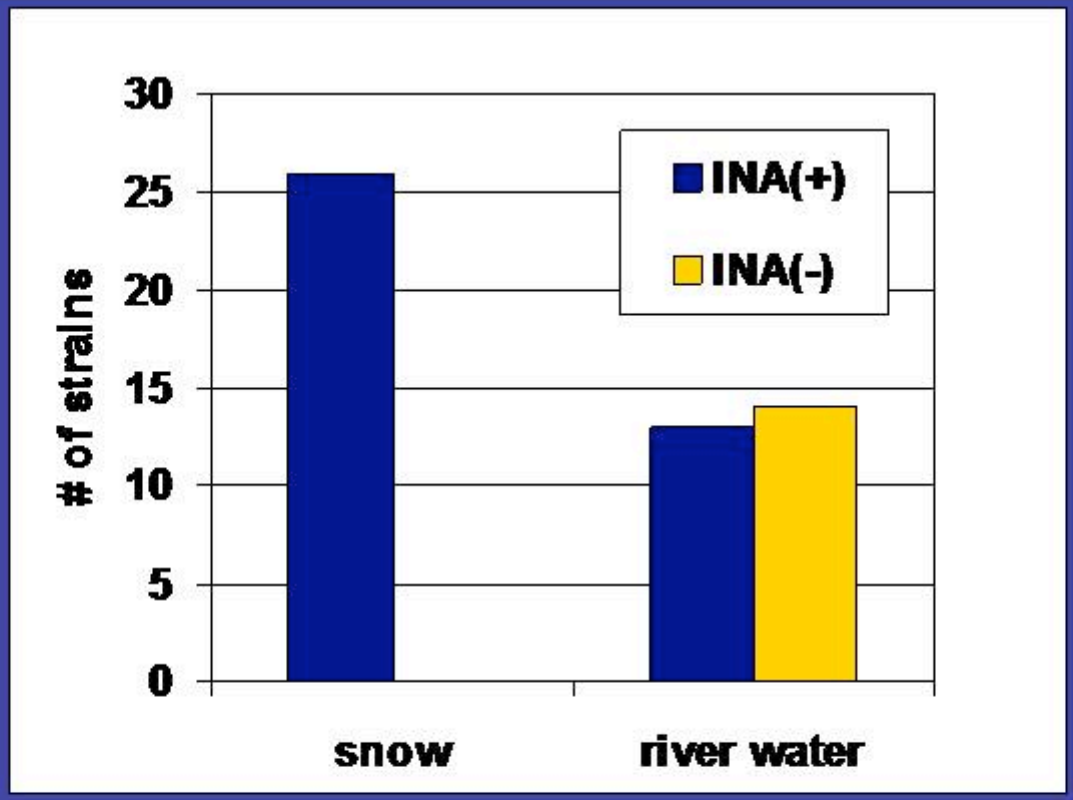
Xanthomonas translucens

Seed Transmission verified in field test.

Infected seed served as point locus for secondary spread 10 days later.

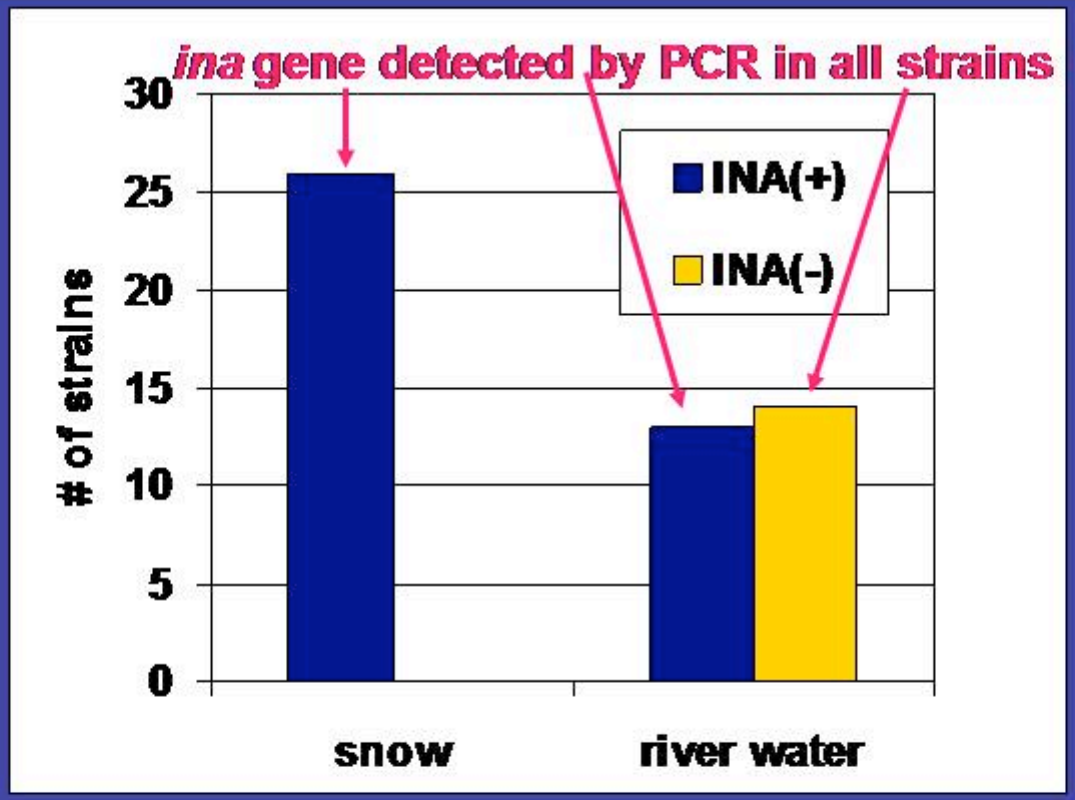


Snow and Ice Nucleation Activity Correlate

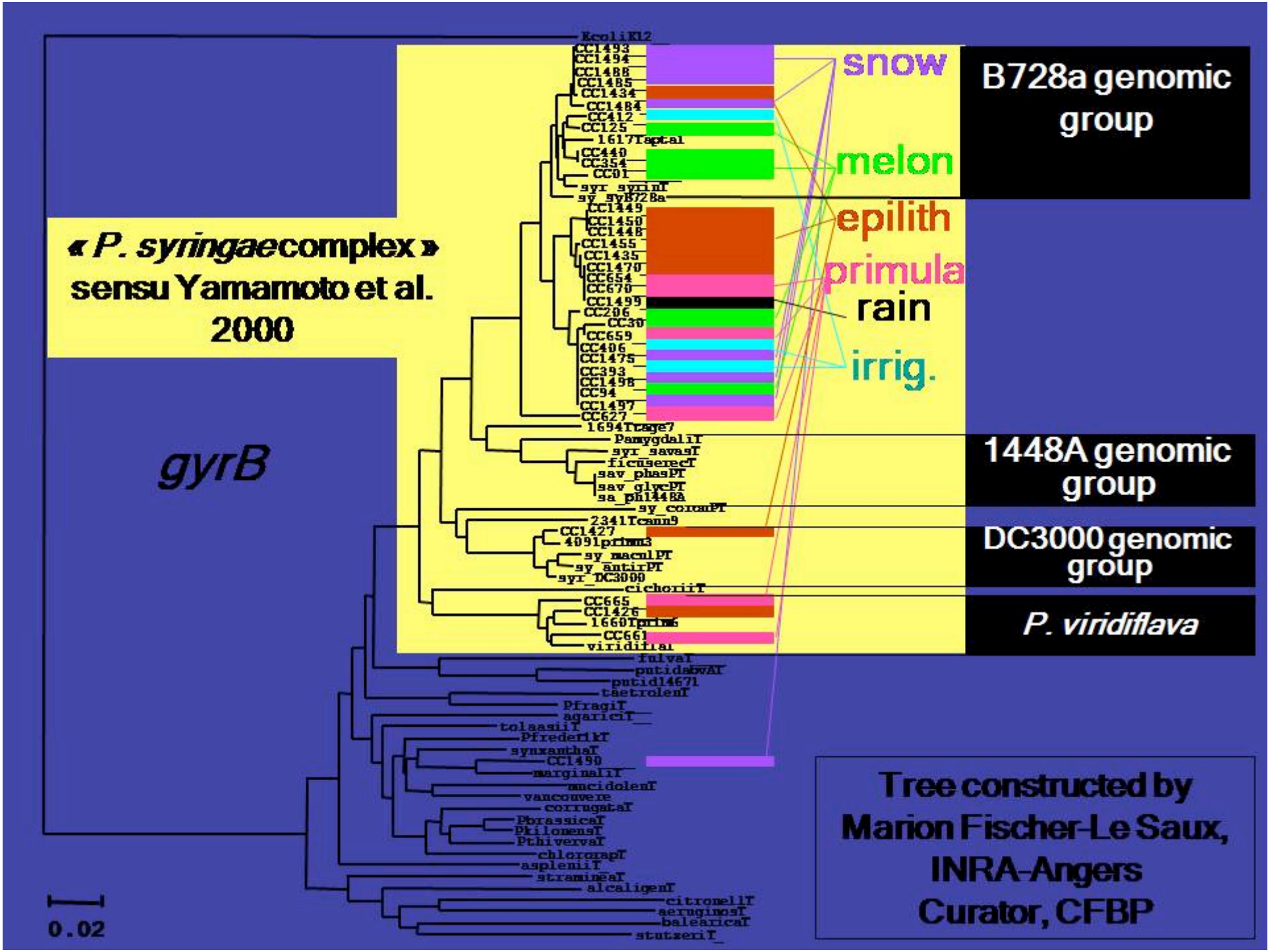


Ice nucleation activity of the 3 main genotypes of *P. syringae* common to snow and river water

Snow and Ice Nucleation Activity Correlate



Ice nucleation activity of the 3 main genotypes of *P. syringae* common to snow and river water


« P. syringae complex »
sensu Yamamoto et al.
2000
gyrB
snow
melon
epilith
primula
rain
irrig.
B728a genomic group
1448A genomic group
DC3000 genomic group
P. viridiflava
Tree constructed by
Marion Fischer-Le Saux,
INRA-Angers
Curator, CFBP
0.02

The cloud guys
with hypotheses
clouded by incomplete data
And bacteriologists with
half a genome in limbo
And global warming
creating flood and famine
With dryness of wit
these poor scientists
Put their theories to the grindstone
and hope for new blood
new minds to drive this machine

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