Landcare Research
Manaaki Whenua

Pathogens don't carry passports

Taxonomic aspects of the Psa story

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Landcare Research, Auckland

Landcare Research Link Seminar, Wellington, 28 May 2013

Taxonomy capability

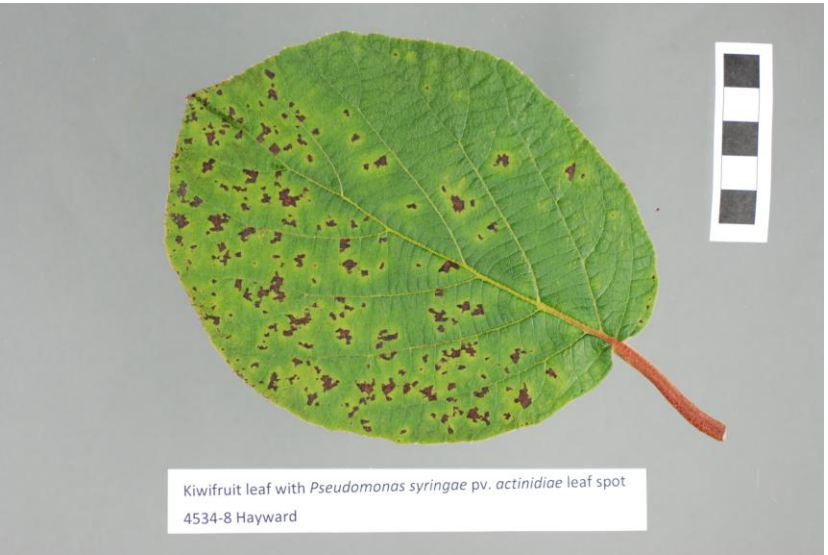
- With Landcare Research for 11 years
  - PhD 2006: rhizobia
  - Postdoc 2011: *Colletotrichum*
- Fungi & Bacteria team: 5 scientists, 5 technicians
- In Auckland
  - Co-located with MPI PHEL

Outline

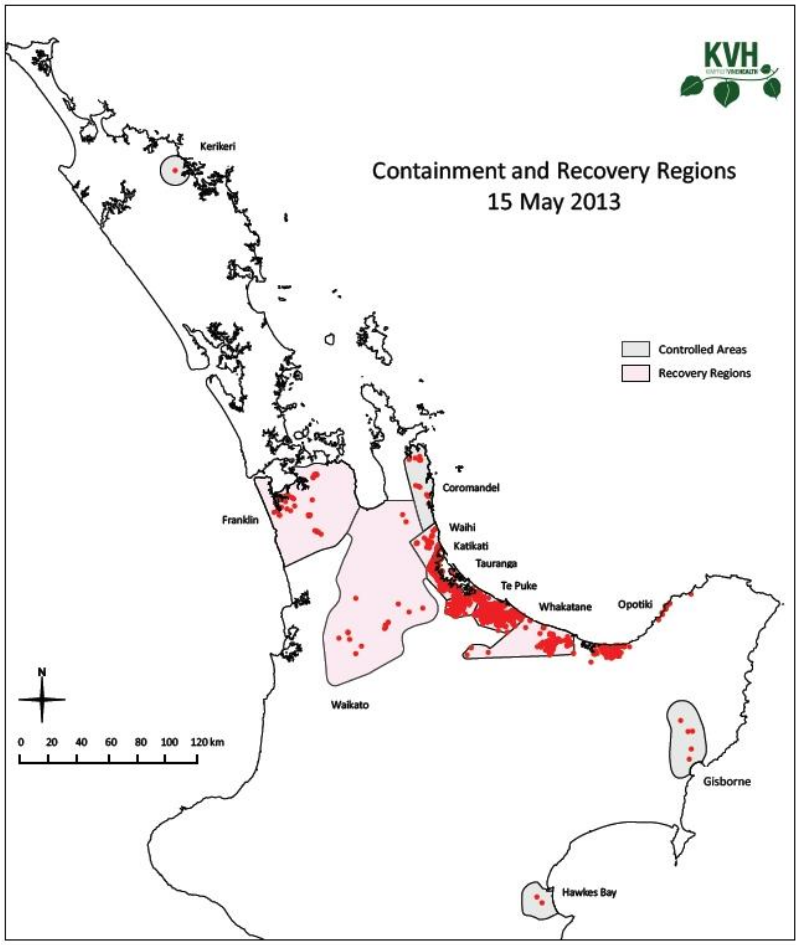
- Pathogens don’t carry passports
  - How do we know what they are?
- How taxonomy + collections + databases can help inform policy decisions
- With reference to the recent Psa kiwifruit disease
  - But relevant to many plant pathogens and fungi
  - PTA, Myrtle rust, Citrus canker, *Xylella* etc.

What is Psa?

- A bacterial disease of kiwifruit
  - Leaf spots, canker, death
- ***P****seudomonas* ***s****yringae* pv. ***a****ctinidiae*
  - Trinomial name?
  - bacterium



Severe kiwifruit disease



- **Psa-V Statistics**
  - 22 May 2013
- **2102** orchards have Psa-V.
- **71%** of New Zealand's kiwifruit hectares
- Not Nelson

Why Landcare Research?

- MPI
  - Initial incursion response
  - Regulations
- Plant and Food Research (PFR)
  - Industry links
  - Developed kiwifruit cultivars (Royalties)
  - Plant pathologists
- KVH
  - Independent org. managing Psa

Why Landcare Research?

- History
- DSIR split into CRIs in 1992
- The PDD of DSIR was split between Crop & Food, Hort Research, AgResearch, and Landcare Research
- Landcare retained:
  - National collections (CHR, PDD, NZAC, **ICMP**)
  - Taxonomists

DSIR Bacteriology 1988

Psa response

Genome sequence

- November 2010 from Te Puke
- MAF ID'd bacterium as Psa
- But which strain?
  - Asian
  - Italian
- Asked by MAF to do whole genome sequence
  - Sequenced in 3 days
  - Capability from in-house research tool (454)
  - Microsatellite discovery

Genome analysis

Gene	Japan 84, Italy 94	Italy 08/09	NZ 2010
avrPto1	-	-	
avrD1	+	+	
avrAE1	+	+	
hopA1	-	+	
hopB1	-	-	
hopC1	-	-	
hopD1	+	+	
hopF2	-	-	
hopG1	-	-	
hrpK1	+	+	
hopAF1	±	-	
hopAN1	+	+	
Coronatine	±	-	
Phaseolotoxin	+	-	

Genome analysis

Gene	Japan 84, Italy 94	Italy 08/09	NZ 2010
avrPto1	-	-	-
avrD1	+	+	+
avrAE1	+	+	+
hopA1	-	+	+
hopB1	-	-	-
hopC1	-	-	-
hopD1	+	+	+
hopF2	-	-	-
hopG1	-	-	-
hrpK1	+	+	+
hopAF1	±	-	-
hopAN1	+	+	+
Coronatine	±	-	-
Phaseolotoxin	+	-	-

ICMP: International Collection of Microorganisms from plants

- National coll. of living bacteria and fungi
  - All of NZ, stakeholders
  - Liquid N_2
- 18,800 cultures
- Important collection of plant pathogenic bacteria
  - “type strain” of Psa (Takikawa 1989)

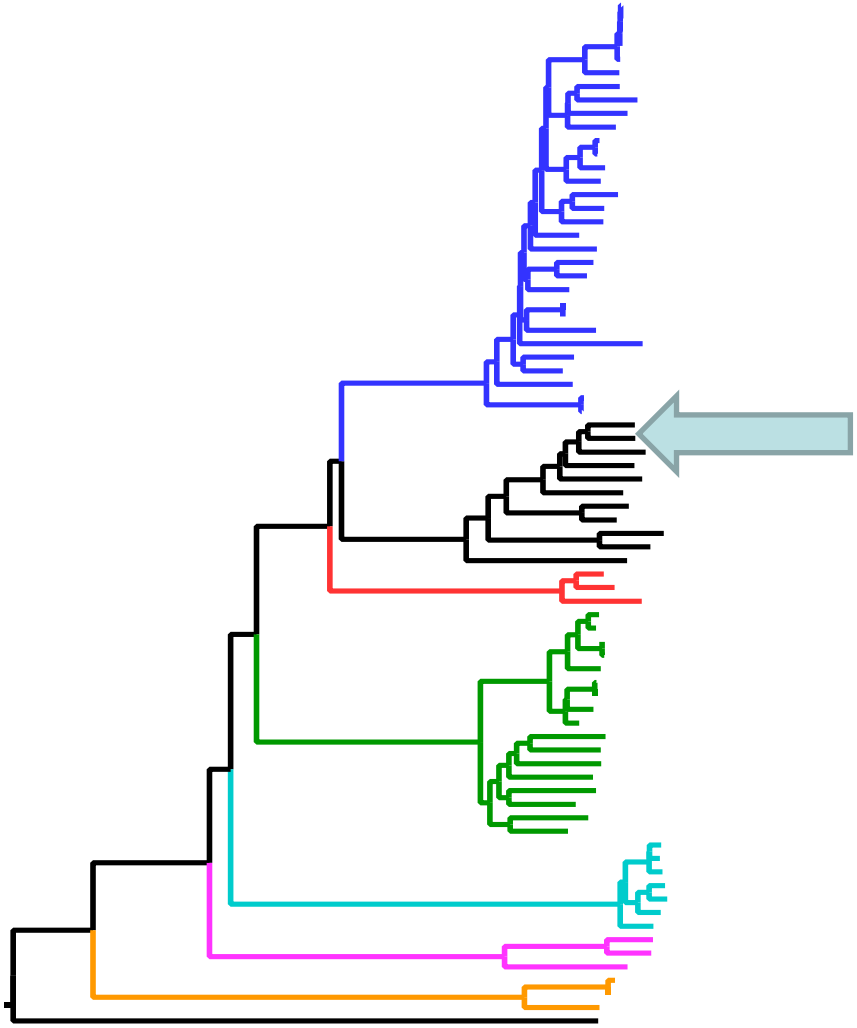
New or Old disease in NZ?

- Was Psa-V a recent introduction?
- Or been here for a long time
  - changing conditions caused severe disease?
- Screened 40 years of ex- kiwifruit bacteria in the ICMP looking for Psa
  - Found no matches from 143
- Value of collections to NZ
  - Keep collecting NZ material

Pseudomonas syringae Taxonomy

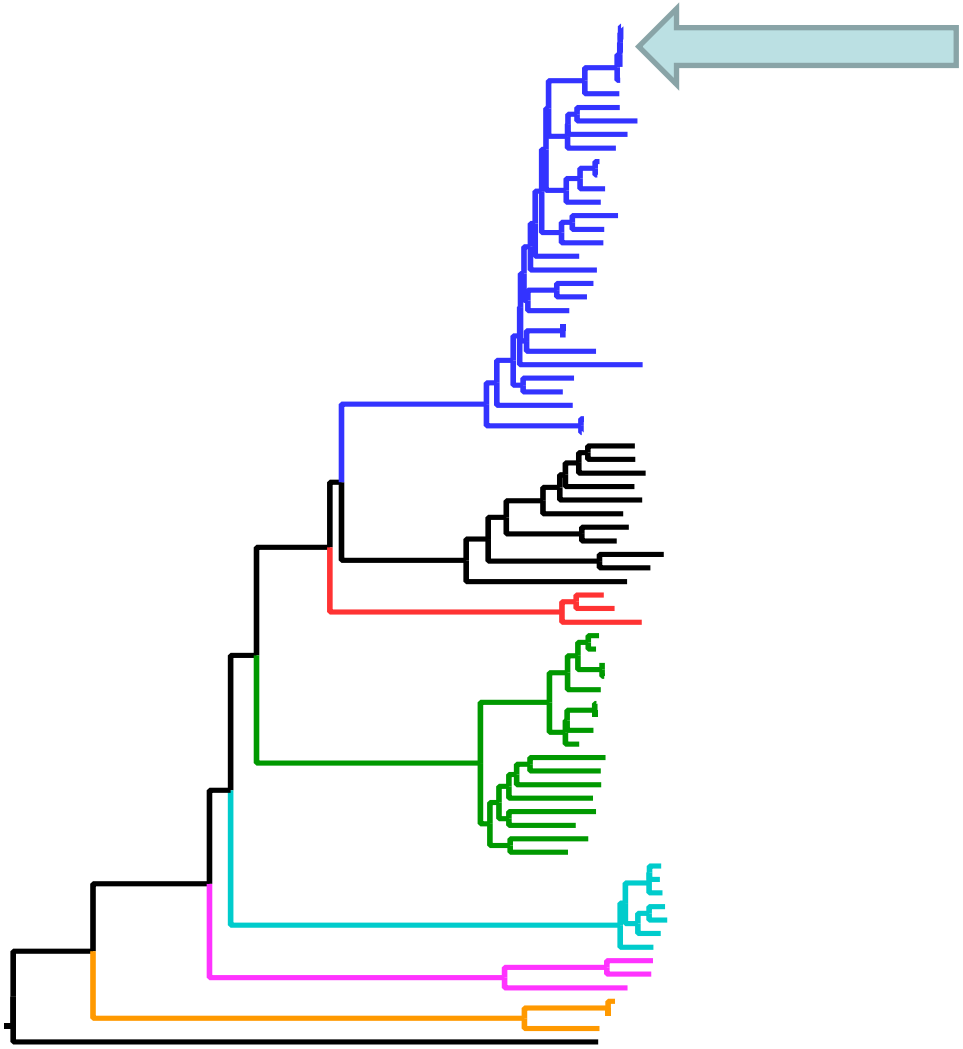
- V. important group of plant pathogens
  - 11 species and 64 pathovars (26 in NZ)
  - Pathovar = Pathological variants
  - Inconsistent confusing taxonomy
  - 7 “Genomospecies”
- *Pseudomonas syringae* pv. *actinidiae*
- Bacteriological Code & Pathovar Standards
  - Rules of nomenclature
  - pv. system developed in NZ

P. syringae complex



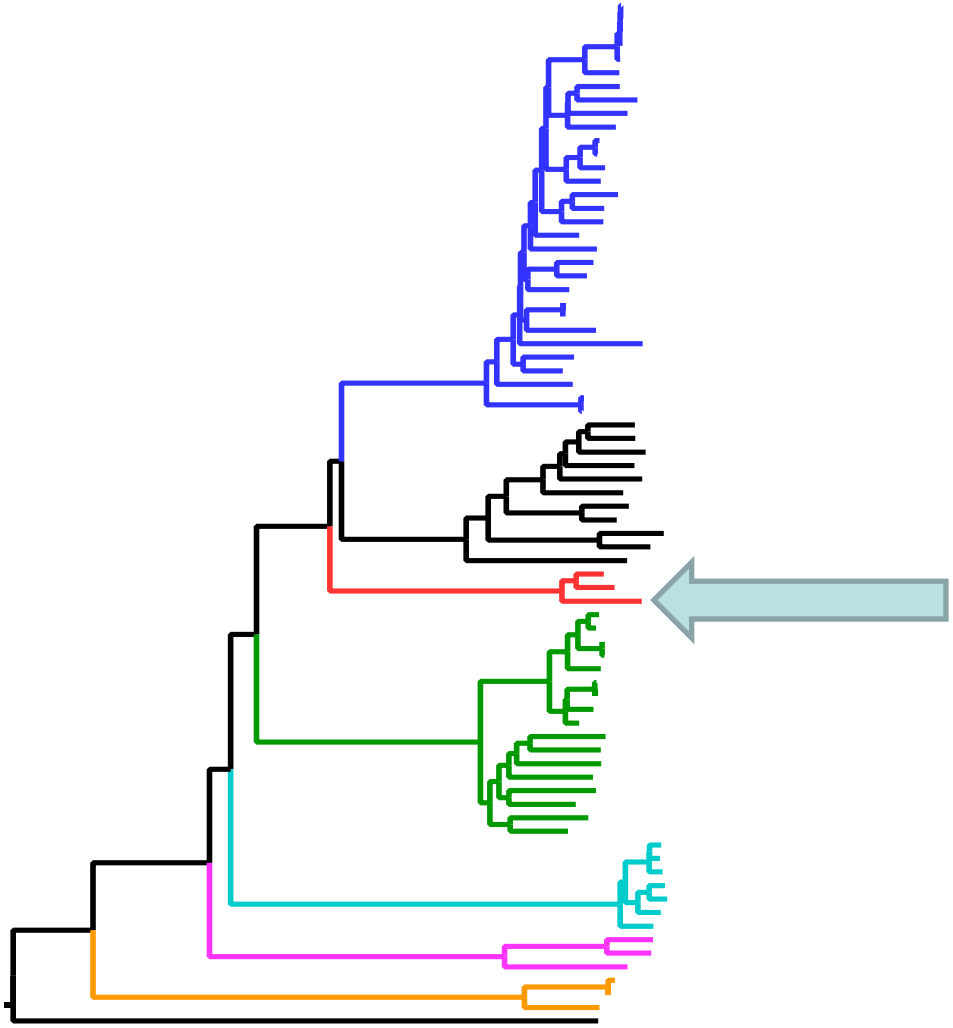
Pseudomonas syringae pv. *syringae*
- Lilac Bacterial blight

P. syringae complex



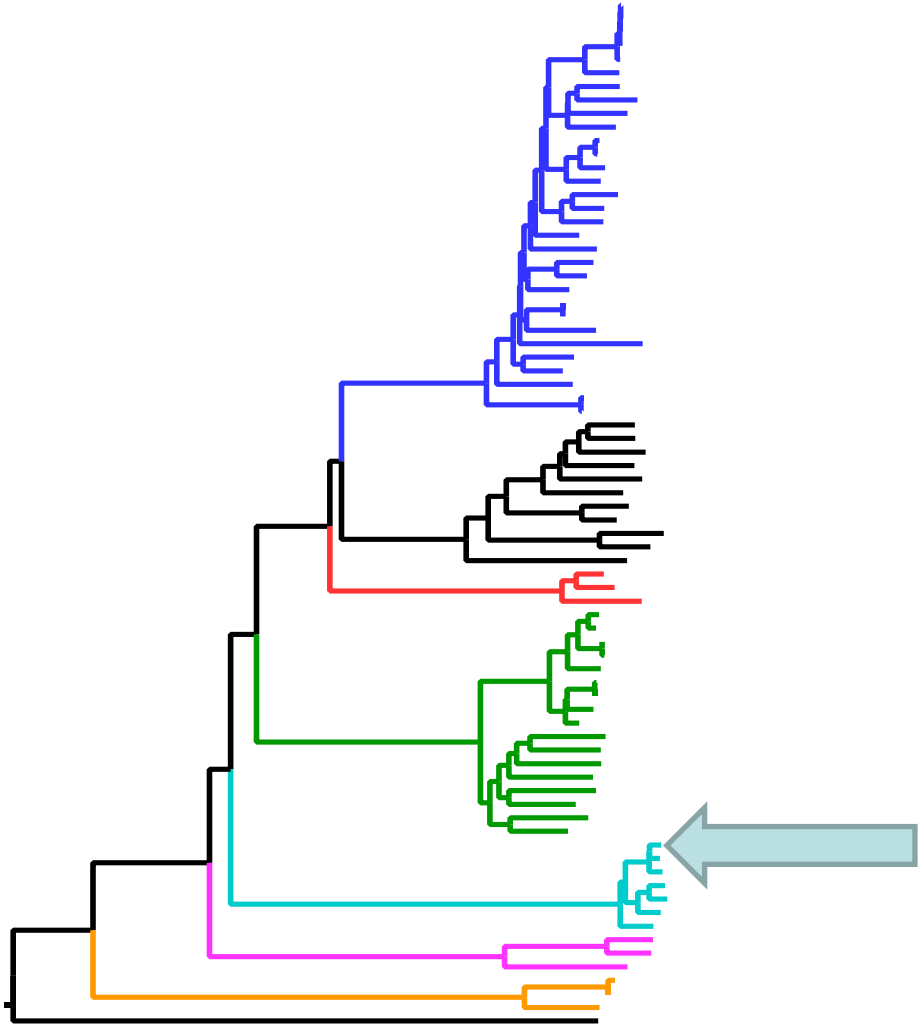
Pseudomonas savastanoi pv. *savastanoi*
- Olive knot disease

P. syringae complex



Pseudomonas syringae pv. *helianthi*
- Sunflower leaf spot

P. syringae complex

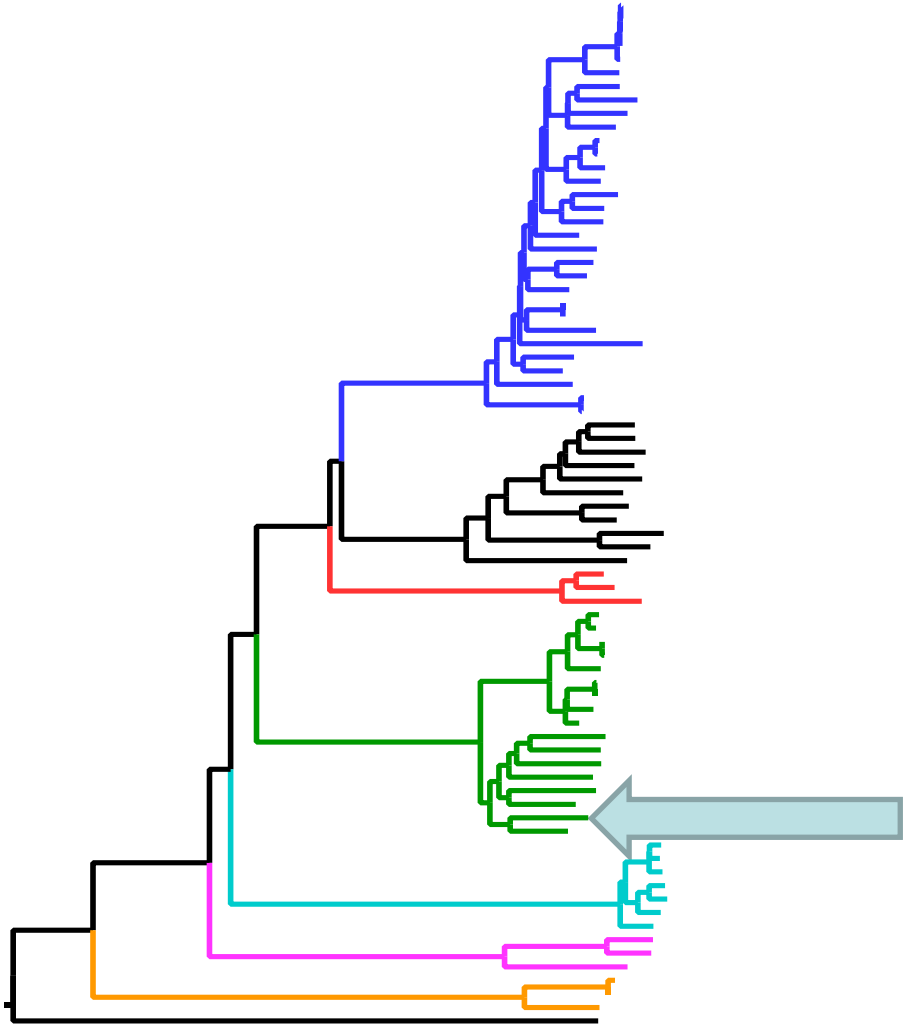


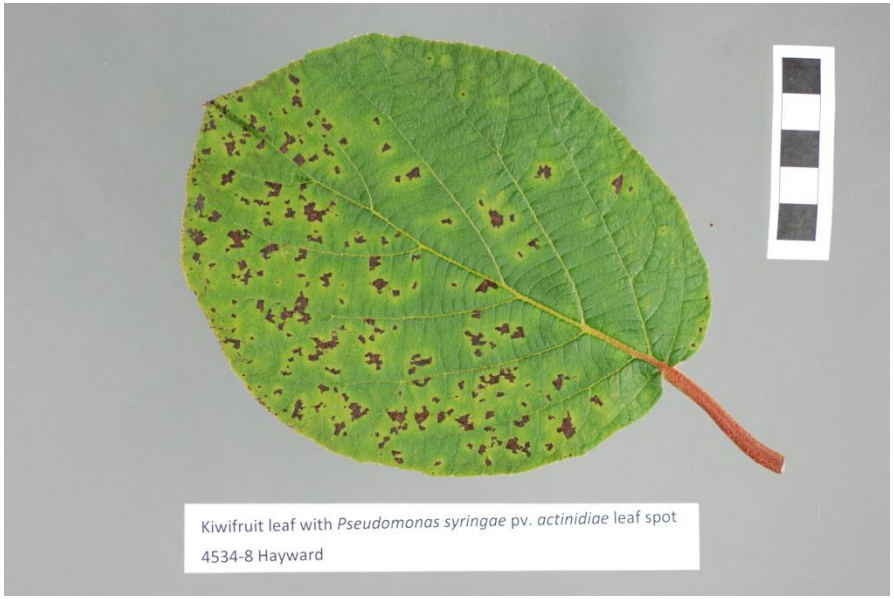
Pseudomonas syringae pv. *coronafaciens*
- Oat halo blight

P. syringae complex

Pseudomonas cannabina pv. *cannabina*
- Hemp leaf and stem rot

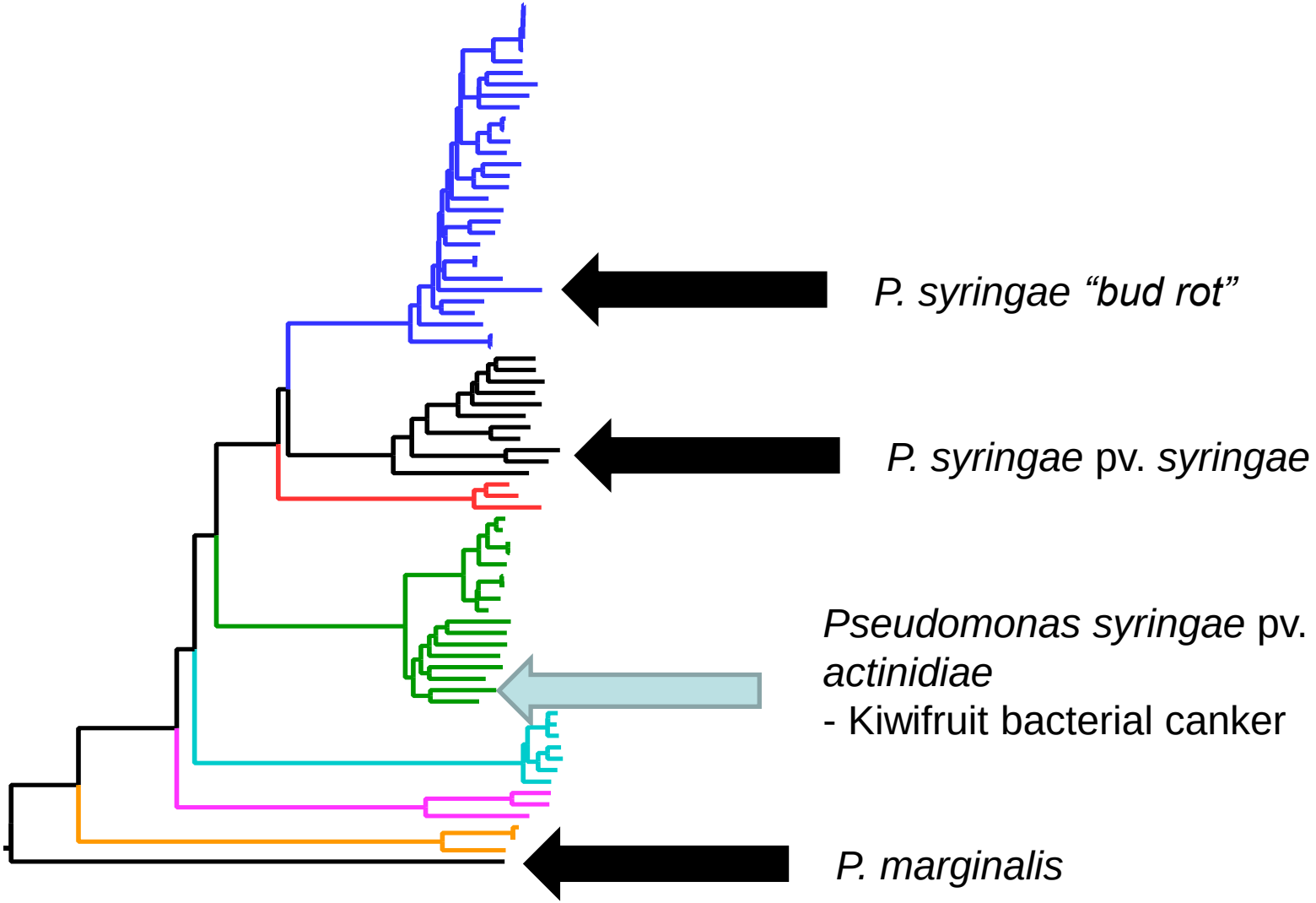
P. syringae complex





Pseudomonas syringae pv. *actinidiae*
- Kiwifruit bacterial canker

P. syringae complex



P. syringae "bud rot"

P. syringae pv. *syringae*

Pseudomonas syringae pv. *actinidiae*
- Kiwifruit bacterial canker

P. marginalis

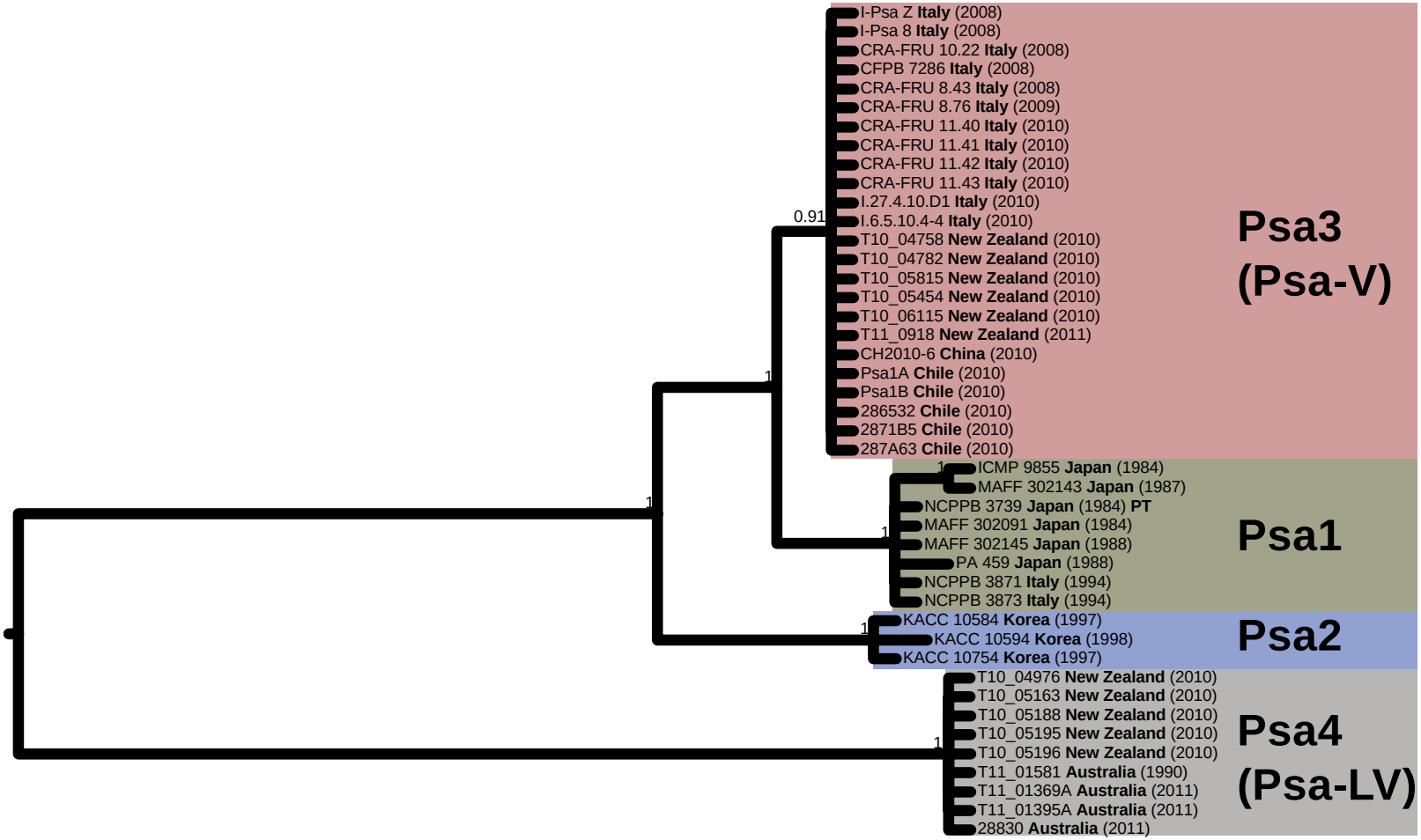
Pseudomonas syringae Taxonomy Research

- Multigene sequencing
  - Novel markers derived from genome sequences
- Taxonomic revision: Will split into 7 species
  - e.g. *P.* ***avellanae*** pv. *actinidiae*
  - Consistent species concept

Different Psa populations in NZ?

- The genome matched ‘Italian’
- Diagnostic test of the time did not differentiate populations
  - Testing from around the country indicated Psa was widespread
  - Impacted policy & biosecurity decisions
- But subsequent sequencing revealed a novel Psa population in NZ
  - Psa-V: strong pathogen
  - Psa-LV: Leaf spots, plants survive, been here a while

Psa global populations



Chapman et al. 2012 – doi:10.1094/PHYTO-03-12-0064-R

Regulation on species?

- Taxonomic hierarchy
  - Species (*P. syringae*)
  - Pathovars (pv. *actinidiae*)
  - Populations (Psa3)
- Which level to regulate at?
- Pathogenicity genes?
  - Psa3 (Psa-V) and Psa4 (Psa-LV) present in NZ
  - Psa1 and Psa2 absent but less virulent
  - Introduction may introduce new path genes

Regulation on species?

- Do pre-emptive work on emerging pathogens
  - If we knew what we know now back in 2010 different decisions could have been made
  - e.g myrtle rust
  - Collb. With B3 / national sci challenges
- Taxonomist provide sensible species concepts
- Investigate population level differences

Diagnostics

Psa diagnostic service

- CRIs have immediate capability
- Did 300+ Psa tests by isolation + seq.
  - Living bacteria gold standard
  - Into the ICMP
- Trained commercial labs
  - Tech transfer
- Now occasional tests
- Did sequencing for MAF
  - weekends

New diagnostic tests

- Helped validate new diagnostic tests
  - Faster, more specific
  - Directly from plant tissue
- Provided positive controls and 150+ DNA
  - genetically similar pathovars
  - other ex-kiwifruit bacteria
  - Based on *P. syringae* taxonomy research
- Validation very important
  - False positives have economic consequences

New plant pathogen facility

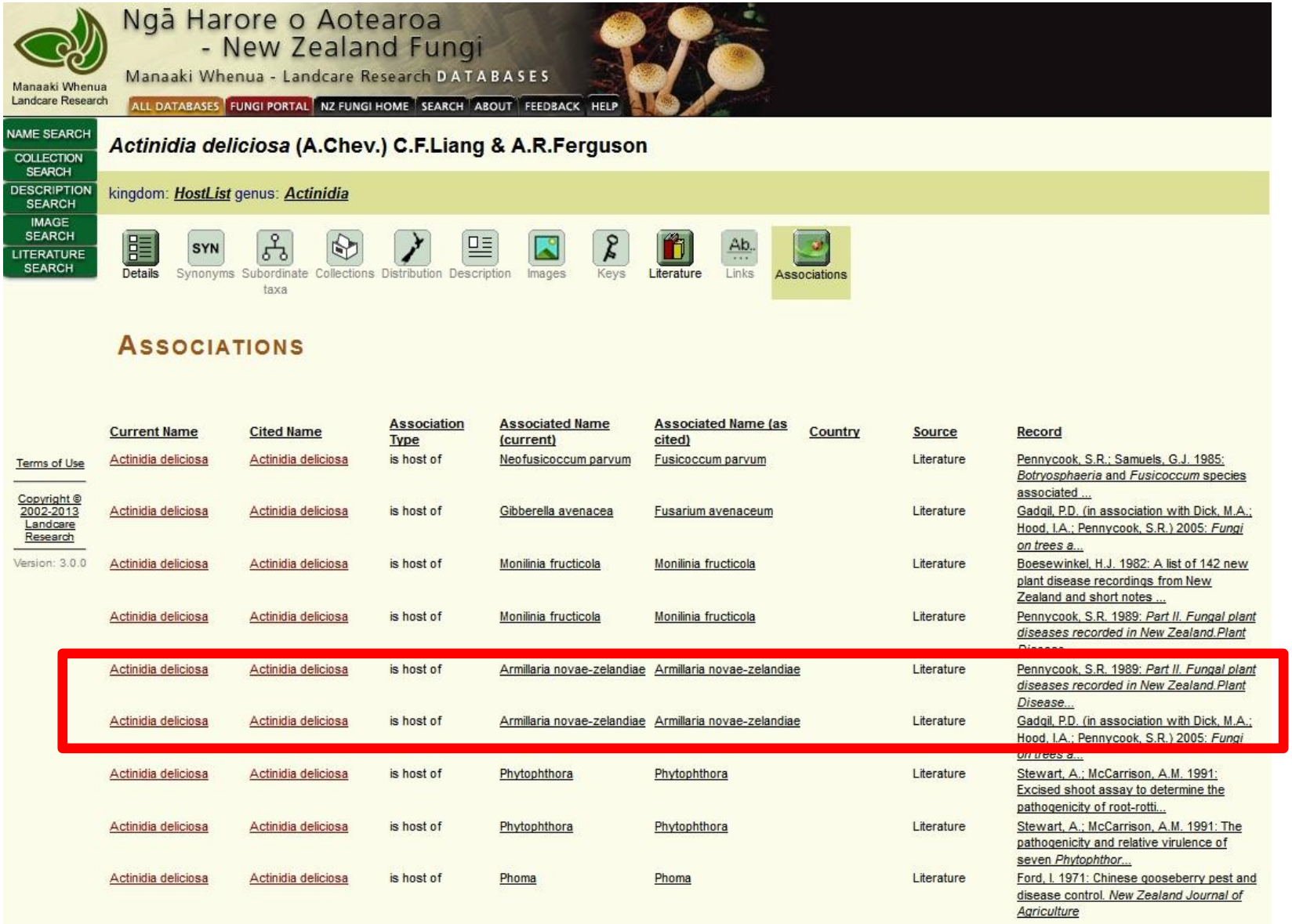
- “PC2+” containment
  - HEPA filter
  - Steam water waste
  - Shower out
- Unique capability
- Enabling new research
  - Testing pathogens and biocontol agents in NZ
- PFR using facility for Psa research

NZfungi database

- Database of Fungi and bacteria
  - Pansectorial, national database
  - Taxonomy (What is the current name?)
  - Biostatus (Is this organism here? Exotic or native?)
  - Associations (What plant is it found on?)
  - Collection specimen data
- Relevant for informing import and export policy and knowing what is in the environment
- http://NZfungi2.LandcareResearch.co.nz/
- Feeds through to NZOR
  - Jerry Cooper Link seminar 26th November 2013

NZfungi database

NZfungi database



Ngā Harore o Aotearoa - New Zealand Fungi

Manaaki Whenua - Landcare Research DATABASES

ALL DATABASES | FUNGI PORTAL | NZ FUNGI HOME | SEARCH | ABOUT | FEEDBACK | HELP

NAME SEARCH | COLLECTION SEARCH | DESCRIPTION SEARCH | IMAGE SEARCH | LITERATURE SEARCH

***Actinidia deliciosa* (A.Chev.) C.F.Liang & A.R.Ferguson**

kingdom: ***HostList*** genus: ***Actinidia***

Details | Synonyms | Subordinate taxa | Collections | Distribution | Description | Images | Keys | Literature | Links | Associations

ASSOCIATIONS

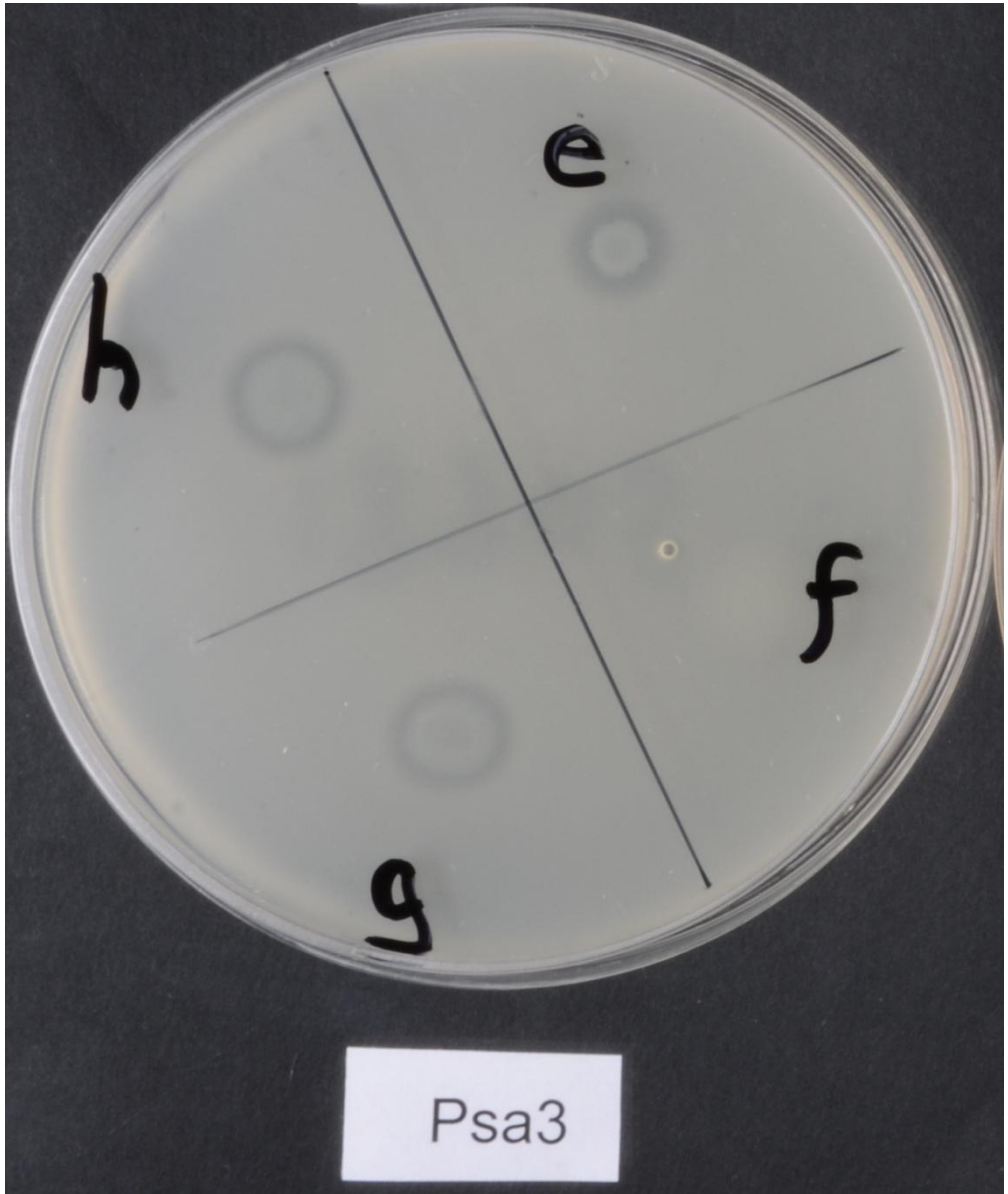
	Current Name	Cited Name	Association Type	Associated Name (current)	Associated Name (as cited)	Country	Source	Record
Terms of Use	Actinidia deliciosa	Actinidia deliciosa	is host of	Neofusicoccum parvum	Fusicoccum parvum		Literature	Pennycook, S.R.; Samuels, G.J. 1985: *Botryosphaeria* and *Fusicoccum* species associated ...
Copyright © 2002-2013 Landcare Research	Actinidia deliciosa	Actinidia deliciosa	is host of	Gibberella avenacea	Fusarium avenaceum		Literature	Gadgil, P.D. (in association with Dick, M.A.; Hood, I.A.; Pennycook, S.R.) 2005: *Fungi on trees a...*
Version: 3.0.0	Actinidia deliciosa	Actinidia deliciosa	is host of	Monilinia fructicola	Monilinia fructicola		Literature	Boesewinkel, H.J. 1982: A list of 142 new plant disease recordings from New Zealand and short notes ...
	Actinidia deliciosa	Actinidia deliciosa	is host of	Monilinia fructicola	Monilinia fructicola		Literature	Pennycook, S.R. 1989: *Part II. Fungal plant diseases recorded in New Zealand Plant Disease...*
	Actinidia deliciosa	Actinidia deliciosa	is host of	Armillaria novae-zelandiae	Armillaria novae-zelandiae		Literature	Pennycook, S.R. 1989: *Part II. Fungal plant diseases recorded in New Zealand Plant Disease...*
	Actinidia deliciosa	Actinidia deliciosa	is host of	Armillaria novae-zelandiae	Armillaria novae-zelandiae		Literature	Gadgil, P.D. (in association with Dick, M.A.; Hood, I.A.; Pennycook, S.R.) 2005: *Fungi on trees a...*
	Actinidia deliciosa	Actinidia deliciosa	is host of	Phytophthora	Phytophthora		Literature	Stewart, A.; McCarrison, A.M. 1991: Excised shoot assay to determine the pathogenicity of root-rotti...
	Actinidia deliciosa	Actinidia deliciosa	is host of	Phytophthora	Phytophthora		Literature	Stewart, A.; McCarrison, A.M. 1991: The pathogenicity and relative virulence of seven *Phytophthor...*
	Actinidia deliciosa	Actinidia deliciosa	is host of	Phoma	Phoma		Literature	Ford, I. 1971: Chinese gooseberry pest and disease control. *New Zealand Journal of Agriculture*

Armillaria novae-zealandiae

Management of Psa?

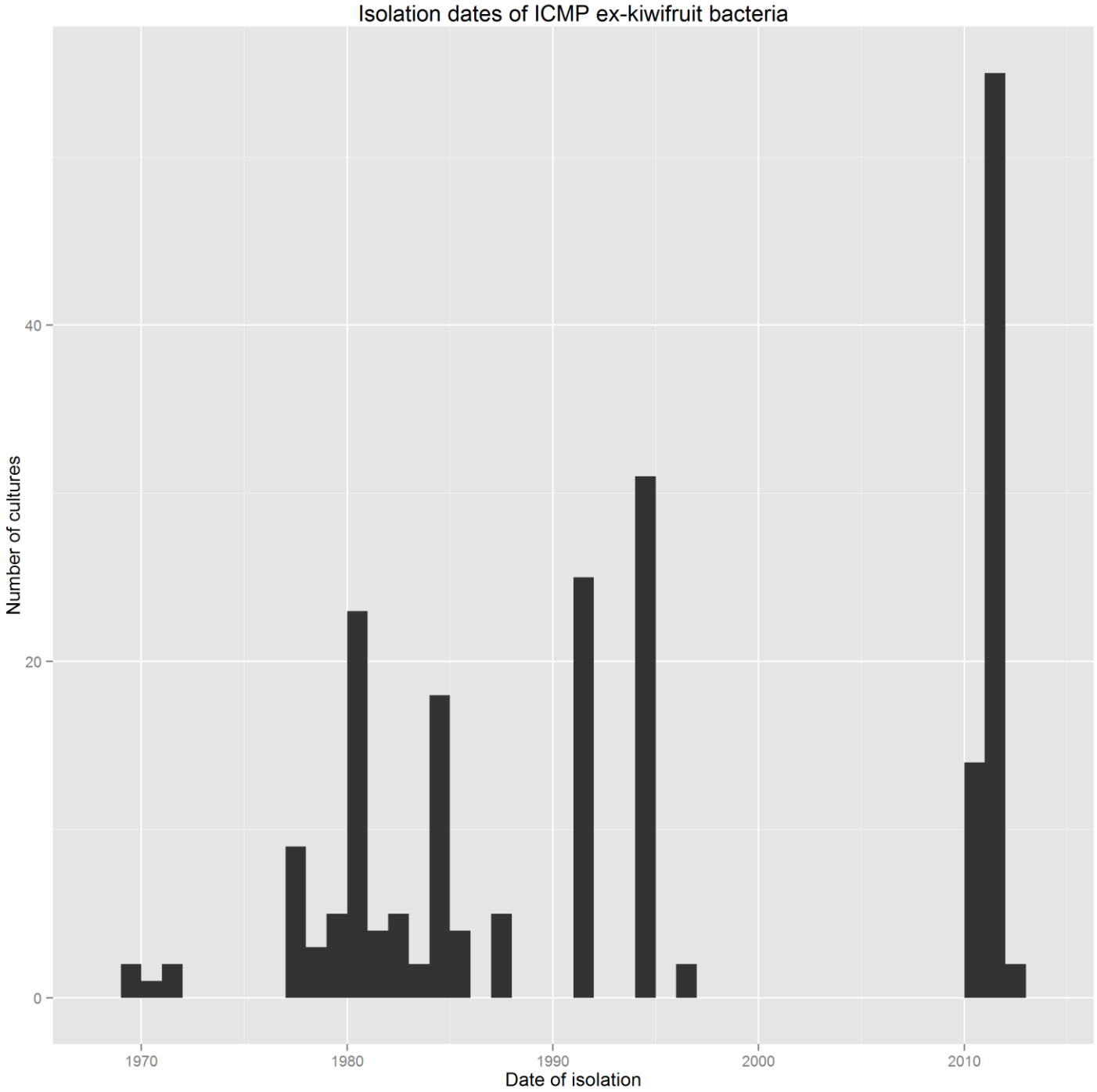
- Psa can be controlled by antibiotics
  - improper use problem
  - Resistance problem
  - EU zero residue problem
  - Problems can be mitigated
- Need a novel biological solution:
  - KVH Investigating biologicals
  - Screened ICMP collection for anti-Psa bacteriocins
  - Found 10 promising candidates (from 200)

Management of Psa?



Conclusions

- Pathogens don't carry passports
- Need taxonomic input to guide policy development
  - Identification & interpretation can be very complicated
    - Species / pathovars / populations / genes
    - Preparedness for emerging pathogens
    - Myrtle rust taxonomy uncertain
    - Taxonomy changing rapidly, need to stay up to date with what we have here
    - Need taxonomic input to guide policy development


Isolation dates of ICMP ex-kiwifruit bacteria
Number of cultures
0
20
40
1970
1980
1990
2000
2010
Date of isolation

Conclusions

- LCR can help with future issues
  - Collections
  - Databases
  - Staff capability

Acknowledgements

- LCR technical staff
  - Maureen Fletcher, Paula Wilkie, Duckchul Park
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  - Brett Alexander, Rob Taylor
- Funding
  - LCR, KVH, MPI