Pseudomonas syringae pv. actinidiae, tri leta po odkritju v Novi Zelandiji: kaj smo se naučili o patogenu, o boleznii in kako se jo obvladuje

Joel L Vanneste

NIB, Ljubljana, Sept 2013
The New Zealand kiwifruit industry in perspective …..

- 2011 exports from New Zealand horticulture industry NZ$ 3.5 billions
- 2011 Exports from kiwifruit industry NZ$ 962 millions (600 mEuros)
- Kiwifruit industry is the largest horticultural export industry in New Zealand
- Last 11 years number of kiwifruit trays exported has doubled
- Av. OGR (Orchard Gate Return) for green kiwifruit NZ$41 830 (26 000 Euros) (export vol 79%, value 67%)
- Av. OGR for gold kiwifruit NZ$90 911 (56 000 Euros) (export vol 21%, value 33 %)
Early November 2010: Few leaf spots....... 

.....and necrosed flowers
Earlier in Italy …..
The follow up in Italy (2009)
Identification of *Pseudomonas syringae* pv *actinidiae* by PCR using the primers PsaF1/R2

All strains of *Psa* tested gave a 280 bp band
None of strains of 25 different pathovars tested gave a 280bp except:
*P. syringae* pv *theae*, *P. syringae* pv *passiflorae*, *P. syringae* pv *morsprunorum*, *P. syringae* pv *ciccaronei*, *P. syringae* pv *helianthi*, *P. syringae* pv *raphiolepidis*, *P. syringae* pv *spinacea*.

Vanneste et al. 2010 New Zealand Plant Protection 63:7-14
November 2010 detection of Psa in New Zealand
Box PCR patterns of Psa strains from New Zealand

Vanneste JL et al. Plant Disease 2013
Box PCR patterns and pathogenicity of Psa strains from New Zealand

Vanneste JL et al. Plant Disease 2013
Box PCR patterns and *cts* haplotypes from *Psa* strains from New Zealand

Vanneste JL et al. *Plant Disease* 2013
Difference in virulence between biovar 3 and biovar 4 of Psa

(Psa V) Biovar 3

(Psa L-V) Biovar 4
Bayesian inference phylogenetic tree of *Pseudomonas syringae* pv. *actinidiae* isolates derived from a partitioned concatenation of seven house-keeping genes.

Chapman, et al. 2012 Phytopathology
Effector genes in strains of *P. syringae pv. actinidiae* from different geographic origins

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Japan</th>
<th>Italy (1992 outbreak)</th>
<th>Korea</th>
<th>New Zealand</th>
<th>Italy (2008–09 outbreak)</th>
<th>Chile</th>
<th>New Zealand</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Psa1</td>
<td>Psa1</td>
<td>Psa2</td>
<td>Psa3</td>
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<td>Psa4</td>
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<td>−</td>
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<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

*Pattern of effector gene presence deduced by BLAST searching the genome sequences of isolate CH2010-6 in GenBank. See main text for details. MLSA = multilocus sequence analysis. + symbol indicates that the gene is present, whereas – symbol indicates that the gene was not detected.*
Bayesian inference phylogenetic tree of *Pseudomonas syringae* pv. *actinidiae* isolates derived from a partitioned concatenation of seven effector genes.

Chapman, et al. 2012 Phytopathology
Similar grouping by MLST using house keeping genes or effector genes.
Phylogeny of Psa based on Whole Genome Sequence

Biovar 1: **Systemic** and produce phaseolotoxin

Biovar 2: **Systemic** and produce coronatine

Biovar 3: **Systemic** and produce no toxin

Biovar 4: **Non systemic** and produce no toxin

Vanneste et al. 2013. Plant Disease
November 2010 detection of biovar 3 Psa in New Zealand
Bay of Plenty December 2010
What is the situation today?  
(18 September 2013)

» Out of 3293 kiwifruit orchards in New Zealand  
  2262 orchards affected by Psa biovar 3

» 10388 hectares of orchards where Psa biovar 3  
  has been identified

» 75 percent of New Zealand kiwifruit hectares  
  are on an orchard affected by Psa biovar 3

» New regions getting infected, Whanganui being  
  latest region declared affected by Psa biovar 3
Cost of Psa in New Zealand (2012 study)

• In less than 2 years over 2000 orchards affected (70%)

• Est of cost over the next 5 years: NZ$ 310 - 410 million (191- 253 mEuros)

• Est cost over a 15 years period NZ$885 million (547mEuros)

• Between 2012-2016, 360 to 470 FTEs lost in the Bay of Plenty alone
Cost of Psa in New Zealand (2013 estimate)

» Response to Psa discovery NZ $ 42 million (26 mEuros)

» In Te Puke alone over 1 000 ha of Hort16A cut out

» Approx. 2100 ha grafted on new variety (G3) NZ$ 60 000/ha for a total of $ 60 million (37mEuros)

» Less than 100% production levels for new cultivars and Hayward

» Reduction of capital land value 450K/ha to 100K/ha for Gold and 250K/ha to 150K/ha for Hayward. Total loss $ 2 billion (1.24 b Euros)
Climatic considerations:

» Disease less active above 20° C, no infection above 25° C

» Most active when average temperature over 10 days is between 10° C and 20° C

Summer Bay of Plenty Ave temp most often below 20° C
Plant 1A: ‘Hort16A’ 3 weeks after inoculation
Plant 1B: ‘Hort16A’ 3 weeks after inoculation
Psa populations in cfu/g

- 1.4 x 10^9
- 2.9 x 10^8
- 2.6 x 10^8
- 6.8 x 10^8 cfu/cm²
- 5.6 x 10^8
- 2.2 x 10^8

Inoculation Site
1.5 x 10^7

Constant Humidity

+= petiole
+= stem
Plant 1A: Constant humidity

$8.3 \times 10^8$ cfu/gm + $1.5 \times 10^7$ cfu/gm + $5.6 \times 10^8$ cfu/gm
+ 2.4 x 10^5 cfu/gm

+ 4.5 x 10^9 cfu/gm
+ 4.5 x 10^9 cfu/gm
Psa populations in cfu/g

Inoculation Site
1.4 x 10^9

P 2 x 10^3

+2.7 x 10^5

48 Hours
Humidity
Plant 1B: 48 hours high humidity

+ $1.4 \times 10^9$ cfu/gm
Other contributing factors to the intensity of the New Zealand outbreak

Narrow genetic base

Monoculture
Epidemiology: where does Psa survive?

- On the leaves
- Shelter belts
- In and on the shoots, leader, and trunk
- Structures
- Leaf litter/cane debris
- Soil/water
- Weeds
Epidemiology: where does Psa survive?

- On kiwfruit leaves
- Shelter belts
- In and on kiwfruit shoots, leader, and trunk
- Structures
- Leaf litter/cane debris or prunings
- Soil/water
- Weeds
Survival on leaf litter (25 May – 1 September)

Tyson et al NZPP 2012
Survival of Psa on symptomatic cane material

Tyson et al NZPP 2012
Unique and shared ortholog groups between biovars

McCann et al. PLoS Pathogen 2013
Effector complement varies considerably between *Psa* biovars.
**Psa V-13 chromosome (biovar 3)**

- **Prophage PSPPH06 family**
- **DnaA**
- **Tn scar (HopA1 C-term)**
- **Mu-like Prophage**
- **rDNA**
- **Exchangable effector locus**
- **Tn 1 (HopA1 N-term)**
- **Tn 2**
- **Tn 3**
- **Tn 4 (Hops Z5, H1)**
- **Tn 5 (HopAM1)**
- **Tn 6 (HopAE1)**
- **Tn 7**
- **Tn 8 (HopAM1)**
- **Tn 9**
- **Tn 10**
- **Tn 11**
- **Tn 12**

*PI with Hops E1, M1 and N1*

*PI with Hops AG1, AH1 and AI1*

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*ICE (Pacific Island)*
ICEs are the key difference between biovar 3 isolates.
### Distribution of Integrative Conjugative Elements in isolates from China

<table>
<thead>
<tr>
<th>Location</th>
<th>Bordatella</th>
<th>MyCt</th>
<th>XerC</th>
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<tr>
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<td>C5</td>
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<td>Mediterranean</td>
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<td></td>
<td></td>
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<td>Mediterranean</td>
<td>Psa-v MI</td>
</tr>
</tbody>
</table>
Inoculation of lower leaf surface

Day 1

Paul Sutherland, PFR
Colonisation of the stomata and bacteria exuding from stomata

Day 5

Paul Sutherland, PFR
Leaf infection
Infected cane (lenticel)

Paul Sutherland, PFR
Paul Sutherland, PFR
Colonisation of the xylem by Psa

Paul Sutherland, Ian Hallett and Midori Jones
Phylogeny of Psa based on Whole Genome Sequence

Biovar 4: Non systemic and produce no toxin

Biovar 2: Systemic and produce coronatine

Biovar 3: Systemic and produce no toxin

Biovar 1: Systemic and produce phaseolotoxin

Vanneste et al. 2013. Plant Disease
Coronatine structural and functional homologue of jasmonic acid
Signalling pathways in Arabidopsis
Incidence of Psa on ‘Bruno’ seedlings treated with Methyl-Jasmonate 7 days before inoculation
Signalling pathways in Arabidopsis

Environmental cues

Pathogen

Signalling molecule

$\text{C}_2\text{H}_4$

$\text{cev1}$

$\text{ETRI}$

$\text{EIN2}$

$\text{ERF1}$

$\text{JA}$

$\text{Auxin}$

$\text{SA}$

Signal transduction and crosstalk

$\text{COII}$

$\text{TIR1}$

$\text{AXRI}$

Output responses

$\text{PDF1·2}$

$\text{Thi2·1}$

$\text{VSP}$

Root growth

Defences

Defences and Wound responses

Defences
Bruno’ seedlings treated with SA or ASM 7 days before inoculation.
Field experiments
Disease incidence 21 days after inoculation with Psa

**HORT16A**

**Bruno**

**Hayward**
In conclusion

- The recent outbreak of Psa biovar 3 is affecting every major kiwifruit producing country in the world
- This is changing How, Where and Which kiwifruit will be grown
- Huge research effort has been initiated
- If we work together the kiwifruit industry might have a bright future