

National Institute for Agrarian and Veterinary Research (INIAV)

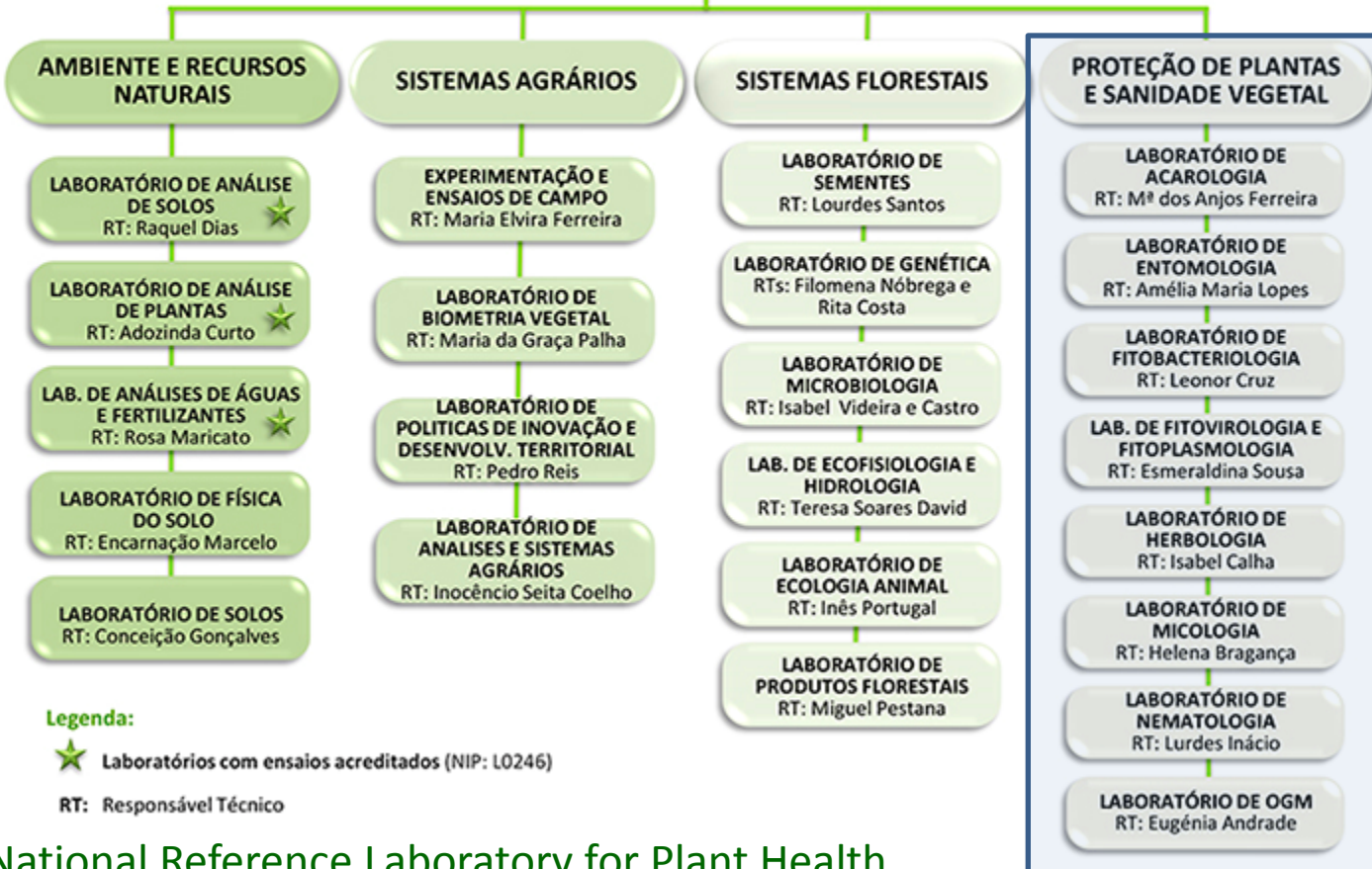
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UEIS - SISTEMAS AGRÁRIOS E FLORESTAIS E SANIDADE VEGETAL

Diretora: Amélia Maria Lopes

Secretariado e apoio
Maria José Broa

Coordenadores das Unidades de Investigação
Inocêncio Seita Coelho | Edmundo Sousa



Legenda:

★ Laboratórios com ensaios acreditados (NIP: L0246)

RT: Responsável Técnico

National Reference Laboratory for Plant Health

Lab Main activities

Research on quarantine and quality phytopathogenic bacteria of national and regional interest (**phenetic diversity, diagnosis and epidemiology**)

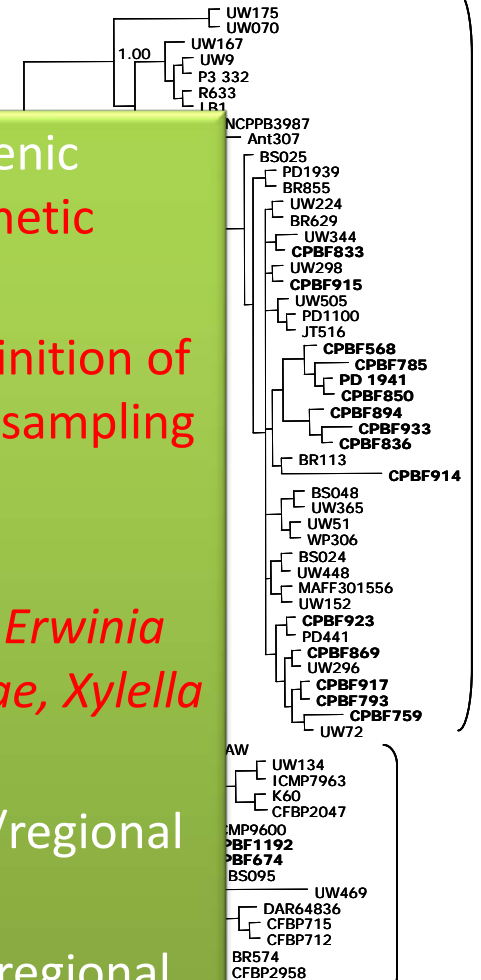
Support to national phytosanitary authority (**definition of national control plans for quarantine bacteria, sampling procedures and diagnostics**)

Analysis of Q-bacteria (*Ralstonia solanacearum*, *Clavibacter michiganensis* subsp. *sepedonicus*, *Erwinia amylovora*, *Pseudomonas syringae* pv. *actinidiae*, *Xylella fastidiosa*; *Huanlonbing*)

Scientific and technical support to official national/regional laboratories (**diagnostic procedures, training**)

Dissemination of knowledge to stakeholders and regional inspectors (**lectures and technical courses**)

Support to community (**Plant Clinics**)



Bacterial canker of kiwi caused by *Pseudomonas syringae* pv. *actinidiae* in Portugal – Disease Importance and pathogen characterization

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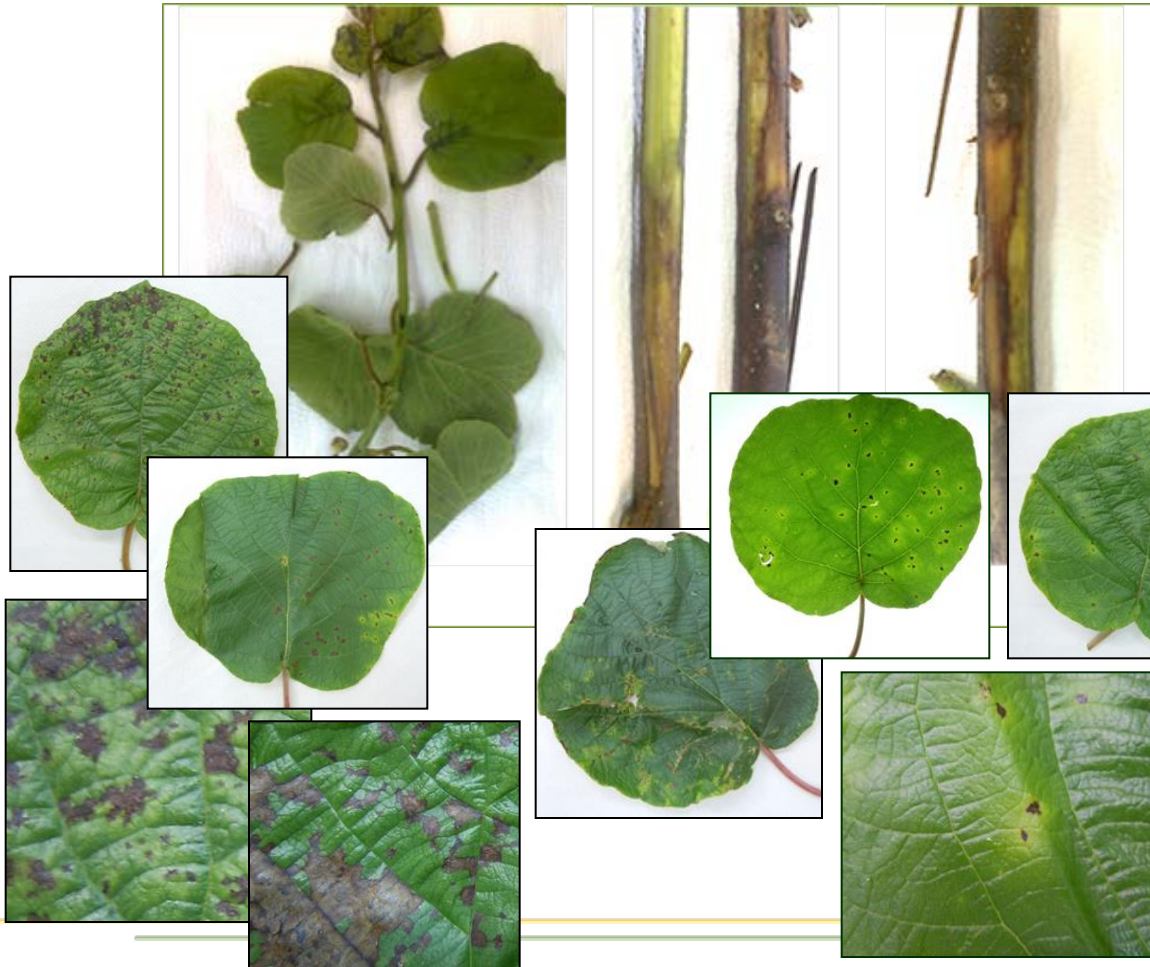
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Symptoms

Leaves



Branches



Symptoms

Flowers



Main risk factors in the area

- ✓ Presence of high levels of inoculum
- ✓ High number of orchards
- ✓ Presence of very susceptible cultivars (*A. chinensis*)
- ✓ Temperature >15°C during blooming
- ✓ Presence of pollinators
- ✓ High HR

Bacterial canker of kiwi

Major and minor hosts

<i>Actinidia chinensis</i> (summer kiwi, chinese kiwi)	<i>Actinidia deliciosa</i> (kiwi, Chinese gooseberry)	<i>Actinidia arguta</i>	<i>Actinidia kolomikta</i>
			

Pseudomonas syringae pv. *actinidiae*

Bacteria → Proteobacteria → Gama-Proteobacteria → Pseudomonadales →
Pseudomonadaceae → *Pseudomonas* sp.
Classified in to 4 biovars based on phenotypic and genomic characteristics

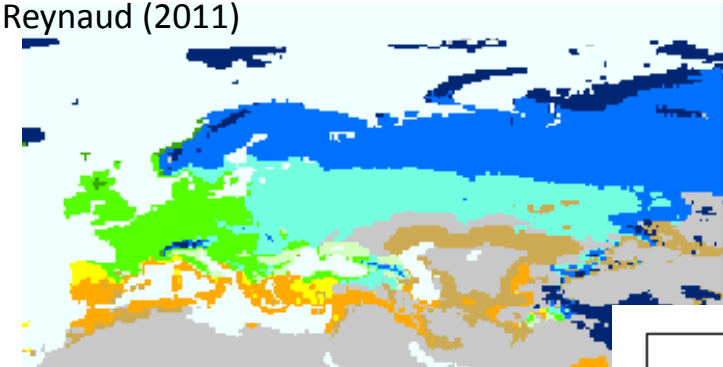
Portuguese situation

Reynaud (2011)

EPPO PRA

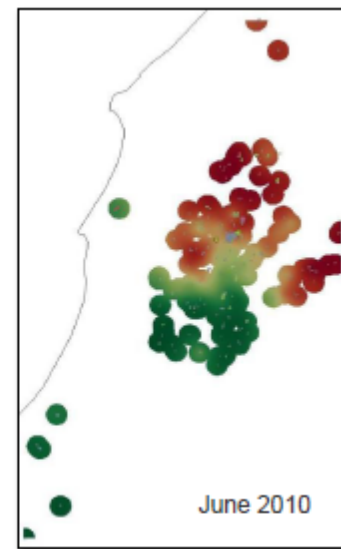
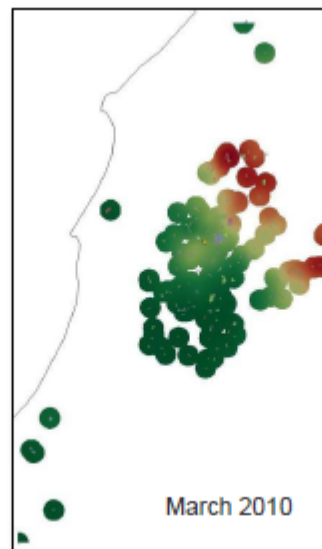
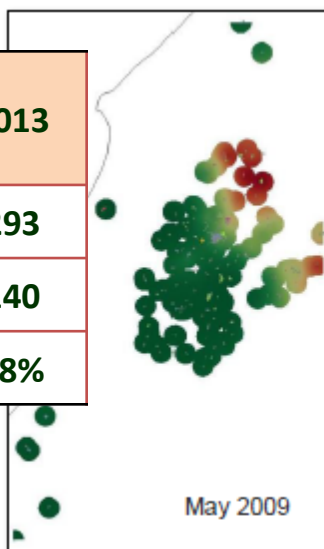
Portugal

Balestra *et al.* 2010d: disease incidence as high as 30% was noted in 2010 and incidence has increased up to 80% in 2011 (Renzi *et al.*, 2011).



ano	2010	2011	2012	2013
nº total	10	175	71	293
pos	6	15	30	140
%	60%	9%	42%	48%

Portuguese Control Plan
P. syringae pv. *actinidiae*



Progression of *P. syringae* pv. *actinidiae* infections in Latina
(Vanneste *et al.* 2011b). (circulo = 1km)

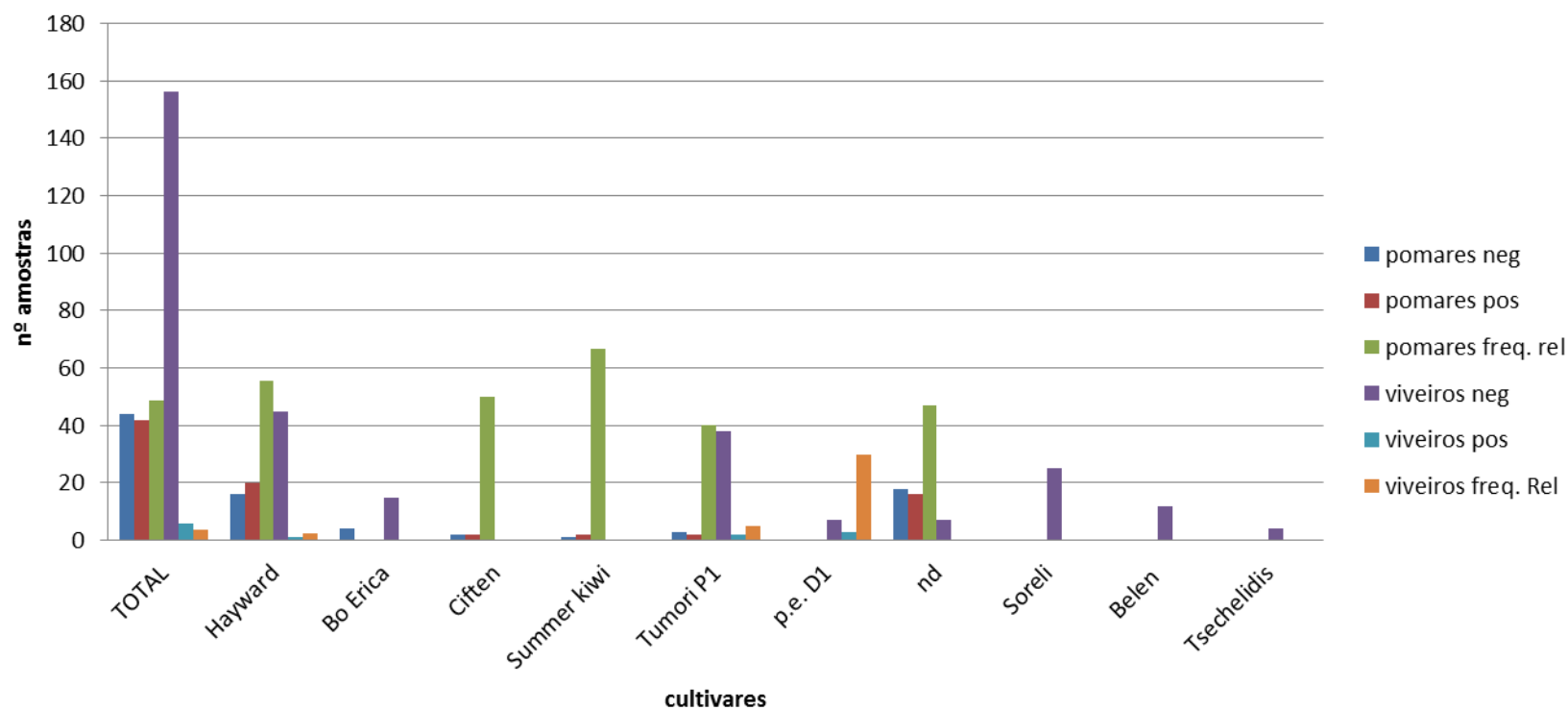
Incidence

03/2010 – First identification (Santa Maria da Feira), in plant material imported sent by Direcção Regional de Agricultura do Norte (DRAPN)

2010-2012	Orchards			Nurseries		
	neg	pos	freq. rel	neg	pos	freq. Rel
TOTAL	44	42	48,84	156	6	3,70
Hayward	16	20	55,56	45	1	2,17
Bo Erica	4	0	0,00	15	0	0,00
Ciften	2	2	50,00	0	0	0,00
Summer kiwi	1	2	66,67	0	0	0,00
Tumori P1	3	2	40,00	38	2	5,00
p.e. D1	0	0	0,00	7	3	30,00
nd	18	16	47,06	7	0	0,00
Soreli	0	0	0,00	25	0	0,00
Belen	0	0	0,00	12	0	0,00
Tsechelidis	0	0	0,00	4	0	0,00

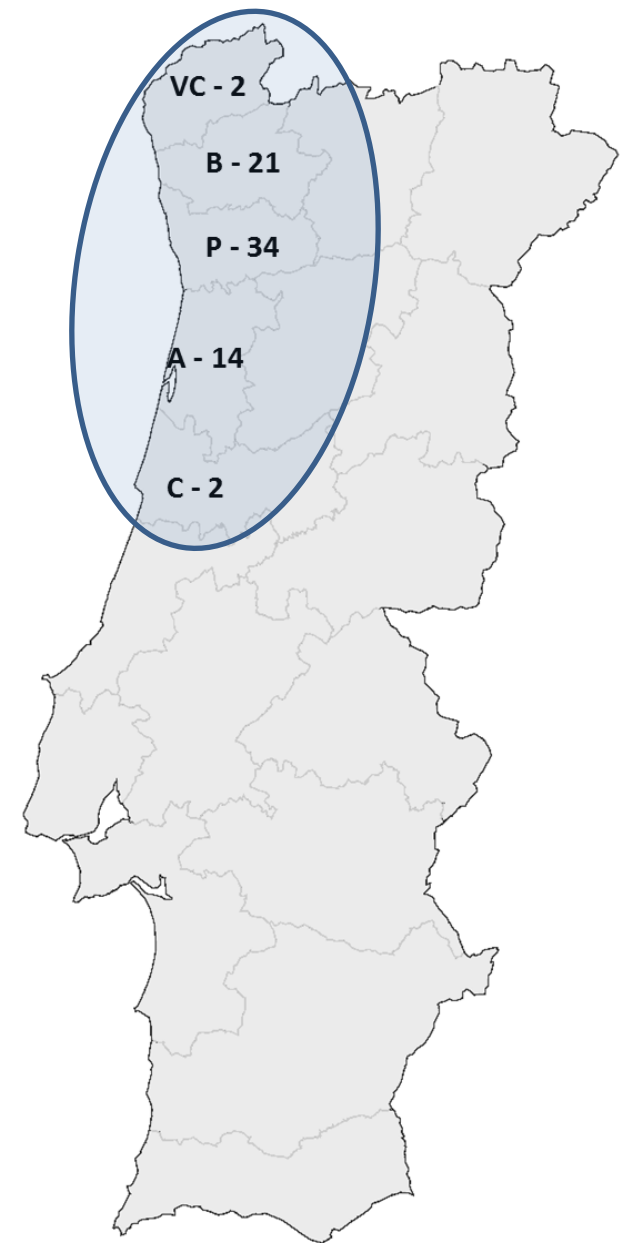
Portuguese situation

Incidência de *P.s. pv. actinidiae* em pomares e materiais de propagação em Portugal (2010-2012)



Sampling

- ✓ Kiwi area in north Portugal – 1500ha
- ✓ Commission Decision n.º 2012/756/EU
- ✓ Definition of portuguese contaminated and disease free areas
- ✓ Definition of a sampling strategy
- ✓ National Control Plan - surveillance of orchards, nurseries and garden centres
- ✓ Training courses for farmers and inspectors
- ✓ Research



Diagnosis

Samples from orchards, nurseries and imported propagation materials
aprox. 1000 (2010-2013)

- ✓ 84 selected isolates from North and Center regions
- ✓ Diagnosis - internal method based on OEPP PM7/120(1)
 - ✓ Extraction from leaves, branches, fruits and roots
 - ✓ Isolation on KMB
 - ✓ Conventional PCR

Scortichini et al., 2002 or Rees-Gerge et al., 2010

PAV 1 GGCGACGATCCGTAAGTGGTCTGAGA 760 bp

P 22 TTCCGAAGGCACTCCTCTATCTCTAAAG

Gallelli et al., 2011

KN-F (5' – CACGATACATGGGCTTATGC – 3') 492 bp

KN-R (5' – CTTTTTCATCCACACTCCG – 3')

AvrDdpx-F (5' – TTTCGGTGGTAACGTTGGCA – 3') 230 bp

AvrDdpx-R (5' – TTCCGCTAGGTGAAAAATGGG – 3')

Organisation Européenne et Méditerranéenne pour la Protection des Plantes
European and Mediterranean Plant Protection Organization

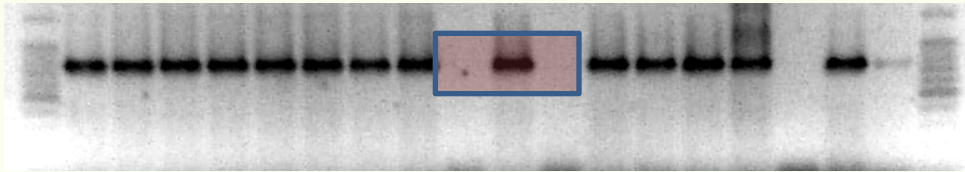
Normes OEPP EPPO Standards

Diagnostic protocols for regulated pests
Protocoles de diagnostic pour les
organismes réglementés

Identification

PCR *Scor*

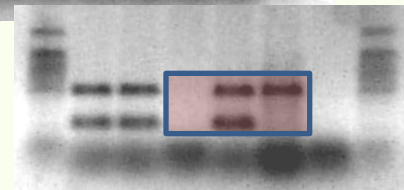
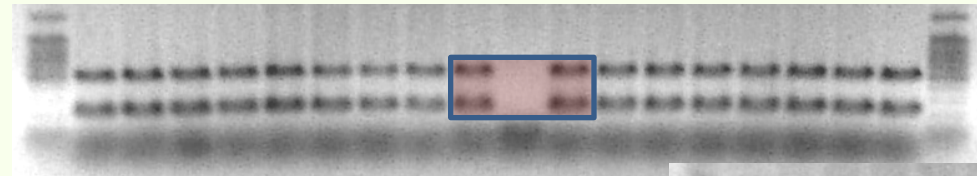
760 bp



PCR *Gal*

492 bp

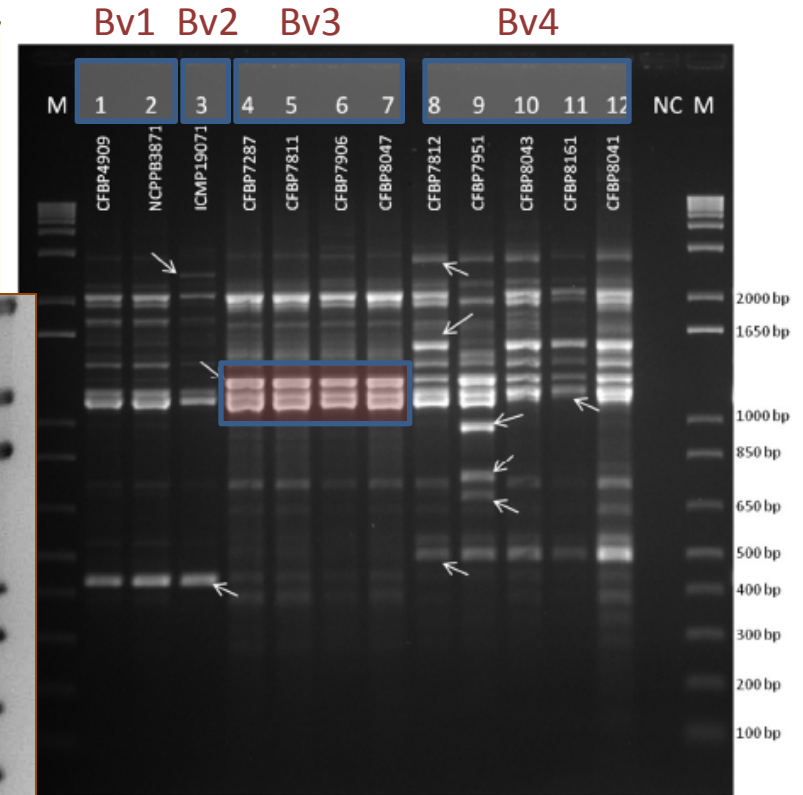
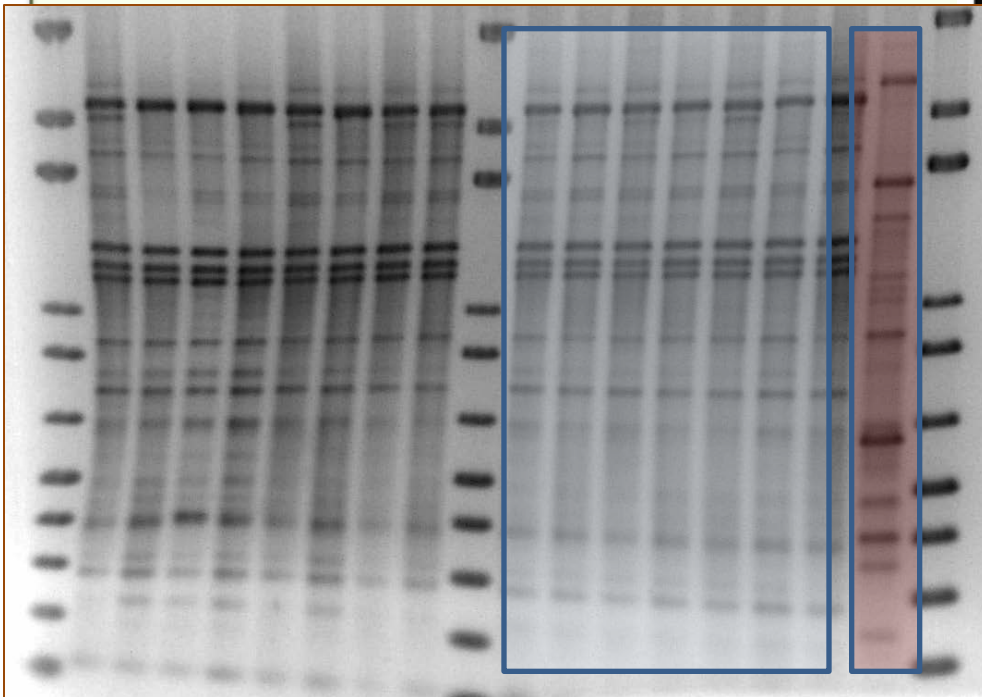
230 bp



Biovar	symptoms	esculin	coronatin	phaseolotoxin	PCR <i>Scor</i>	PCR <i>Gal</i> (bands)
1	ramos/folhas	-	-	+	+	2
2	ramos/folhas	-	+	-	+	2
3	ramos/folhas	-	-	-	+	2
4	folhas	+	-	-	+	1

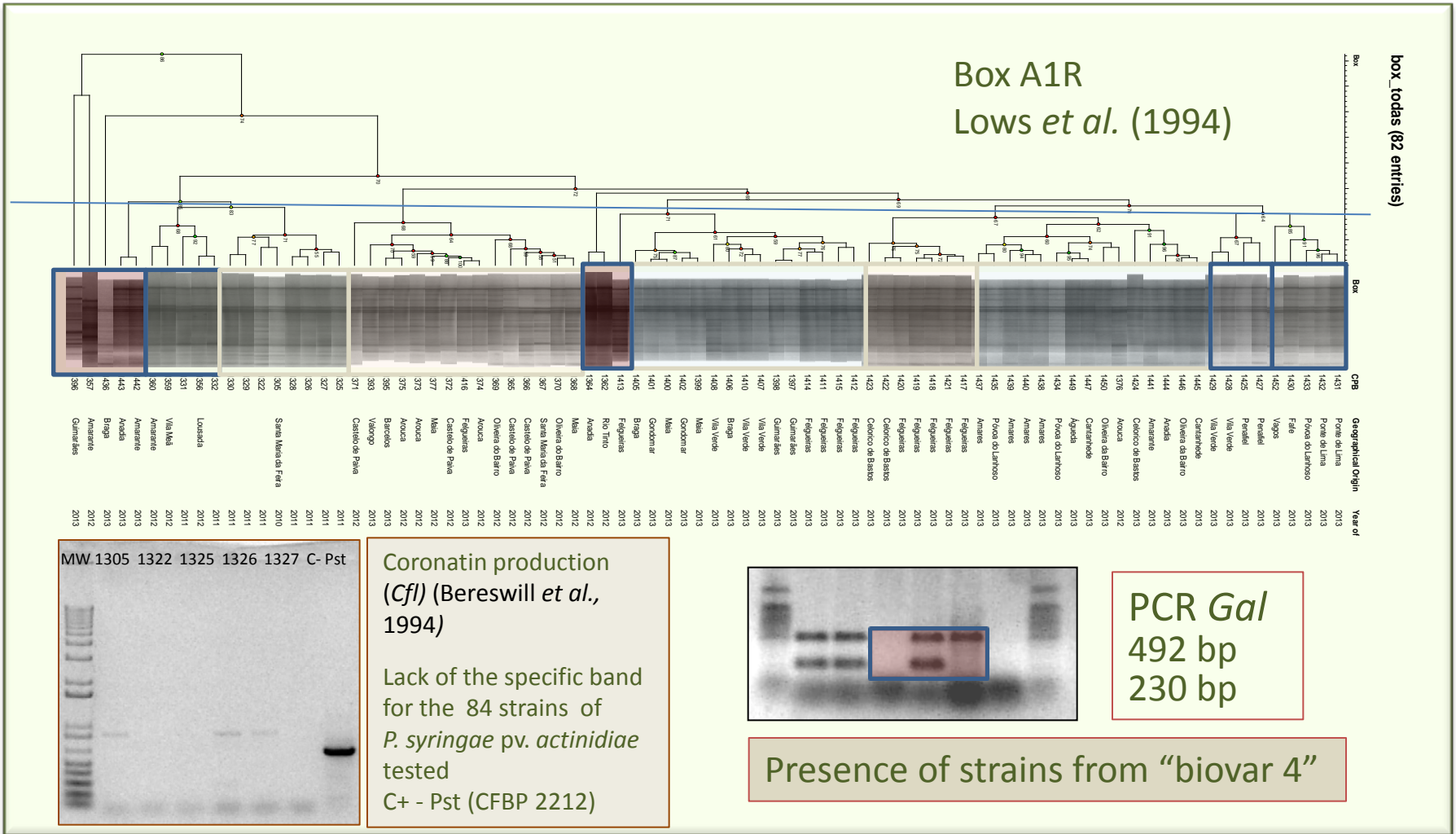
Genomic characterization

- L1 – 1Kb plus
- L2 – Psa 1365
- L3 – Psa 1366
- L4 – Psa 1367
- L5 – Psa 1368
- L6 – Psa 1369
- L7 – Psa 1370
- L8 – Psa 1371
- L9 – Psa 1372
- L10 – 1Kb plus
- L11 – Psa 1373
- L12 – Psa 1374
- L13 – Psa 1375
- L14 – Psa 1416
- L15 – Psa 1377
- L16 – Psa 1393
- L17 – Psa 1395
- L18 – Psa 1396
- L19 – 1Kb plus



(Cunty et al., 2015)

Genomic characterization



Diagnosis

Decision scheme

1 . Sample preparation

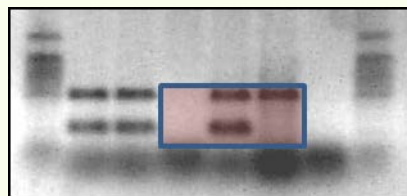
Orchard sub-samples of male and female plants

2 – Screening tests

Isolation on KMB and conventional PCR

(Scortichini et al 2002; Rees-George et al., 2010)

3 – Identification of colonies by Galleli et al. (2011)



4 – Confirmation by Real - time PCR (Galleli et al., 2013) (EUPHRESKO II – PSADID)

EUPHRESCO II - European Phytosanitary Research Coordination II



Development and harmonization of methods for diagnosis, detection and identification of *Pseudomonas syringae* pv. *actinidiae* (2013-2015)

Resultados previstos:

- ✓ Implementation of new tools for the diagnosis of Psa in symptomatic a asymptomatic plant material
- ✓ Validation of a sampling protocol
- ✓ Epidemiological knowledge of *Pseudomonas syringae* pv. *actinidiae* in distinct areas of Europe

	Partner	Country
1	MIPAAF	Italy
2	FR-DGAL	France
3	ES-IVIA	Spain
4	PT-INIAV	Portugal
5	NZ-MPI	New Zealand

Phylogenetic Characterization

Italy

1st Focus – 1992

2nd Focus – 2008

Estirpes com características fisiológicas (produção de faseolotoxina e coronatina) e genómicas distintas (genes constitutivos e de virulência)

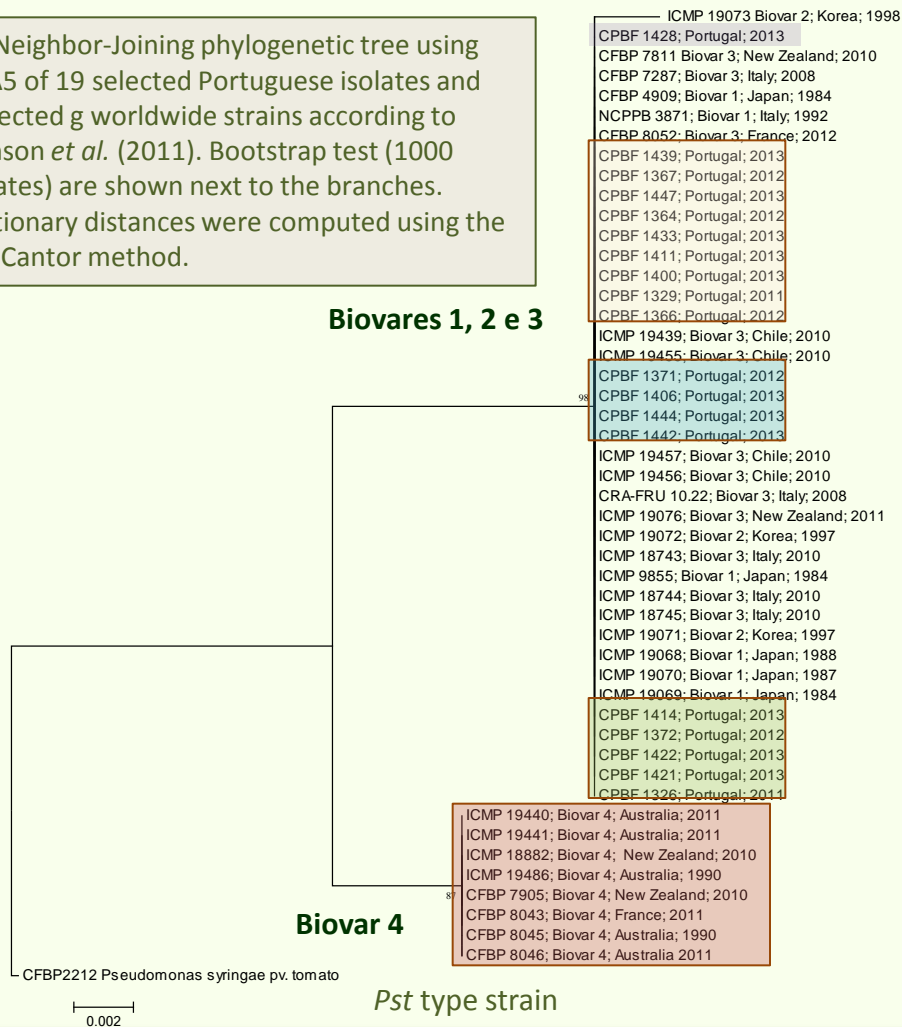
Portugal

1st Identification – 2010

Identification of the bacterium in orchards with more than 10 years

Sequenciação de 1 isolado Português obtido em 2010

rpoD Neighbor-Joining phylogenetic tree using MEGA5 of 19 selected Portuguese isolates and 30 selected *Pst* worldwide strains according to Parkinson *et al.* (2011). Bootstrap test (1000 replicates) are shown next to the branches. Evolutionary distances were computed using the Jukes-Cantor method.



Conclusions

- ✓ Between 2010 and 2013 more than 100 isolates of *Pseudomonas syringae* pv. *actinidiae* were collected from orchards nurseries and imported propagation materials.
- ✓ The use of two conventional PCR protocols allowed identifying all known biovars of *Pseudomonas syringae* pv. *actinidiae*.
- ✓ The use of primers directed to genes responsible for the production of the toxins coronatin (*Cfl*) and/or phaseolotoxin (*argK*) allow to exclude the presence of biovar 2 among Portuguese strains.
- ✓ BOX PCR fingerprinting profiles were characteristic of biovar 3 for most of the strains tested
- ✓ The phylogenetic tree generated by *rpoD* confirms the association of the selected strains as belonging to biovar 3.
- ✓ The lack of *avrD1* amplification indicates the presence of a small population of biovar 4 strains allocated recently to *Pseudomonas syringae* pv. *actinidifoliorum*.



Obrigada!

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