



# 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

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RT: Rosa Maricato

LABORATÓRIO DE FÍSICA DO SOLO  
RT: Encarnação Marcelo

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RT: Lurdes Inácio

LABORATÓRIO DE OGM  
RT: Eugénia Andrade

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



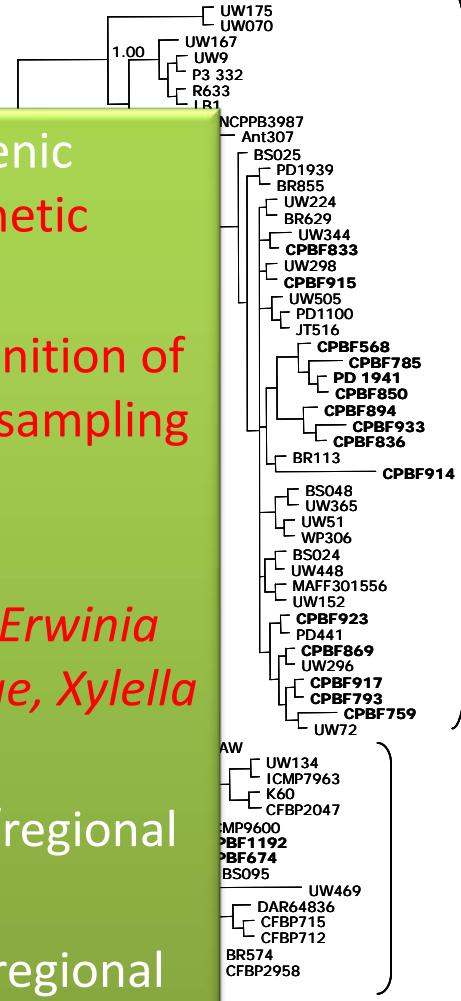
Analysis of Q-bacteria (*Ralstonia solanacearum*, *Clavibacter michiganensis* subsp. *sepedonicus*, *Erwinia amylovora*, *Pseudomonas syringae* pv. *actinidiae*, *Xylella fastidiosa*; *Huanlonbing*)



Scientific and technical suport 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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<sup>2</sup> DRAPN –Divisão de Apoio ao Sector Agroalimentar, Senhora da Hora, Porto;

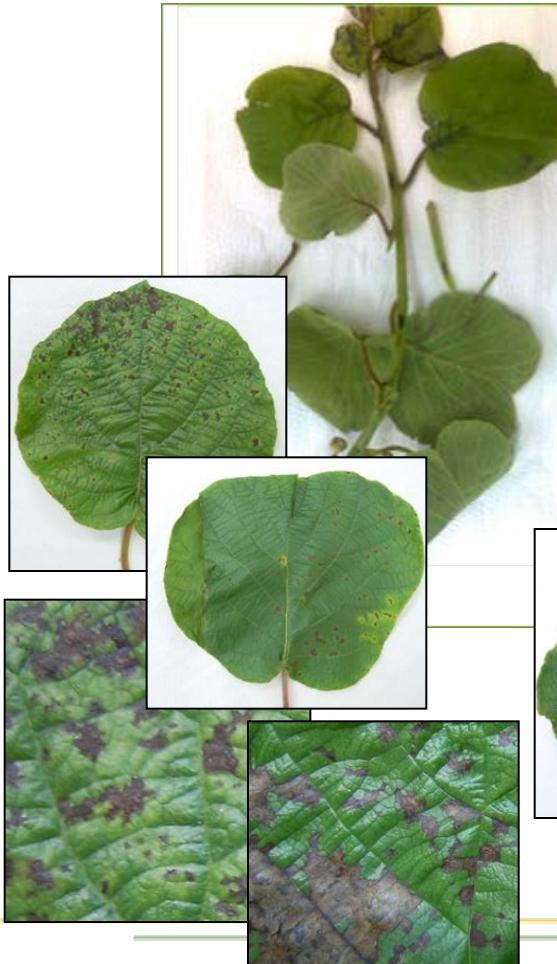
<sup>3</sup> Universidade de Lisboa, Faculdade de Ciências, Centro de Biodiversidade Genómica Integrativa e Funcional (BioFiG);

<sup>4</sup> Universidade do Porto, Faculdades de Ciências, Campo Alegre, Porto.

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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  $>15^{\circ}\text{C}$  during blooming
- ✓ Presence of pollinators
- ✓ High HR

# Bacterial canker of kiwi

## Major and minor hosts

*Actinidia chinensis*  
(summer kiwi,  
chinese kiwi)



*Actinidia deliciosa*  
(kiwi, Chinese  
gooseberry)



*Actinidia arguta*



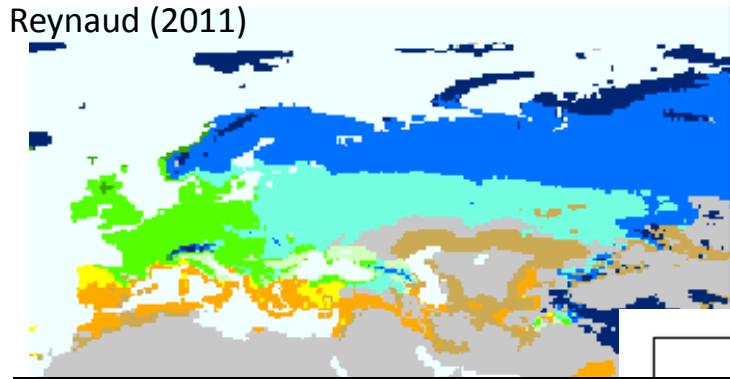
*Actinidia kolomikta*



## *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

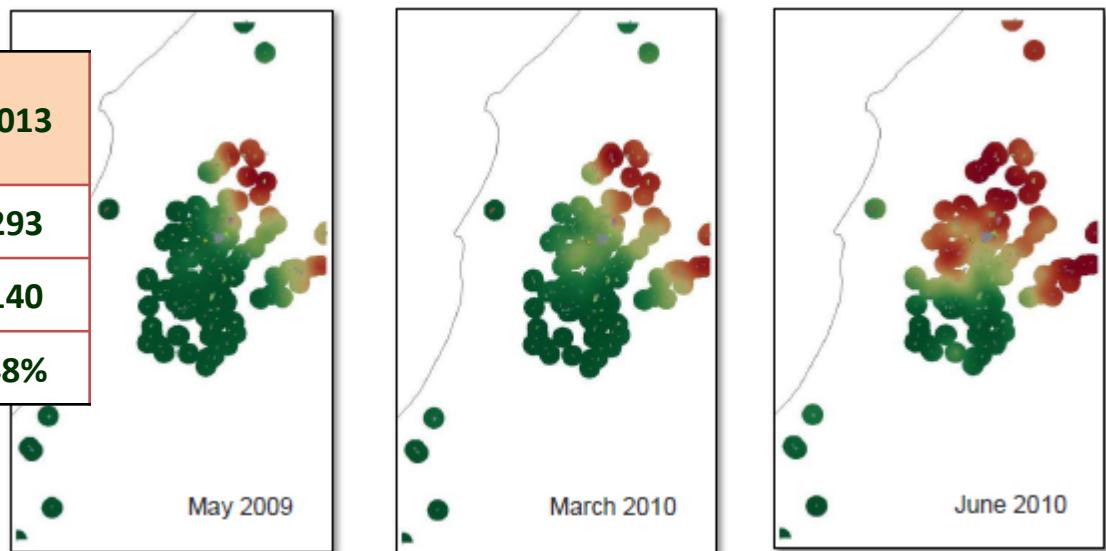


EPPO PRA  
Portugal

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	<b>293</b>
pos	6	15	30	<b>140</b>
%	60%	9%	42%	<b>48%</b>

Portuguese Control Plan  
*P. syringae* pv. *actinidiae*



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

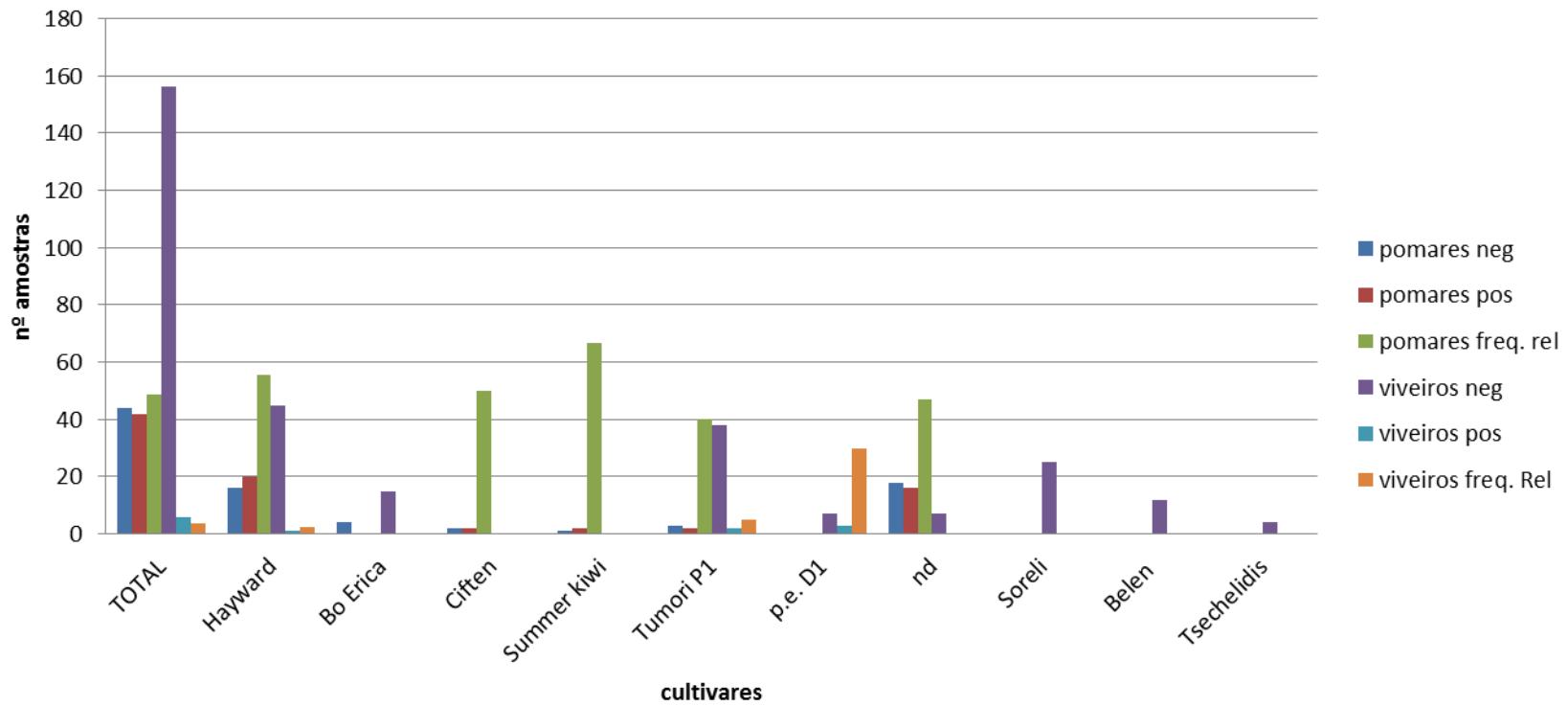
# Incidence

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

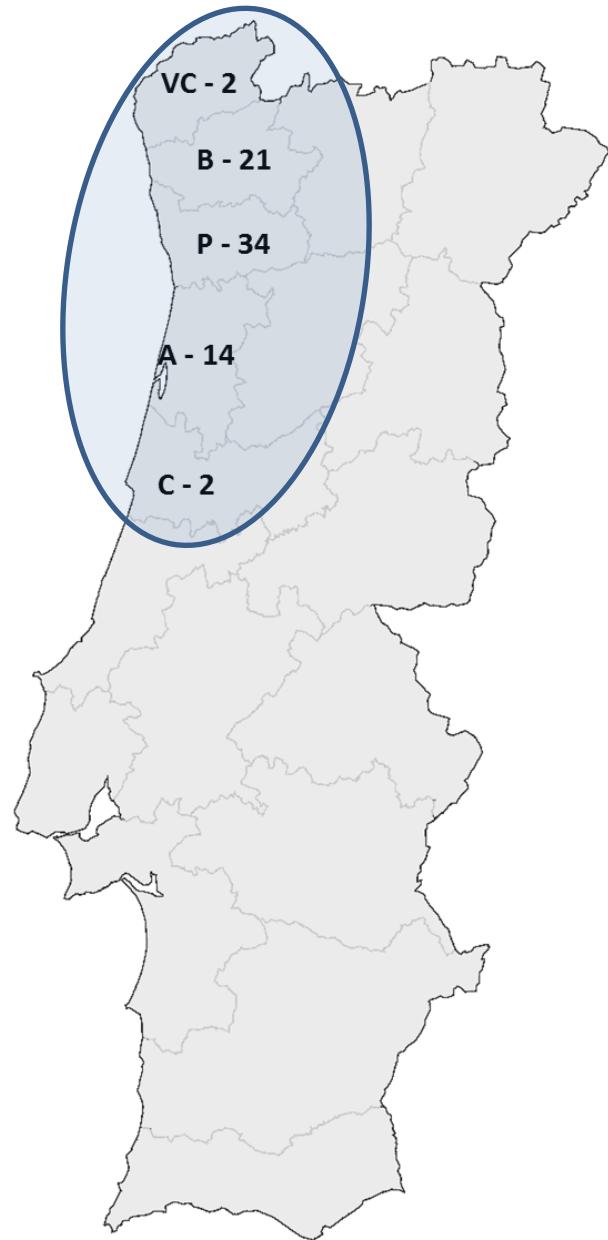
# Portuguese situation

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



# Sampling

- ✓ Kiwi area in north Portugal – 1500ha
- ✓ Comission Decision n.<sup>o</sup> 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 GGCGACGATCCGTAACGGTCTGAGA **760 bp**

P 22 TTCCCGAAGGCACTCCTCTATCTCTAAAG

Gallelli *et al.*, 2011

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

KN-R (5' – CTTTCATCCACACACTCCG – 3')

AvrDdpx-F (5' – TTTGGTGGTAACGTTGGCA – 3') **230 bp**

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

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

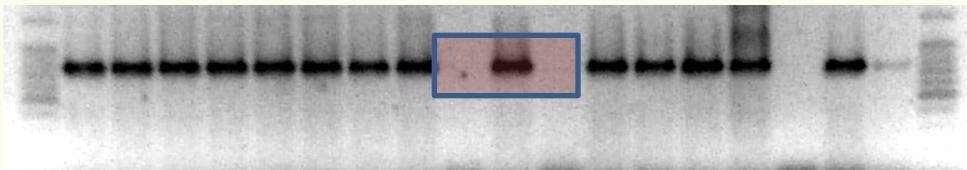
## Normes OEPP EPPO Standards

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

# Identification

PCR *Scor*

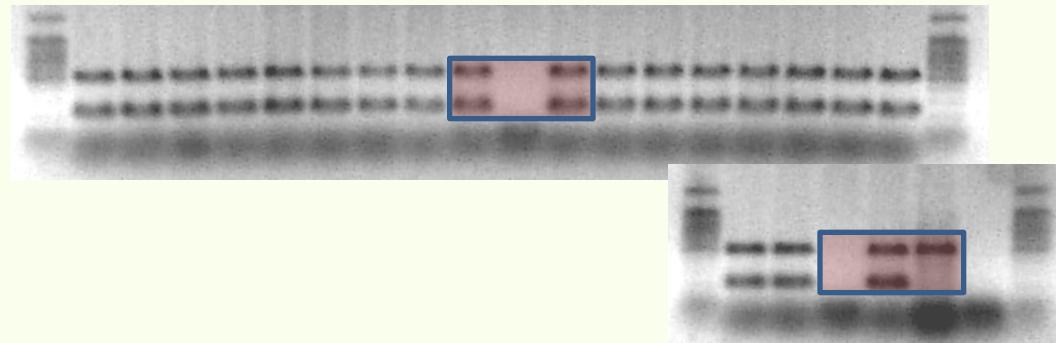
760 bp



PCR *Gal*

492 bp

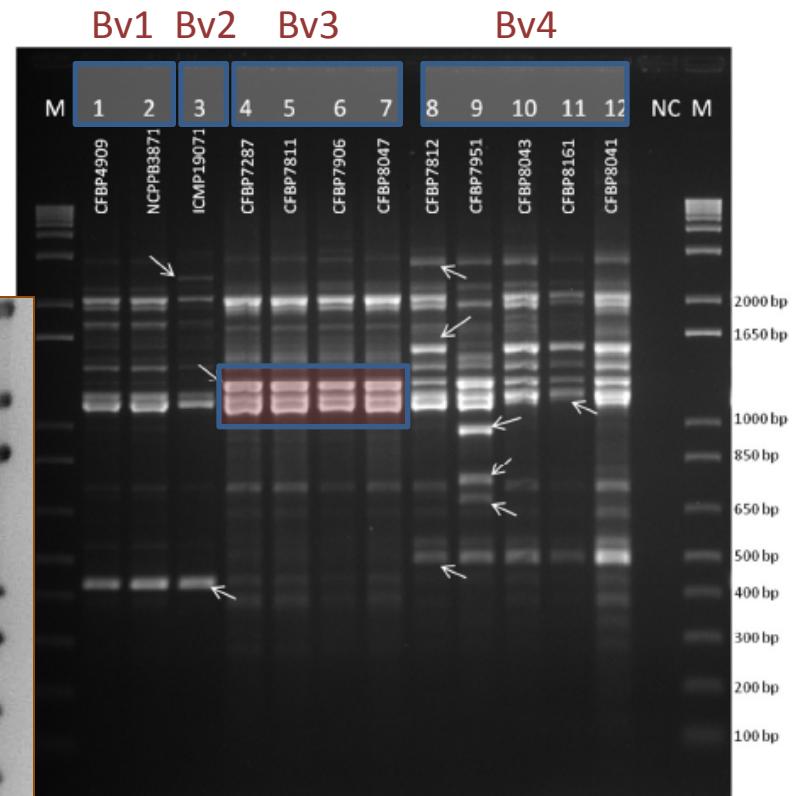
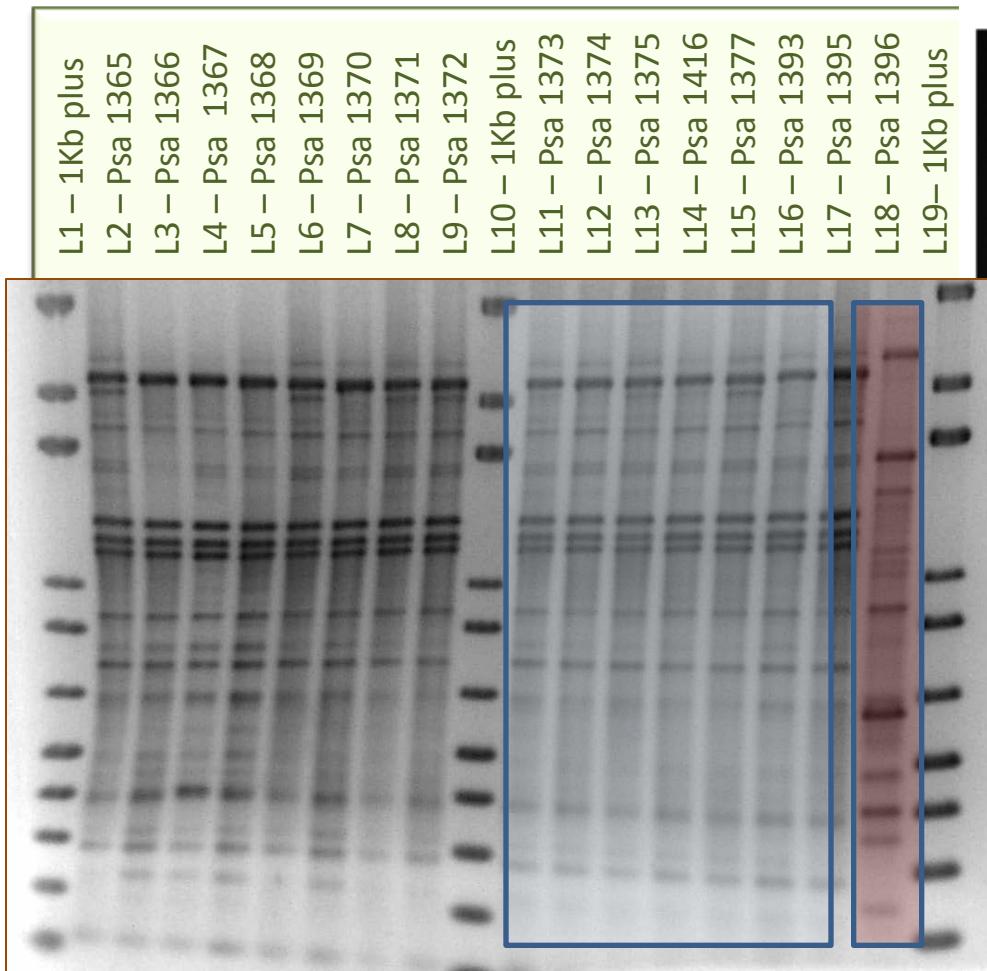
230 bp



Leoni

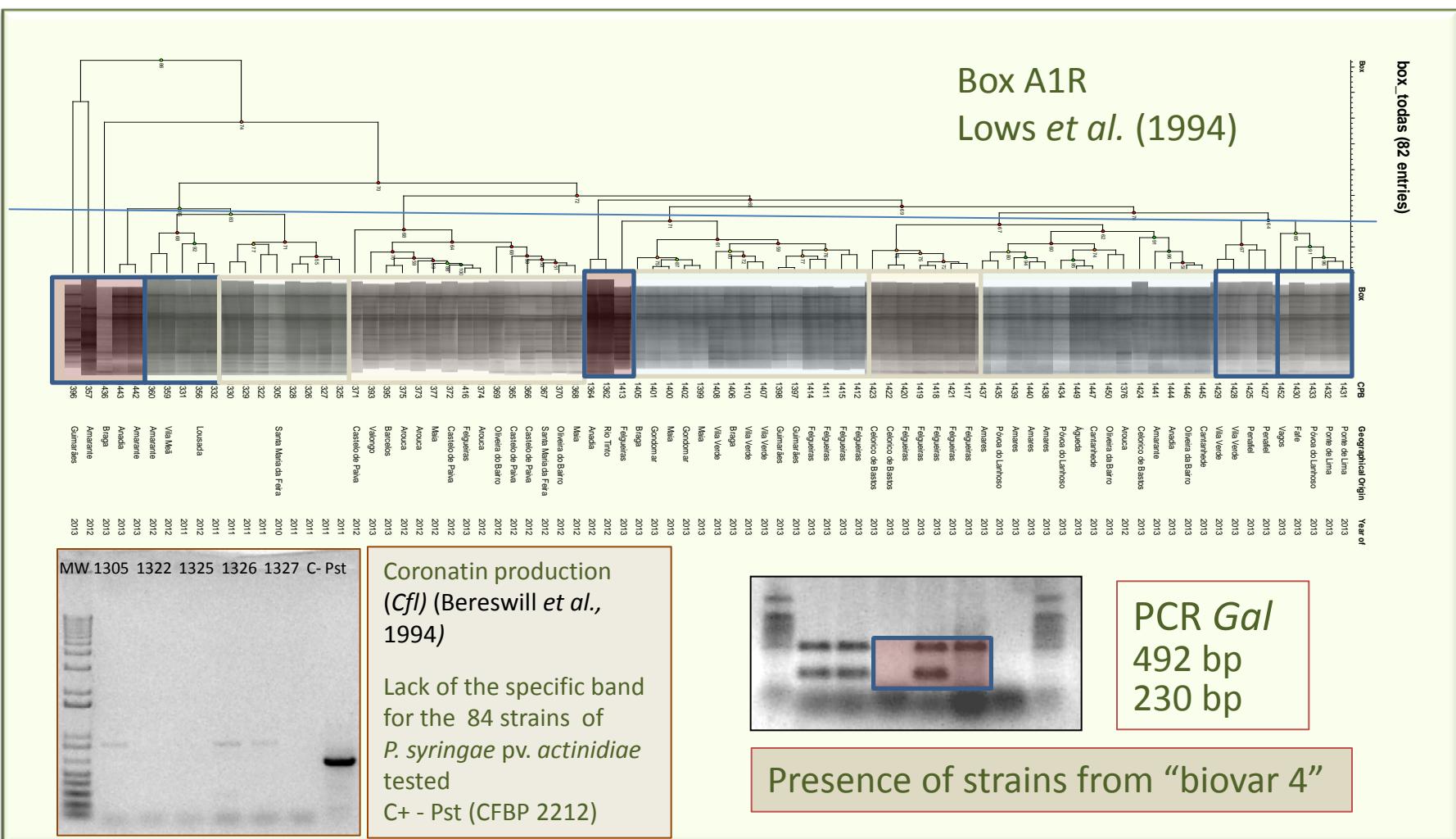
Biovar	symptoms	esculin	coronatin	phaseolotoxin	PCR <i>Scor</i>	PCR <i>Gal</i> (bands)
1	ramos/folhas	-	-	+	+	2
2	ramos/folhas	-	+	-	+	2
3	ramos/folhas	-	-	-	+	2
4	folhas	+	-	-	+	1

# | Genomic characterization



(Cunty et al., 2015)

## Genomic characterization



# Diagnosis

## Decision scheme

### 1 . Sample preparation

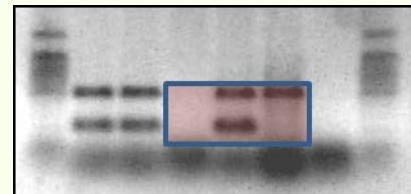
Orchard sub-samples of male and female plants

### 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) (EUPHRESCO 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 and 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

# I 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

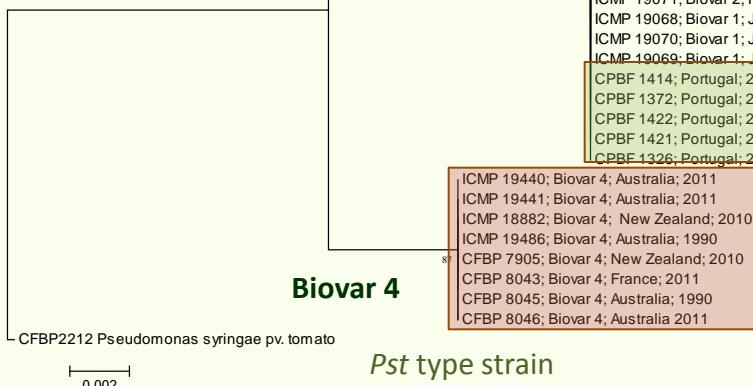
*rpoD* Neighbor-Joining phylogenetic tree using MEGA5 of 19 selected Portuguese isolates and 30 selected worldwide strains according to Parkinson *et al.* (2011). Bootstrap test (1000 replicates) are shown next to the branches. Evolutionary distances were computed using the Jukes-Cantor method.

### Biovar 1, 2 e 3

ICMP 19073 Biovar 2; Korea; 1998  
CPBF 1428; Portugal; 2013  
CFBP 7811 Biovar 3; New Zealand; 2010  
CFBP 7287; Biovar 3; Italy; 2008  
CFBP 4909; Biovar 1; Japan; 1984  
NCPPB 3871; Biovar 1; Italy; 1992  
CFBP 8052; Biovar 3; France; 2012  
CPBF 1439; Portugal; 2013  
CPBF 1367; Portugal; 2012  
CPBF 1447; Portugal; 2013  
CPBF 1364; Portugal; 2012  
CPBF 1433; Portugal; 2013  
CPBF 1411; Portugal; 2013  
CPBF 1400; Portugal; 2013  
CPBF 1329; Portugal; 2011  
CPBF 1366; Portugal; 2012  
ICMP 19439; Biovar 3; Chile; 2010  
ICMP 19455; Biovar 3; Chile; 2010  
CPBF 1371; Portugal; 2012  
CPBF 1406; Portugal; 2013  
CPBF 1444; Portugal; 2013  
CPBF 1442; Portugal; 2013

ICMP 19457; Biovar 3; Chile; 2010  
ICMP 19456; Biovar 3; Chile; 2010  
CRA-FRU 10.22; Biovar 3; Italy; 2008  
ICMP 19076; Biovar 3; New Zealand; 2011  
ICMP 19072; Biovar 2; Korea; 1997  
ICMP 18743; Biovar 3; Italy; 2010  
ICMP 9855; Biovar 1; Japan; 1984  
ICMP 18744; Biovar 3; Italy; 2010  
ICMP 18745; Biovar 3; Italy; 2010  
ICMP 19071; Biovar 2; Korea; 1997  
ICMP 19068; Biovar 1; Japan; 1988  
ICMP 19070; Biovar 1; Japan; 1987  
ICMP 19069; Biovar 1; Japan; 1984  
CPBF 1414; Portugal; 2013  
CPBF 1372; Portugal; 2012  
CPBF 1422; Portugal; 2013  
CPBF 1421; Portugal; 2013  
CPBF 1326; Portugal; 2011

ICMP 19440; Biovar 4; Australia; 2011  
ICMP 19441; Biovar 4; Australia; 2011  
ICMP 18882; Biovar 4; New Zealand; 2010  
ICMP 19486; Biovar 4; Australia; 1990  
CFBP 7905; Biovar 4; New Zealand; 2010  
CFBP 8043; Biovar 4; France; 2011  
CFBP 8045; Biovar 4; Australia; 1990  
CFBP 8046; Biovar 4; Australia 2011



# 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 alocated recently to *Pseudomonas syringae* pv. *actinidifoliorum*.



# Obrigada!

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