Characterization of *Pseudomonas syringae* pv. *actinidiae* genetic diversity in Portugal

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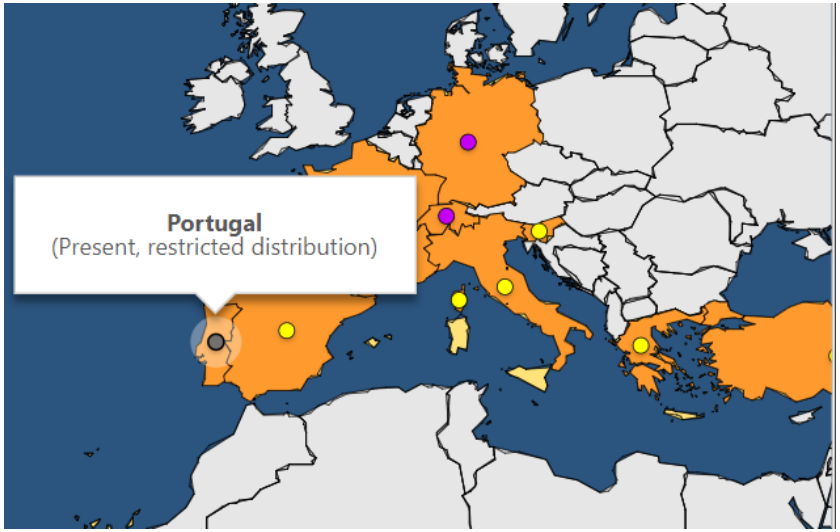
PSA IN PORTUGAL

First report: 2010



New Disease Reports (2010) 22, 10. [doi:10.5197/j.2044-0588.2010.022.010]

New Disease Reports

First report of bacterial canker of *Actinidia deliciosa* caused by *Pseudomonas syringae* pv. *actinidiae* in Portugal

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SCAP

Identificação e caracterização de *Pseudomonas syringae* pv. *actinidiae* (Psa) na Região do Entre Douro e Minho (Portugal)

Identification and characterization of *Pseudomonas syringae* pv. *actinidiae* (Psa) in Entre Douro and Minho region (Portugal)

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Short Notes

Widespread distribution of kiwifruit bacterial canker caused by the European *Pseudomonas syringae* pv. *actinidiae* genotype in the main production areas of Portugal

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S03.04

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

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Previous studies revealed that Psa isolates analysed from Entre Douro e Minho region were identical to the **biovar 3** (Renzi *et al.*, 2012; Moura *et al.*, 2015)

Portuguese Psa population from Entre Douro e Minho region presents **some degree of genetic variability** (Moura *et al.*, 2015).

Field Surveys

Objective: Analyse the diversity of Psa populations in Portugal.

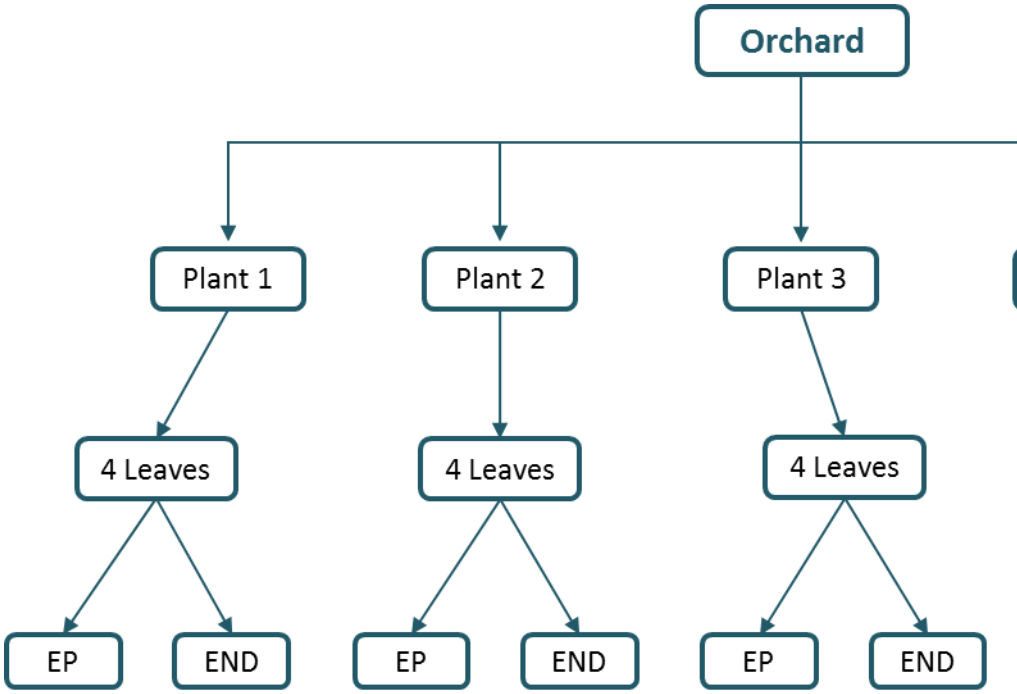
Orchard	Localization	Cultivar	Age (years)	First detection of Psa	Psa disease severity degree
A	Viana do Castelo	*A. deliciosa* cv. "Hayward"	7	2010	1
B	Guimarães	*A. deliciosa* cv. "Erika"	5	2015	2
C	Albergaria-a-Nova	*A. deliciosa* cv. "Hayward"	16	2016*	1
D	Montemor-o-Velho	*A. deliciosa* cv. "Hayward"	4	2015	3
E	Montemor-o-Velho	*A. deliciosa* cv. "Hayward"	30	2016	2

*This study

Spring and Autumn

2016

METHODS



Isolation according to EPPO, 2014

Spring and Autumn

METHODS

Isolates

Presumptive **identification** of Psa

(Gallelli *et al.*, 2011)

Non Psa isolates

Poster

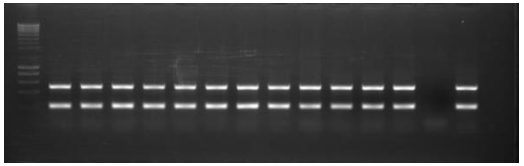
Psa isolates

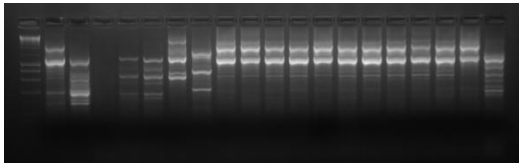
Characterization by BOX-PCR

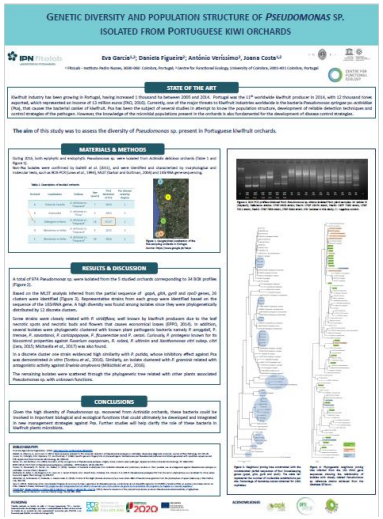
47 Profiles

MLST

gapA, gltA, gyrB, rpoD



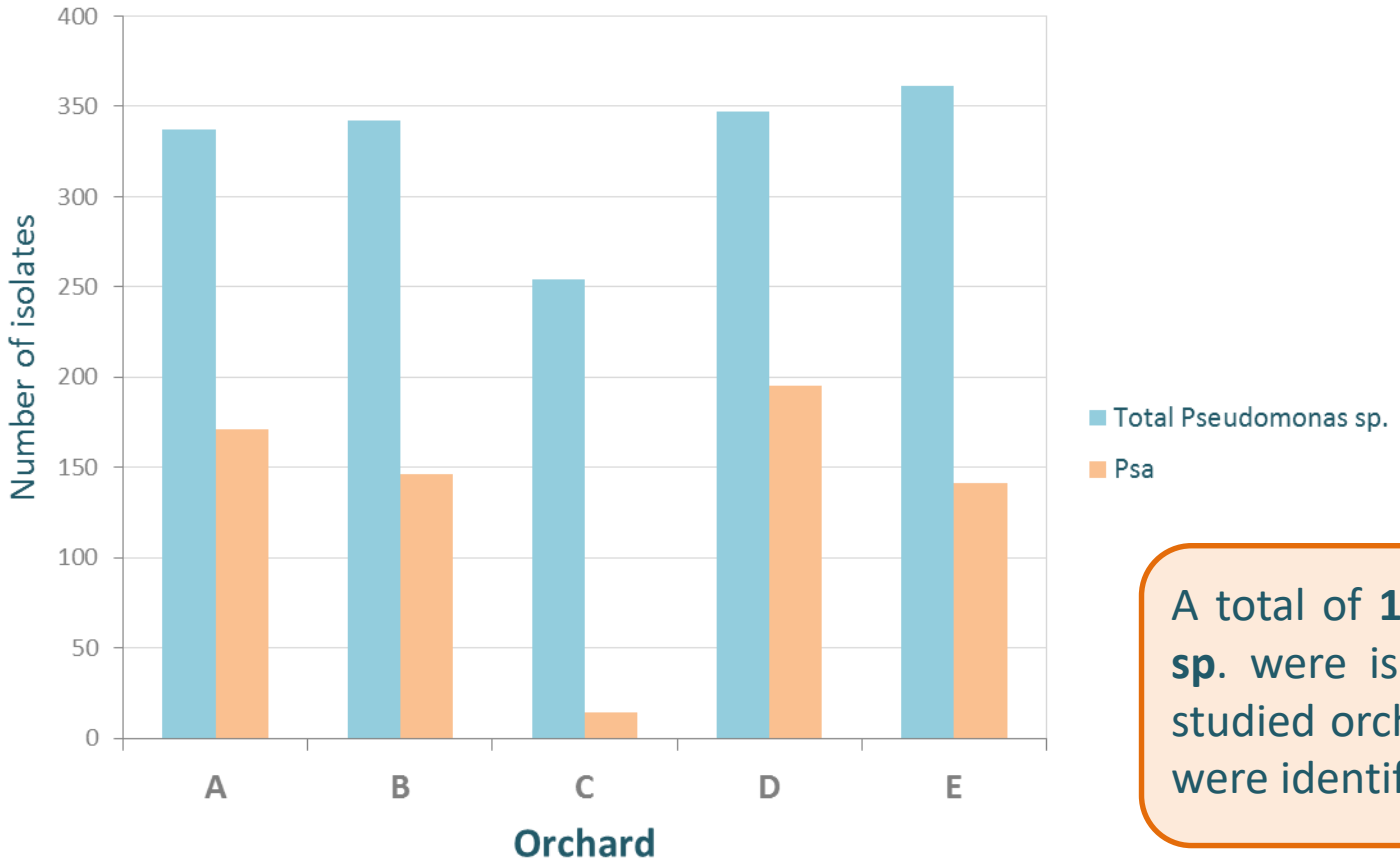



GENETIC DIVERSITY AND POPULATION STRUCTURE OF *PSEUDOMONAS* SP. ISOLATED FROM PORTUGUESE KIWI ORCHARDS

RESULTS

Objective 1

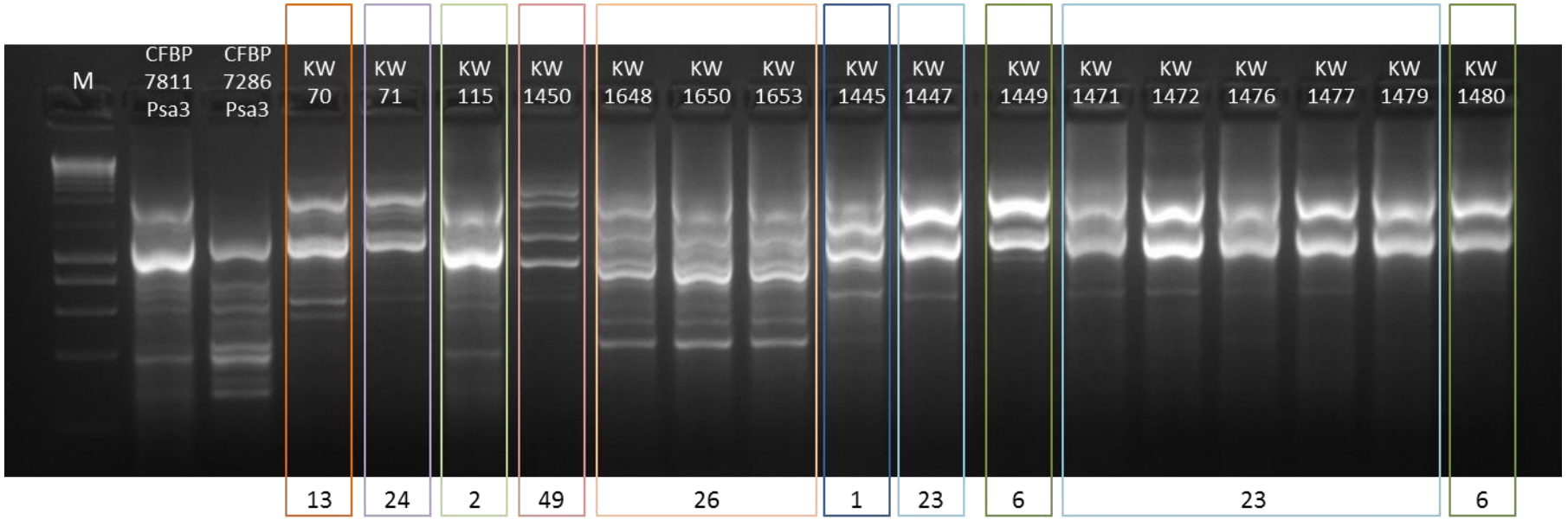
Isolate the maximum number of ***Pseudomonas* sp.** from kiwifruit orchards.



A total of **1641 *Pseudomonas* sp.** were isolated from the 5 studied orchards of which **667** were identified as **Psa**.

Objective 2

Determine the **diversity of Psa** populations.

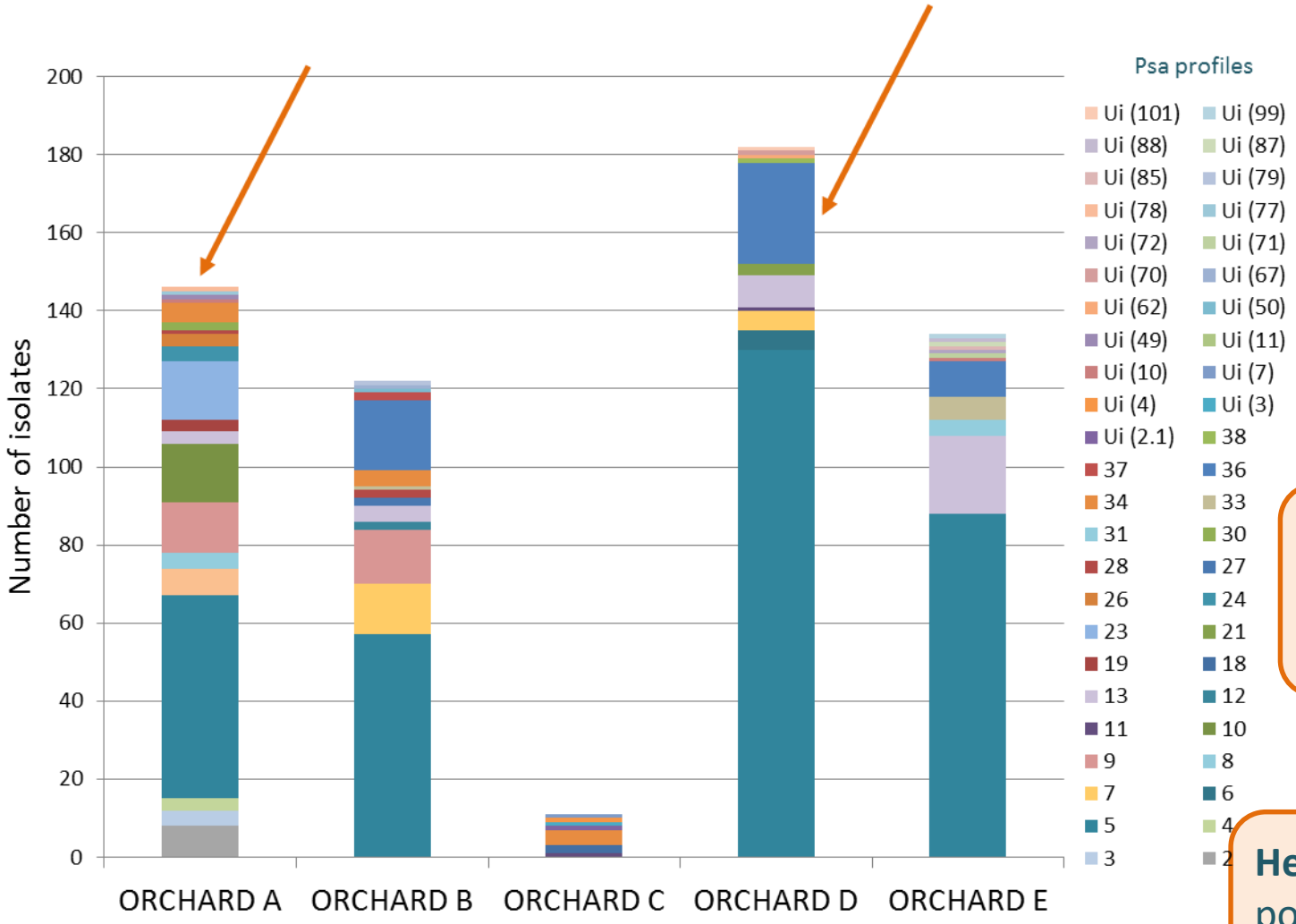


Profiles	Nº of isolates	Profiles	Nº of isolates	Profiles	Nº of isolates	Profiles	Nº of isolates	Profiles	Nº of isolates	Profiles	Nº of isolates
2	8	**10**	15	**24**	4	**36**	53	**Ui (49)**	1	**Ui (78)**	1
3	4	**11**	2	**26**	3	**37**	2	**Ui (50)**	1	**Ui (79)**	1
4	3	**12**	2	**27**	2	**38**	1	**Ui (62)**	1	**Ui (85)**	1
5	327	**13**	35	**28**	3	**Ui (2.1)**	1	**Ui (67)**	1	**Ui (87)**	1
6	12	**18**	2	**30**	2	**Ui (3)**	1	**Ui (70)**	1	**Ui (88)**	1
7	18	**19**	3	**31**	4	**Ui (4)**	1	**Ui (71)**	1	**Ui (99)**	1
8	4	**21**	3	**33**	7	**Ui (7)**	1	**Ui (72)**	1	**Ui (101)**	1
9	27	**23**	15	**34**	13	**Ui (10)**	2	**Ui (77)**	1		

A total of **47** BOX profiles were identified.

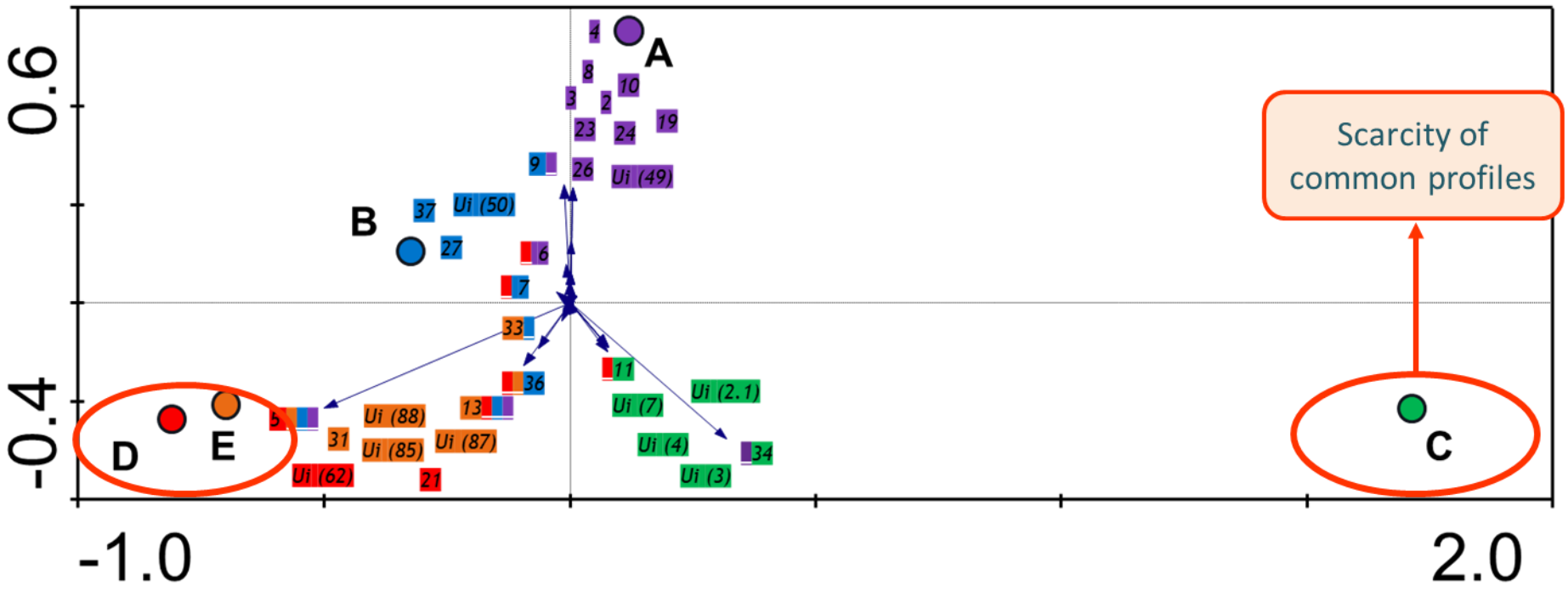
Objective 3

Determine the **diversity** of Psa populations **between orchards**.



Not a single profile was common to all orchards.

Heterogeneity in Psa population was found between orchards.



Principal component analysis – inter-species correlate – of Psa profiles from each studied orchard. Purple A: total plant isolates from orchard A; Blue B: total plant isolates from orchard B; Green C: total plant isolates from orchard C; Red D: total plant isolates from orchard D; Orange E: total plant isolates from orchard E. Numbers correspond to Psa profiles. Colours identify Psa profiles that have more weight in each condition.

	A	B	C	D	E
A					
B	12.50				
C	3.33	0.00			
D	5.26	17.39	7.14		
E	4.35	12.90	0.00	18.75	

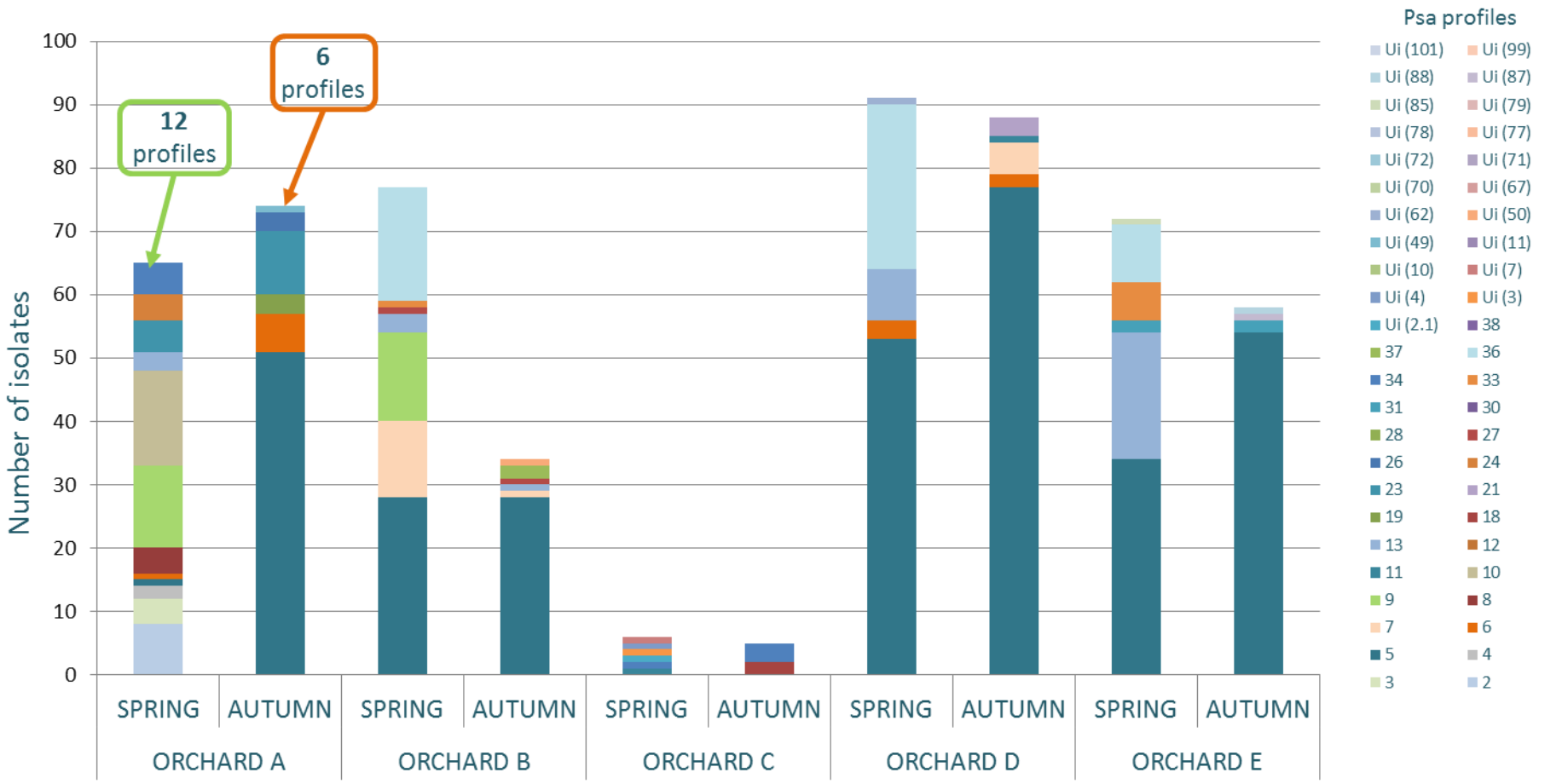
Jacard similarity index, colour scale (%)

20	15	10	5	0

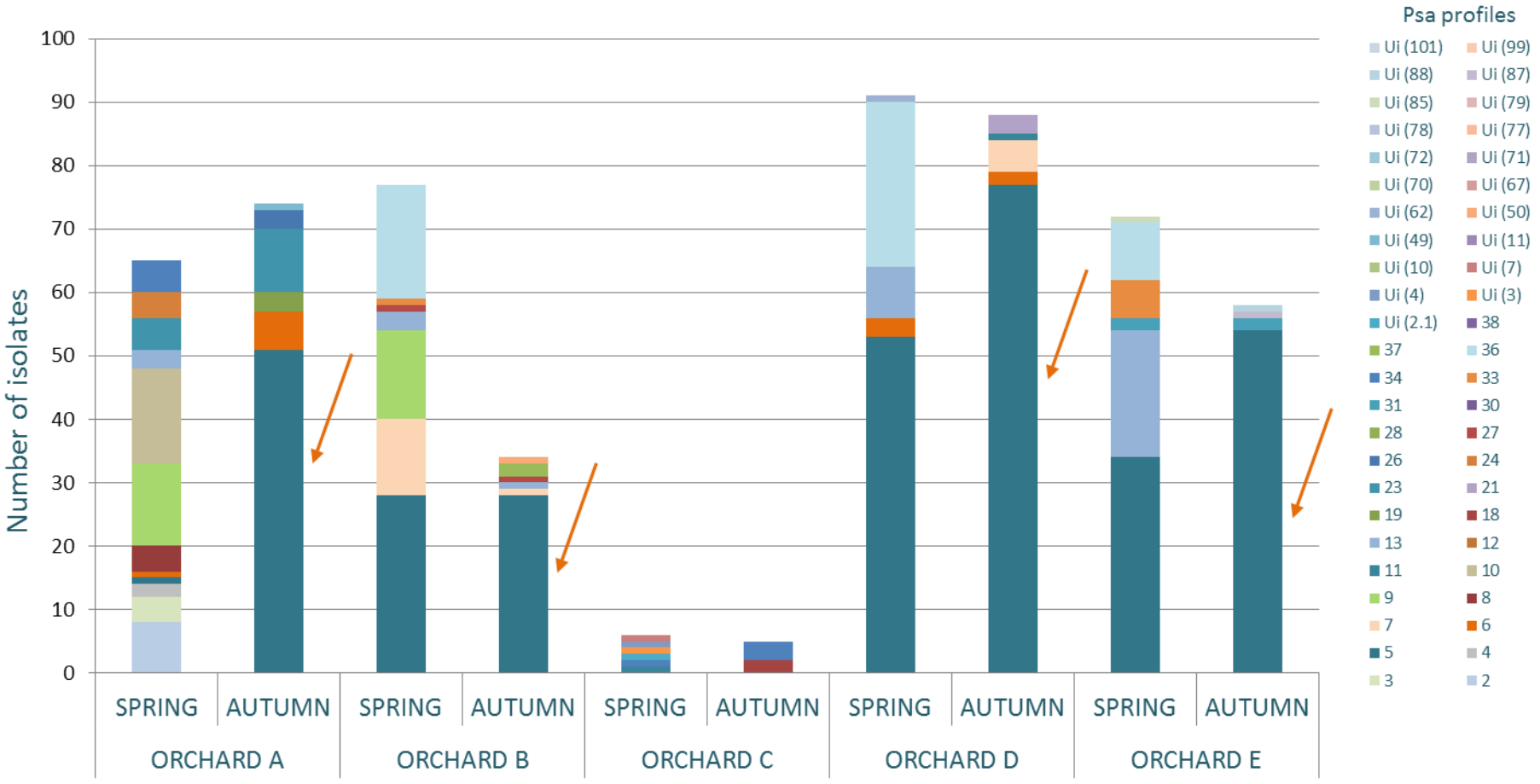
Beta diversity matrix, determined by Jaccard similarity index for the five studied orchards (A, B, C, D and E).

Objective 4

Determine the **diversity** of Psa populations **over time**.



The **diversity** among Psa populations determined in **spring was higher** when compared to those determined in autumn.



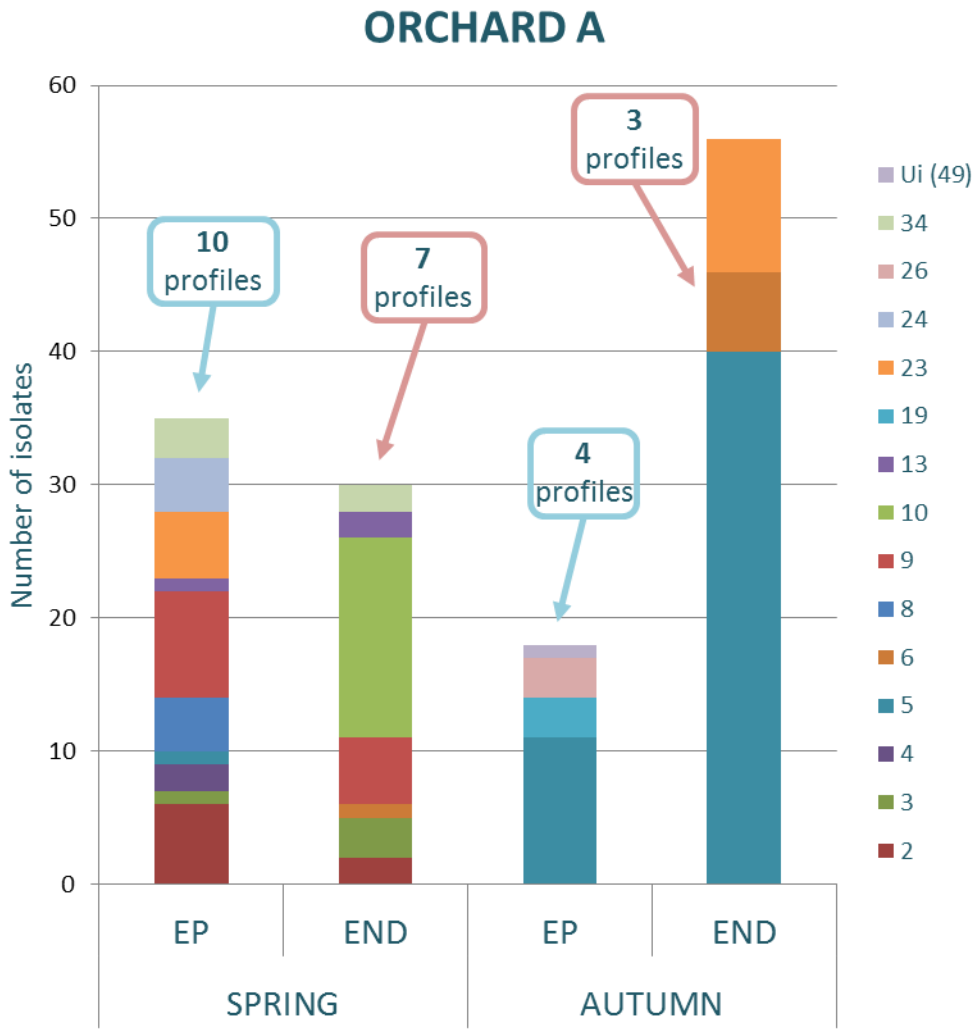
Dominant population in Autumn:

Psa profile 5

Psa profile 5 could be more resilient or better adapted to summer conditions.

Objective 5

Diversity of **endophytic** and **epiphytic** Psa strains over time.



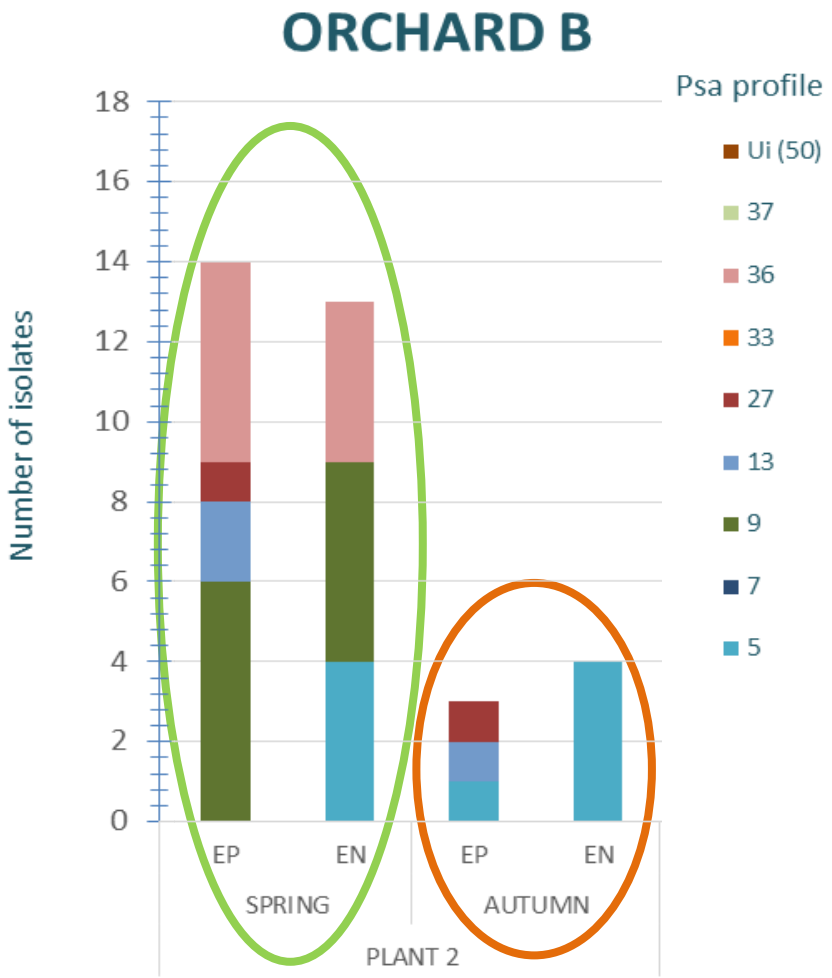
Psa population **heterogeneity** was found **between endophytic and epiphytic** isolates.

Psa population varied **over time**.

Higher diversity was found among **epiphytic** Psa populations when compared to endophytic Psa populations.

Objective 6

Diversity of **endophytic** and **epiphytic** Psa strains in the **same plant, over time**.



Structure of Psa populations **varied over time in the same plant**.

Objective 7

Psa diversity between **North** and **Centre** region.

Index	North		Centre	
	Orchard A	Orchard B	Orchard D	Orchard E
Dmg	3,40	2,33	2,07	2,60
H'	1,43	1,13	0,77	1,11
J'	0,77	0,73	0,57	0,69
1-D	0,67	0,60	0,40	0,56

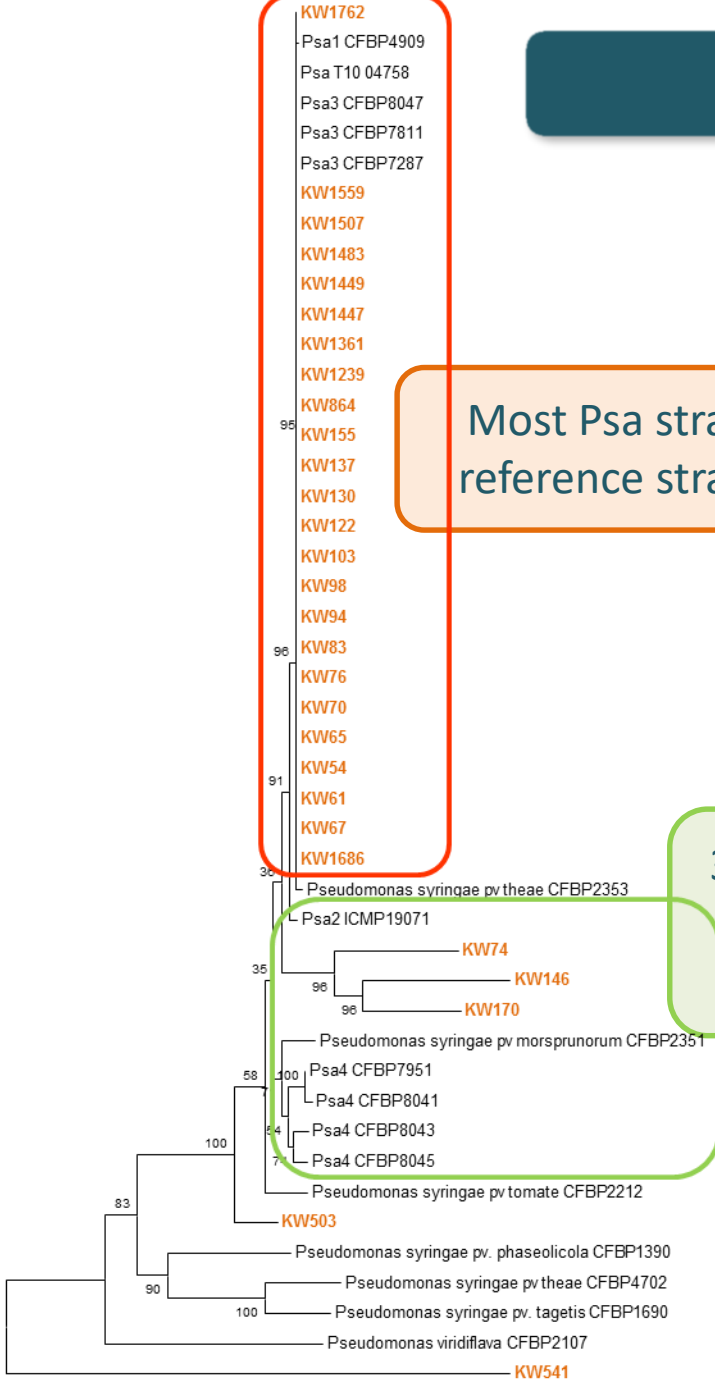
Higher diversity

Dominant profiles

Orchards from the **North** region presented **higher Psa diversity** than the orchards from the Centre region.

Presence of **dominant profiles** was more evident in **Centre region**.

Phylogenetic analysis



Most Psa strains were clustered with reference strains from Psa1 and Psa3.

3 isolates were closely related with Psa2 and Psa4 (*P. syringae* pv. *actinidifoliorum*).

Neighbour joining tree constructed with the concatenated partial sequences of four housekeeping genes (*gapA, gltA, gyrB* and *rpoD*).

CONCLUSIONS

- Psa populations present in Portuguese orchards were **heterogeneous**.
- This **heterogeneity** was found **within orchards** and **between orchards**.
- The **structure** of Psa populations **varied over time in the same plant**.
- The **diversity** among Psa populations determined in **spring was higher** when compared to autumn.
- The **decrease in Psa diversity** in autumn was accompanied by an **increase in dominant populations**, such as Psa profile 5, suggesting that this clone could be more resilient or better adapted to summer conditions.
- Orchards from the **Centre region presented less Psa diversity** due to the presence of dominant profiles.

ACKNOWLEDGES

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