# Genetic diversity of *Pseudomonas syringae* pv. *actinidiae:* seasonal and spatial population dynamics

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## State of the Art

*Pseudomonas syringae* pv. *actinidiae* (Psa) is a gram-negative bacterium responsible for the bacterial canker in *Actinidia deliciosa* and *A. chinensis*, a quarantine disease threatening the kiwifruit industry sustainability. No curative methods have yet been described and control strategies are based mostly on preventive measures (1). Psa has been the subject of several studies in attempt to know the population structure and currently the existence of at least six different Psa types (also known as biovars) capable of infecting *Actinidia* spp., with different levels of virulence was confirmed (2,3). The predominant Psa population isolated since the 2008 outbreak in Italy, and currently spread throughout European orchards, belongs to the pandemic biovar 3, responsible for substantial economic losses worldwide (4). This pandemic biovar derived from a diverse Psa population in China, presents a highly stable core genome and has undergone clonal expansion (5). Recent studies in biovar 3 strains isolated in Europe suggested the existence of variant strains, with reduced virulence, co-existing with virulent strains in mixed populations (6). The knowledge of the structure and evolution of Psa populations present in the orchards is fundamental for the development of disease control strategies.

## Materials & Methods

Four orchards of *A. deliciosa* from different areas of continental Portugal (Fig. 1) were selected for the presence of Psa based on the region, age, degree of Psa severity and cultivar (Table I). The orchards were sampled twice in 2016, once during the spring and again in the autumn. Leaves were collected from the same kiwifruit plants and the endophytic and epiphytic Psa diversity was assessed independently.

Psa isolates were identified with duplex-PCR (7) and the DNA fingerprinting performed using BOX-PCR (8). Molecular tests, such as multiplex-PCR (9), phytotoxins - coronatin (10) and phaseolotoxin (11) and MLST (12) were performed for biovar identification and characterization. Six of our biovar 3 isolates were sequenced using Illumina HiSeq platform with NovaSeq 6000 S2 PE150 XP system (Eurofins Genomics Europe Sequencing GmbH, Germany).

**Table 1** Characteristics and climatic conditions of the studied Portuguese kiwifruit orchards.

| Orchard | Localization | Cultivar | Age (years) | Psa detection | Psa disease severity degree[1] | Average annual temperature (T°)[2] | Annual cumulative rainfall (mm)[2] | Number of cold hours (h)[2] |
|---|---|---|---|---|---|---|---|---|
| A | North | Erica | 7 | 2010 | 1 | 13.5 | 1800 | 478 |
| B | North | Hayward | 5 | 2015 | 2 | 12.5 | 1800 | 1031 |
| C | Centre | Hayward | 4 | 2015 | 3 | 14.5 | 1100 | 541 |
| D | Centre | Hayward | 30 | 2016 | 2 | 14.5 | 1100 | 440 |

1 Adapted from a symptomatology scale (0 – asymptomatic plants to 4 – completely dry plants) created by Cunty et al., 2015;
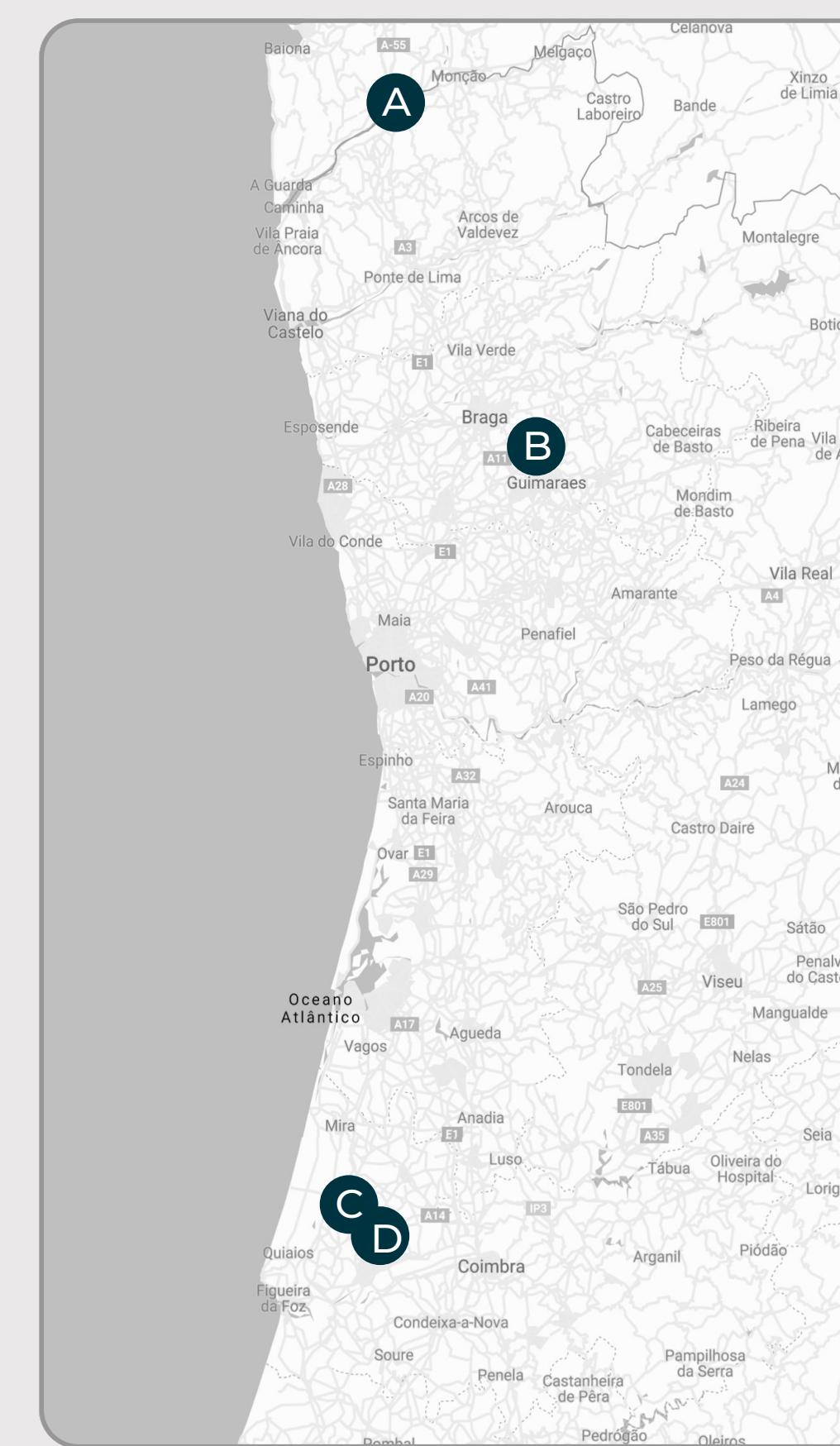2 Accessed online: www.ipma.pt - Portuguese Institute for Sea and Atmosphere, I.P. (IPMA, I.P).



**Figure 1** Geographical localization of the four selected kiwifruit orchards in Portugal.
**A** Valença do Minho (Viana do Castelo;
**B** São Esteves de Briteiros (Guimarães);
**C** Catarruchos (Coimbra);
**D** Cabeça-Alta (Coimbra).
**Source:** https://www.google.pt/maps

## The aim of this study was to determine the genetic structure of endophytic and epiphytic population of *Pseudomonas syringae* pv. *actinidiae* (Psa)
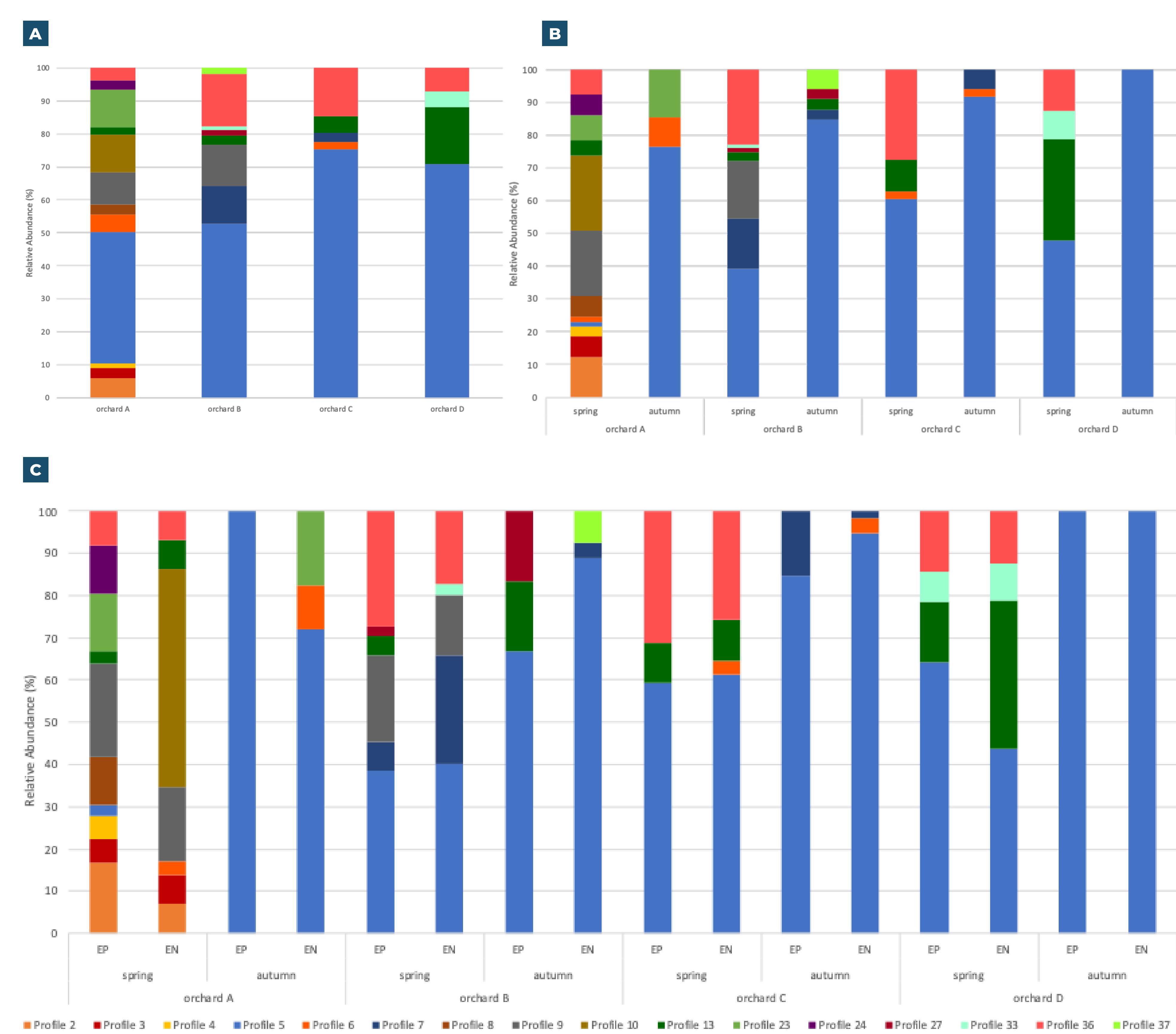


**Figure 2** Diversity of Psa isolates determined by BOX PCR profiling. **A** in orchard A to D; **B** in spring and autumn from the analysed orchards; **C** in epiphytic (EP) and endophytic (EN) isolates from spring and autumn from each of the analysed orchards; **D** in epiphytic (EP) and endophytic (EN) isolates from spring and autumn from the three representative plants from each orchard.
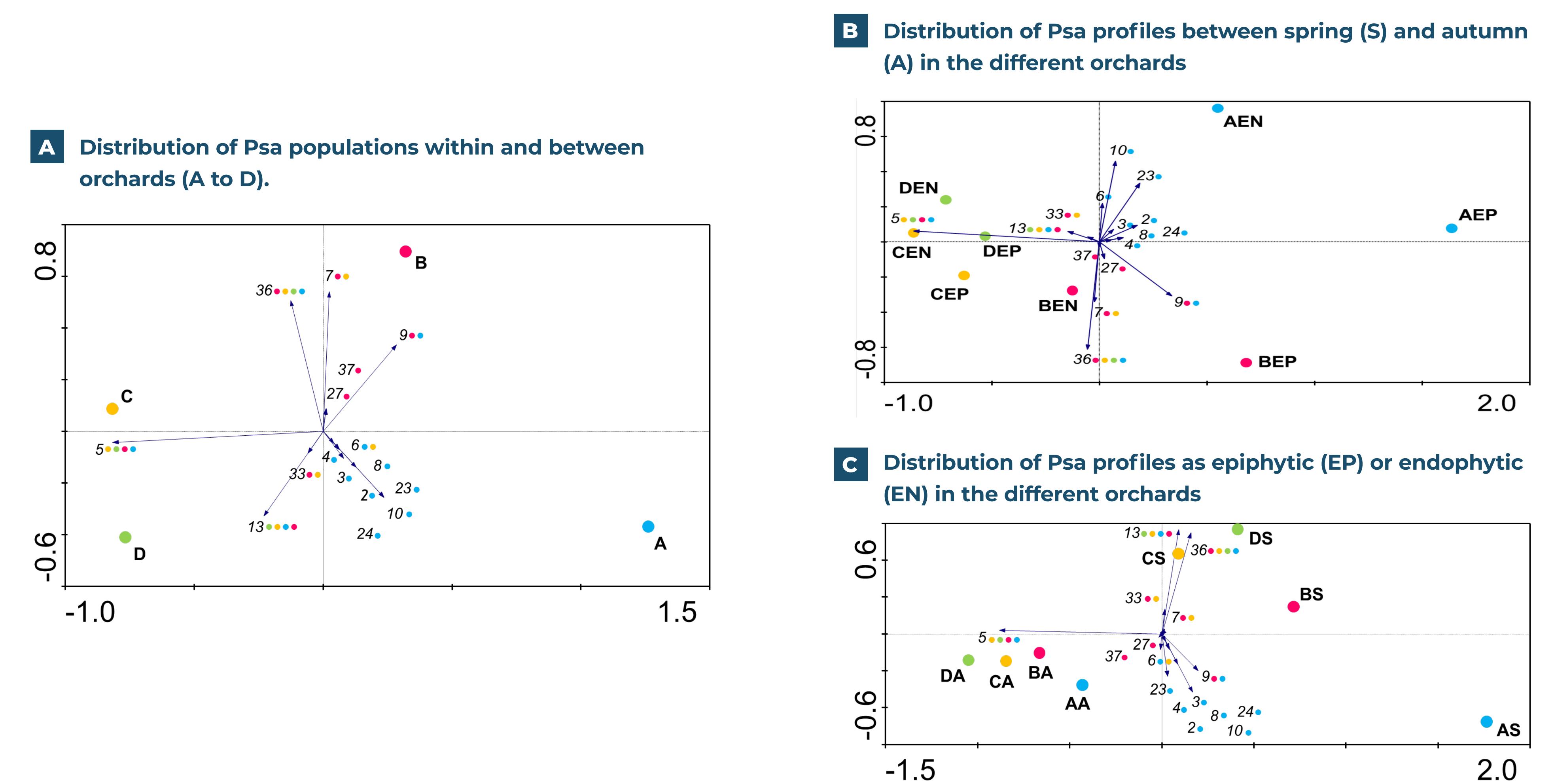
## Results

### Diversity of Psa populations in Portuguese kiwifruit orchards

The Psa population genetic structure was characterized from 600 isolates obtained from several Portuguese orchards with distinct abiotic conditions in consecutive seasons. Based on BOX-PCR fingerprinting analysis we determined that Psa population was highly heterogeneous with several co-existing populations (Fig. 2). These results were confirmed by whole genome analysis (Fig. 4).

Our data supports the existence of mixed Psa populations within each orchard (Fig. 2A, 3A). In addition, the overall Psa diversity was remarkably distinct between kiwifruit orchards, since only three Psa profiles (P) were common to all, namely P5, P13 and P36 (Fig. 2A, 3A).

### The Psa populations varied between seasons and leaf location

Evident changes occurred in the population structure between seasons translated in a notable decrease in Psa diversity in autumn (Fig. 2B, 3B). However, some Psa populations were persistently recovered - namely the dominant ones, such as P5 and P13. This trend was observed for all orchards (Fig. 2B). Moreover, differences between the epiphytic (EP) and endophytic (EN) population were also observed in samples collected simultaneously (Fig. 2C, 3C). This distribution of Psa profiles could be a consequence of changes in abiotic conditions combined with several implemented orchards practices that varied between spring and autumn that could affect directly the Psa population structure, indirectly the community or the physiological status of the plant (6, 13, 14, 15).



**A** Distribution of Psa populations within and between orchards (A to D).

**B** Distribution of Psa profiles between spring (S) and autumn (A) in the different orchards

**C** Distribution of Psa profiles as epiphytic (EP) or endophytic (EN) in the different orchards

**Figure 3** PCoA plots were generated from Psa profiles determined by BOX fingerprinting from the four studied orchards (A, B, C and D) using CANOCO 5 (16). Numbers correspond to Psa profiles and arrows identify the weight that each profile had on the diversity relationship between orchards.
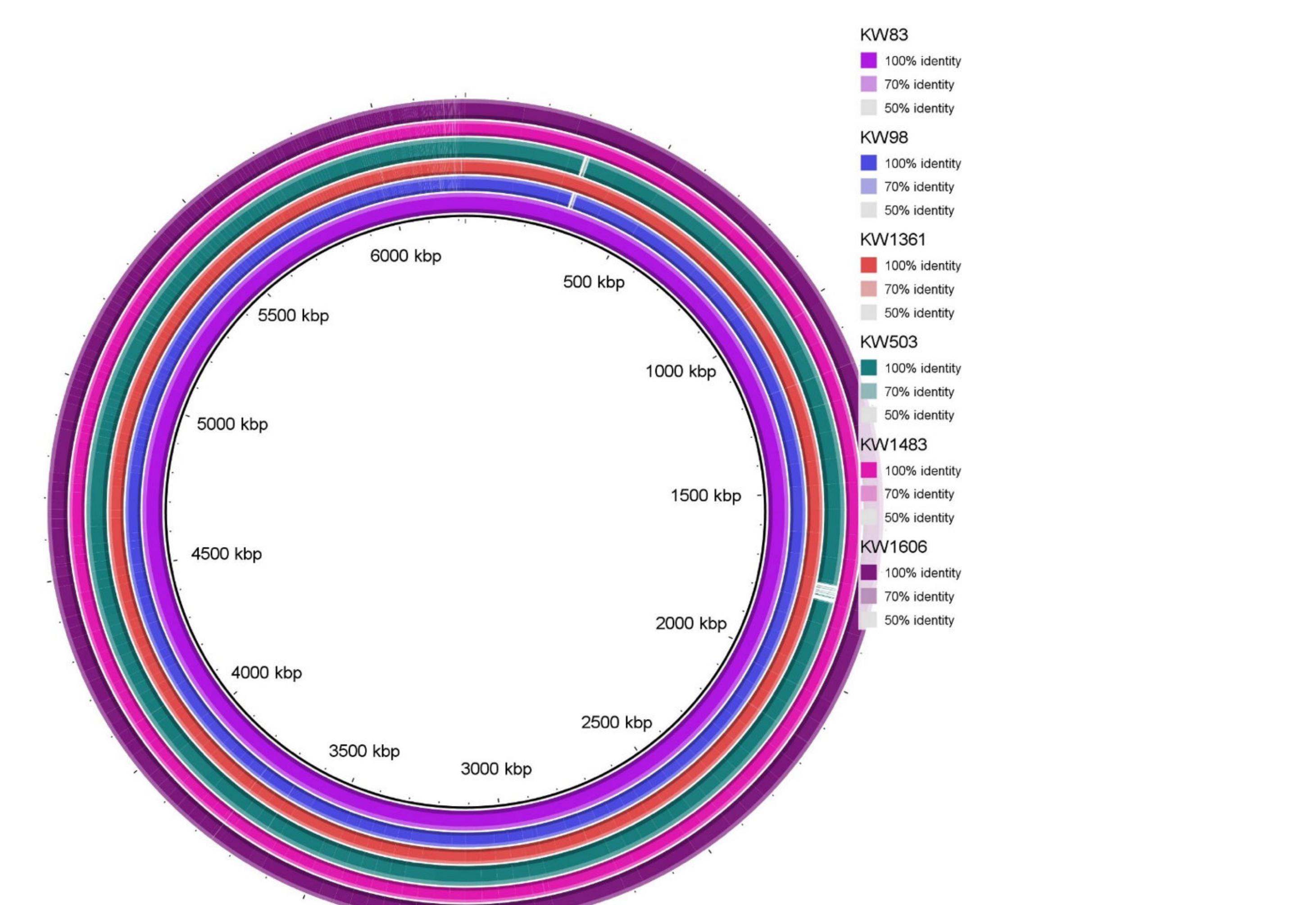
## Conclusions

Obvious changes in population's structure occurred between leaf niches and seasons favoring the dominance of some Psa strains in autumn. In this context Psa populations seem to be selected overtime from a diverse genetic pool according to their fitness. This perspective is important for the understanding of kiwifruit bacterial canker disease occurrence and Psa evolution and it is also relevant when adopting strategies for epidemics management bacterial canker disease occurrence and Psa evolution.

Bibliography:
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**Figure 4** BLAST comparisons between the genome of six Portuguese Psa isolates against reference strain Psa NZ13 (5). The isolates were selected in order to represent different Psa profiles obtained in this study (KW83 - P2; KW1361 - P5; KW1483 - P6; KW503 - P33; KW98 - P36; KW1606 - P37).