Use of plasmid profiles in epidemiological surveillance of *Xylella fastidiosa* outbreak in the Valencian Community, Spain

Lyon, 19 and 20 August 2023

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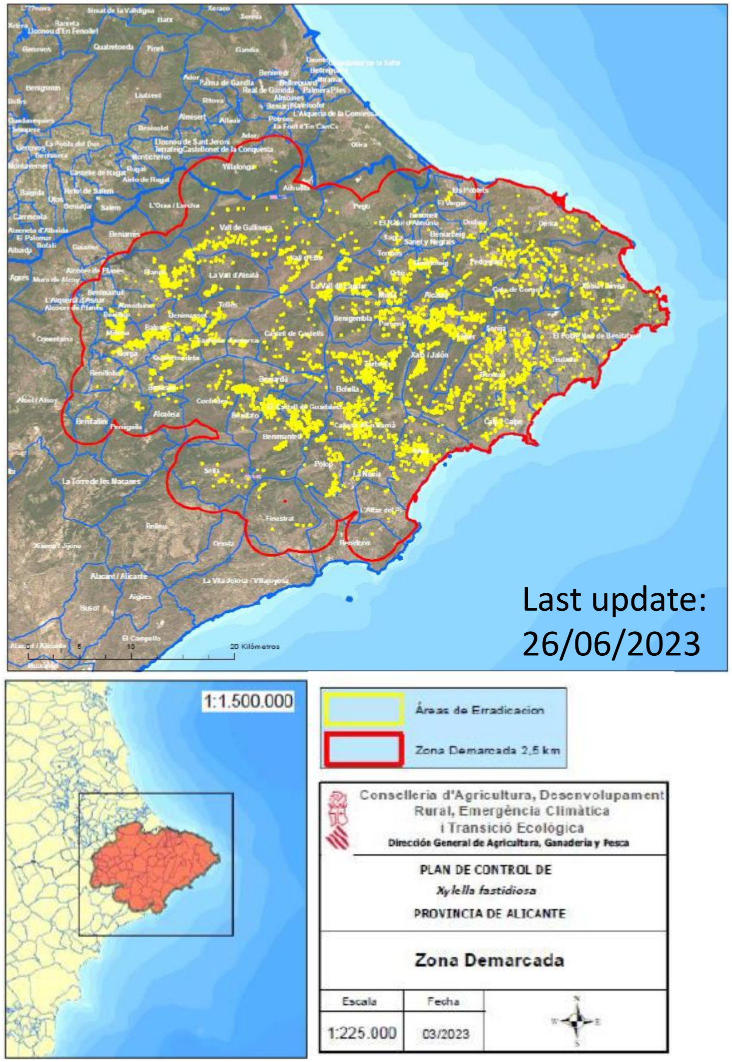
Situation of *Xylella fastidiosa* in the Valencian Community



Last update: 26/06/2023

The current **demarcated area (DA)** covers **145,141 ha,** of which 3,220 ha are infected zone (IZ).

- > 75,700 ha →of shrub pasture, forest and pasture with trees, mainly pine forests.
- > 36,900 ha → cropland: fruit trees, olive crops, citrus and vineyards.

***Xylella fastidiosa* subsp. *multiplex* ST 6**

65	Alicante
5	Valencia

Number of municipalities in the **infected zone** (IZ)

Host plants of *Xylella fastidiosa* in Valencian Community

Plant species	Number of positives
Prunus dulcis	4,953
Calicotome spinosa	113
Helichrysum italicum	96
Polygala myrtifolia	70
Helichrysum stoechas	62
Salvia rosmarinus	50
Phagnalon saxatile	37
Cistus albidus	31
Rhamnus alaternus	31
Prunus armeniaca	27
Prunus domestica	12
Laurus nobilis	10
Lavandula dentata	8
Acacia saligna	7
Lavandula angustifolia	6
Ulex parviflorus	5
Cistus salvifolius	4

Plant species	Number of positives
Genista scorpius	3
Cistus monspeliensis	3
Lavandula latifolia	3
Santolina chamaecyparissus	3
Acer granatense	1
Lavandula intermedia	1
Spartium junceum	1
Viburnum tinus	1
Asparagus acutifolius	1
Total positives	**5,540**

NEW!

26 *Xf*- host plant species

Host plants of *Xylella fastidiosa* in Valencian Community

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Prunus dulcis ←	4,953
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Cistus albidus	31
Rhamnus alaternus	31
Prunus armeniaca	27
Prunus domestica	12
Laurus nobilis	10
Lavandula dentata	8
Acacia saligna	7
Lavandula angustifolia	6
Ulex parviflorus	5
Cistus salvifolius	4
Genista scorpius	3
Cistus monspeliensis	3
Lavandula latifolia	3
Santolina chamaecyparissus	3
Acer granatense	1
Lavandula intermedia	1
Spartium junceum	1
Viburnum tinus	1
Asparagus acutifolius	1
Total positives	**5,540**

NEW!

26 *Xf*- host plant species

~ **90%** samples → almond trees

Xylella fastidiosa subsp. *multiplex* ST 6 from the IZ in Alicante

	ESVL	IVIA5901
Total growth	**+**	**+++**
Biofilm formation	**+**	**+**
Planktonic growth	**++**	**+++**
Setting rate	**+**	**+**
Adhesion force	**+++**	**+**
Twitching speed	**++**	**-**
Twitching 2 dpi	**++**	**-**
Twitching 5 dpi	**-**	**-**
Type I pili	**Present**	**Present**
Type IV pili	**Present**	**Present**
Cell length	**++**	**+**
Virulence in Tobacco	**++**	**+**

Chromosome

Strains isolated from the same almond tree → **>99%** of similarity in their chromosomal sequences (**ANI**)

Plasmid pUCLA-ESVL
(1,289 bp)

Plasmid pXF64-Hb_ESVL
(59,678 bp)

Source: Román-Écija et al (2023)

Objectives

The role of plasmids in the *Xylella fastidiosa* life cycle is still poorly explored or understood, even though plasmids may contribute to its virulence, and adaptation to new environments and host plants

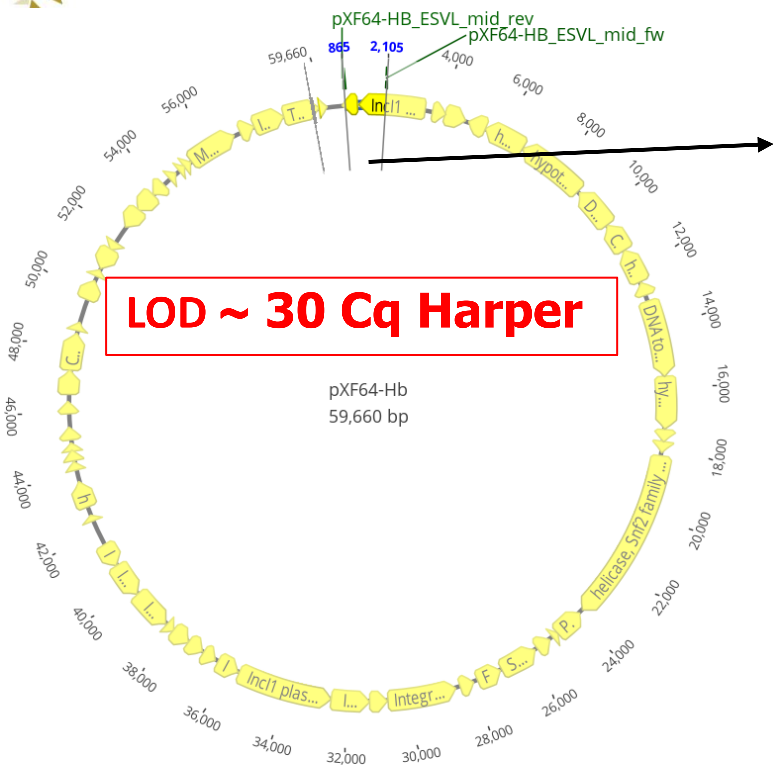
Develop a PCR-based plasmid-typing approach as a tool in the epidemiological surveillance of this *Xylella fastidiosa* outbreak.

Study the geospatial associations of the different plasmid profiles and the implications in the surveillance of the *Xylella fastidiosa* epidemic in the demarcated area of the Valencian Community.

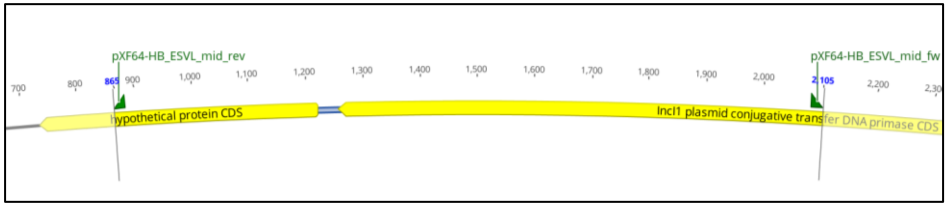
To determine if there is specificity or association of *Xylella fastidiosa* genotypes (defined by its plasmid profile) in the DA of the Valencian Community according to:

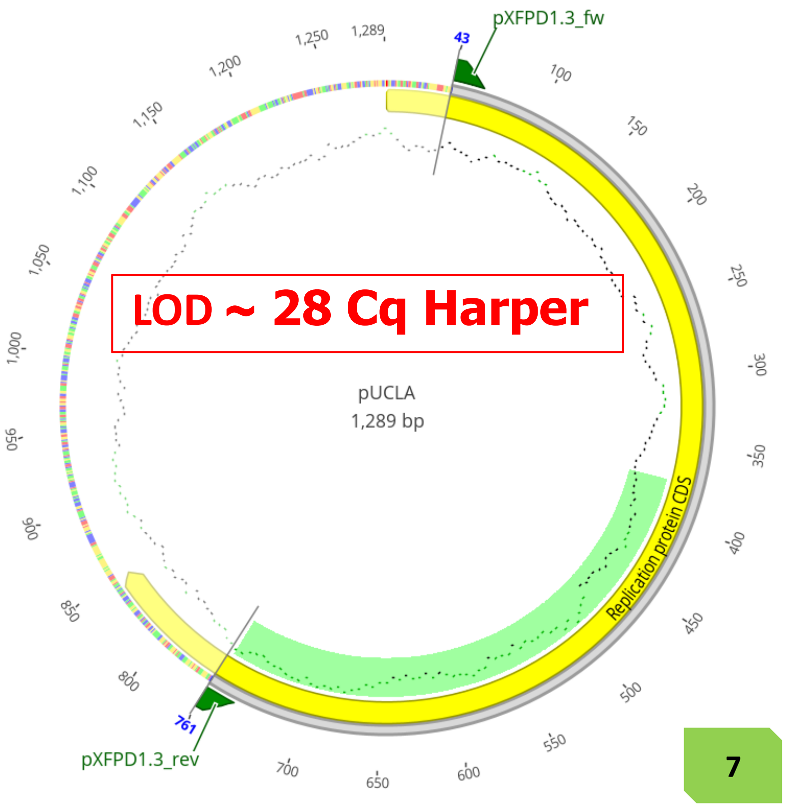
- Location of sampling (geographical association)
- The host plants
- Environmental variables

Materials & Methods: PCR-based plasmid-typing approach



LOD ~ 30 Cq Harper





LOD ~ 28 Cq Harper

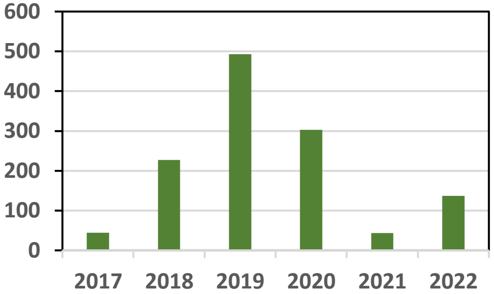
Using Primer BLAST

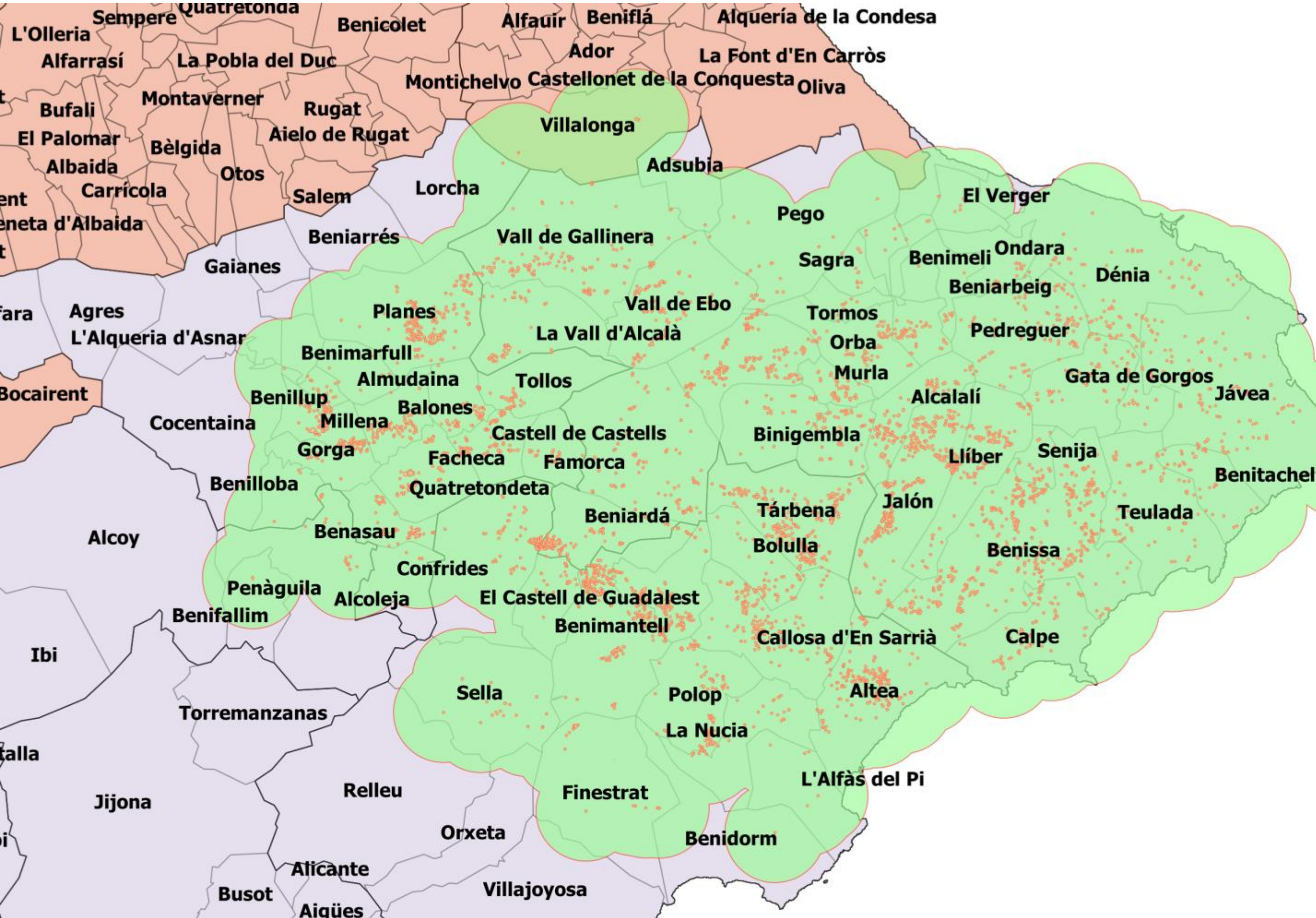
How to use Primer BLAST in Sequence Viewer

pXFPD1.3_fw	GGCAAGTTCATGGGAGCGAC
pXFPD1.3_rv	ATACGTCGCGTTCTCGTGT
pXF64-HB_ESVL_mid_fw	GAATGCGCGATGCAATGAGT
pXF64-HB_ESVL_mid_rv	ATCTGTCCGTTGATGCCGTT

Materials & Methods: Samples analyzed

- **1,249 samples** were selected for the study from a total of >5,500 infected samples
- From **68/70 municipalities** of the **IZ** of the Valencian Community during 6 years
- From **20 different hosts and insect vectors** (**1187** samples from **almond; 95%**)
- With a **Cq of qPCR harper < 27** (When possible)





Almond samples Selected/municipality from the 5000 (+)

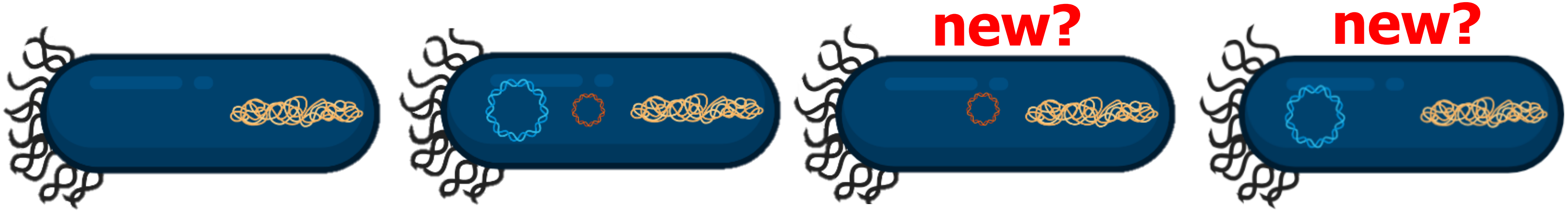
- ≤10 : all
- 10-20: 10
- 20-30: 15
- 30-50: 20
- 60-80: 25
- 80-100: 30
- >100: 40

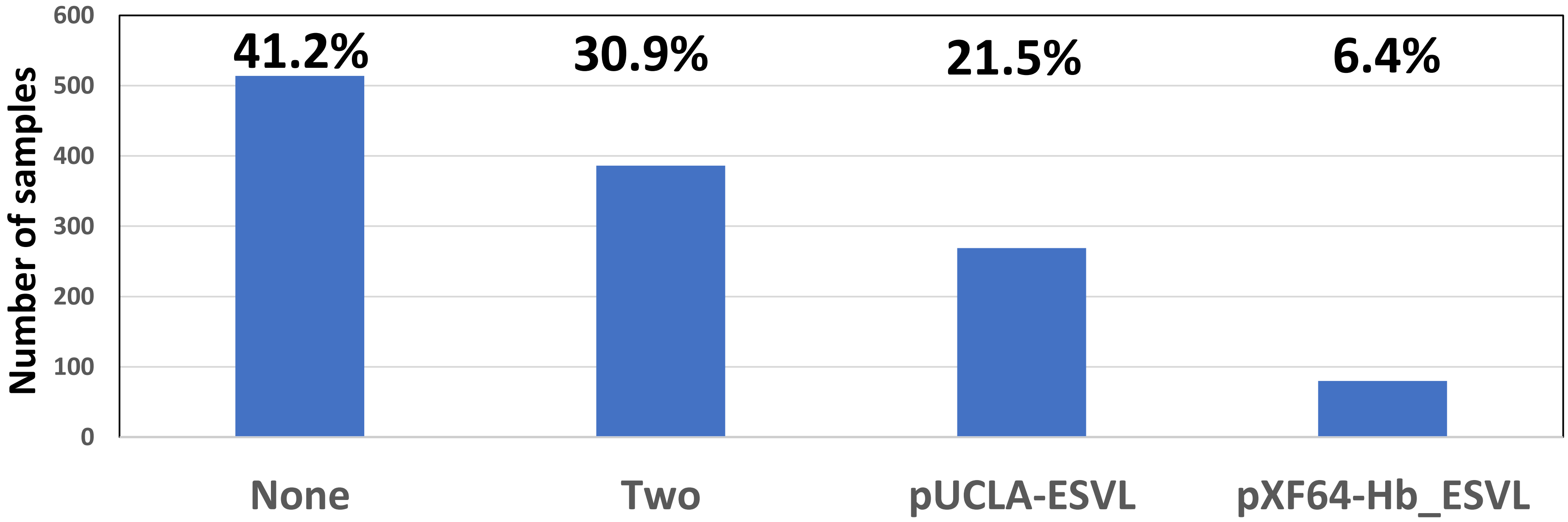
Results: *Xf* plasmid profiles in the Valencian Community

Host Plants	None	pXF64-HB_ESVL	pXFPD1.3	Two Plasmids	Total
Acacia saligna		1			1
Asparagus acutifolius	1				1
Calicotome spinosa	6	1	2	2	11
Cistus albidus	2		1	1	4
Cistus monspeliensis	1				1
Cistus salvifolius	1				1
Genista scorpius	1			1	2
Helichrysum italicum	2		2	1	5
Helichrysum stoechas	1		1		2
Laurus nobilis	2				2
Lavandula dentata	1				1
Lavandula latifolia	1				1
Phagnalon saxatile	1				1
Polygala myrtifolia	1			8	9
Prunus armeniaca		1			1
Prunus domestica	2				2
Salvia rosmarinus			1		1
Santolina chamaecyparissus	2			1	3
Ulex parviflorus	2			1	3
Other hosts	**27**	**3**	**7**	**15**	**52**
Prunus dulcis	**481**	**75**	**262**	**369**	**1187**
Insect vectors	**3**	**2**	**0**	**1**	**6**

Results: *Xf* plasmid profiles in the Valencian Community

- From the total of **1,239 plant samples** analyzed the Xf **genotype with no plasmids** was the most abundant in the **20 different hosts** analyzed,
- The genotype with two plasmids was the most disperse since it was found in **64/70 municipalities** sampled in the **IZ** of the Valencian Community
- Two potential **new plasmid profiles** were detected





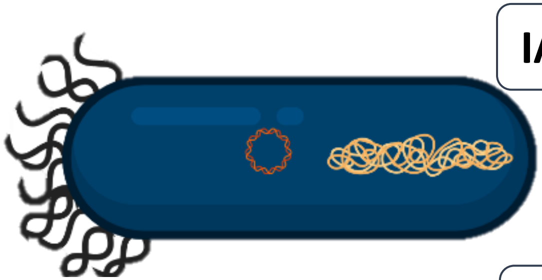
Do those *Xf* plasmid profiles real exist in the IZ of Alicante?

ESVL

N= 12 → 33%

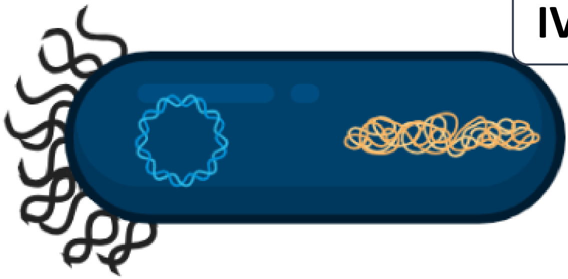
IVIA5901

N=20 → 56%



IAS-AXF235T1

N=3 → 8%



IVIA574/22

N= 1 → 3%

HYBRID ASSEMBLY

Oxford NANOPORE Technologies

illumina

Strain collection

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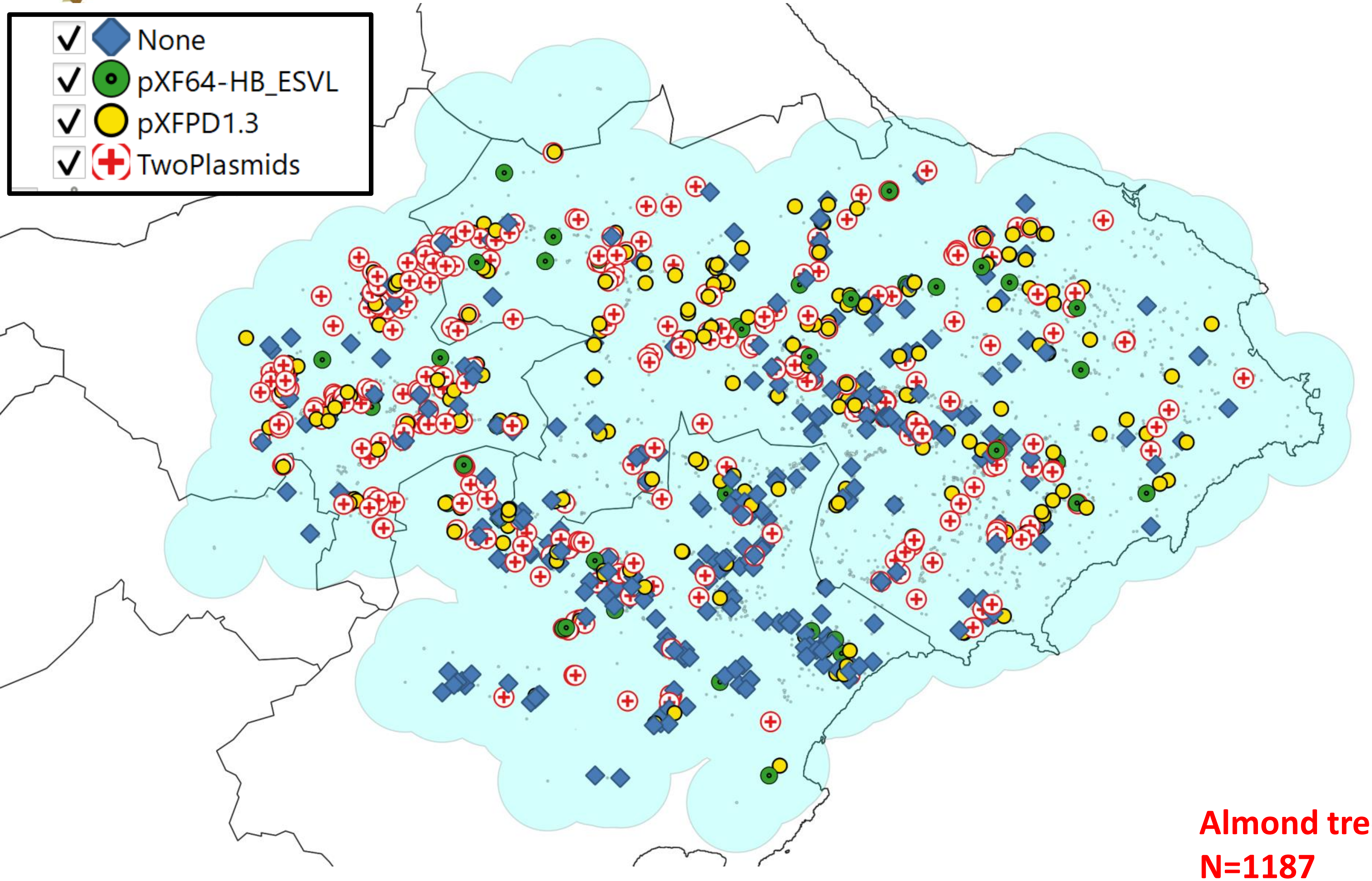
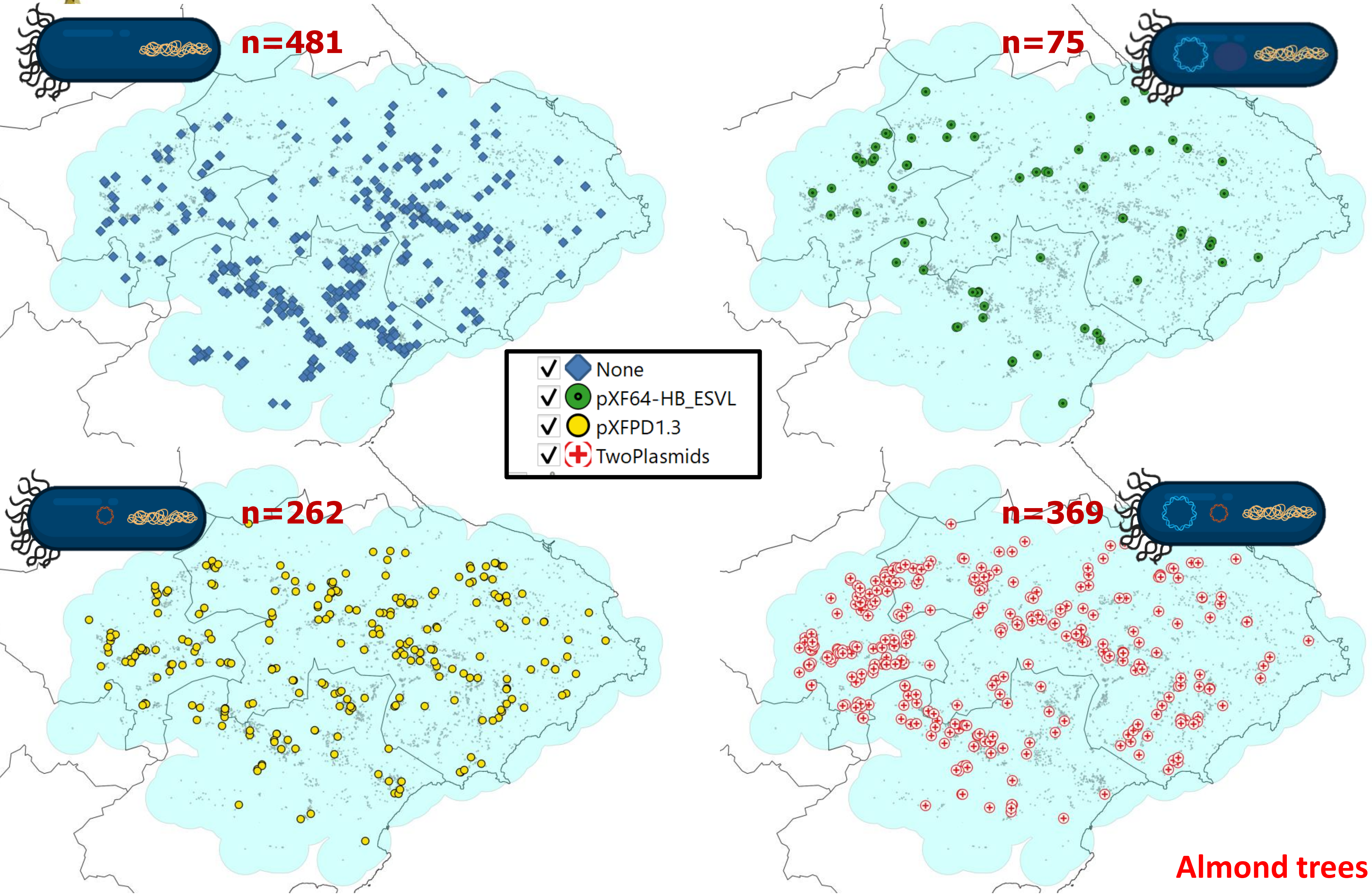
de investigaciones agrarias

Results: Geographycal distribution of *Xf* plasmid profiles



Almond trees
N=1187

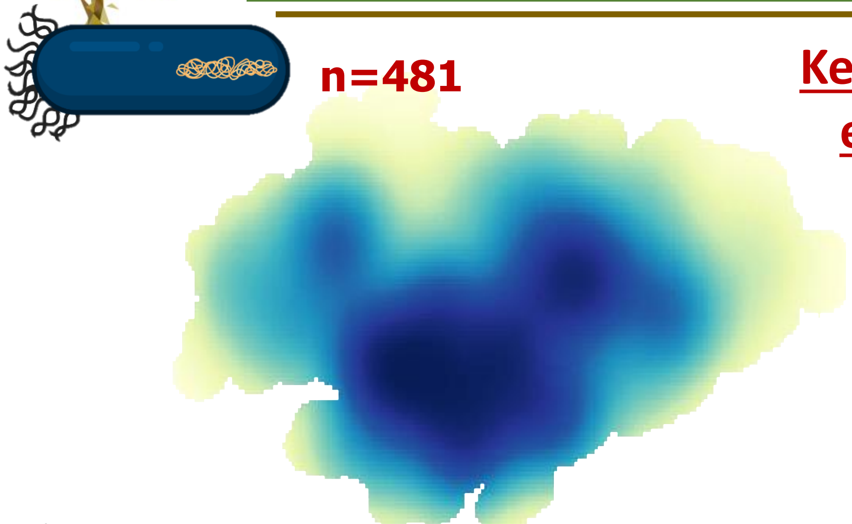
Results: Geographycal distribution of *Xf* plasmid profiles

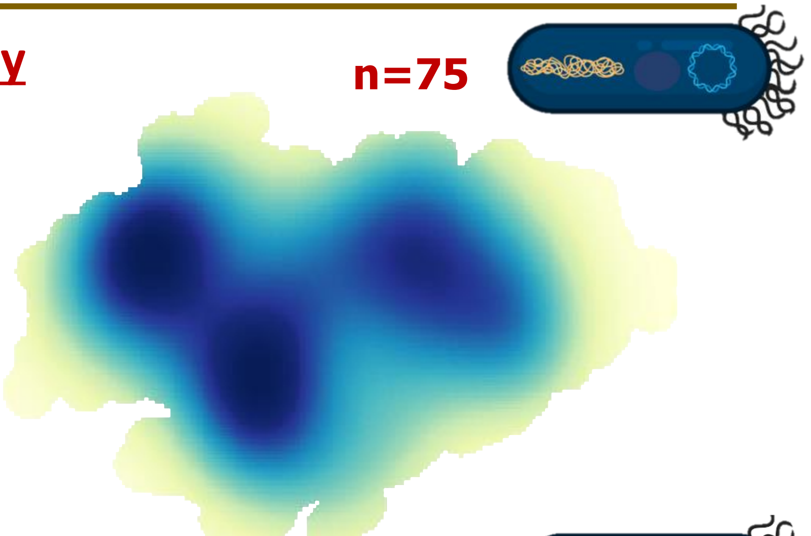


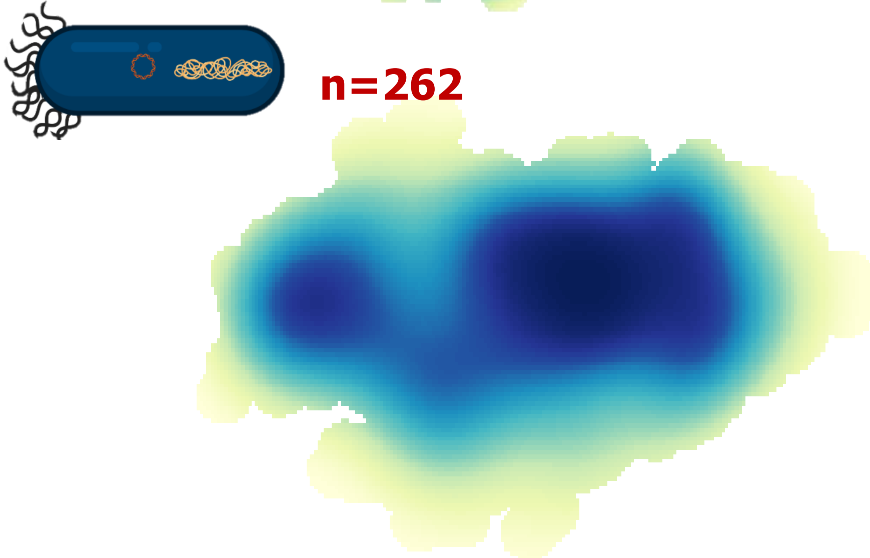
Almond trees

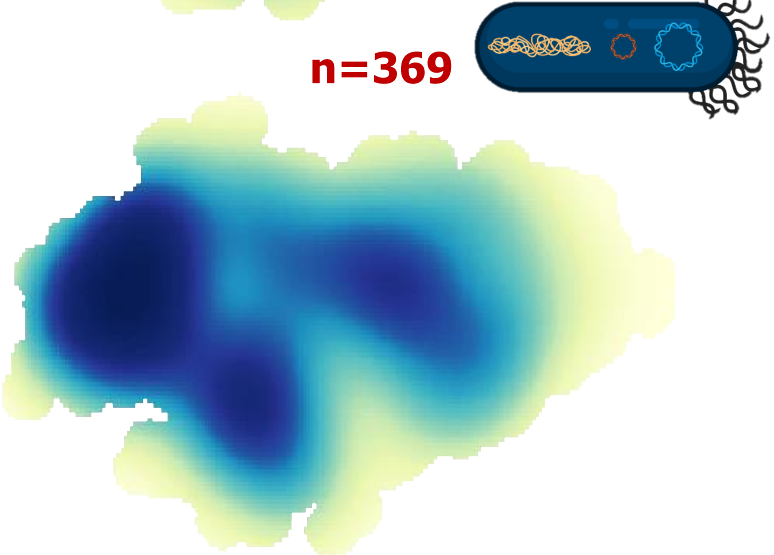
Results: Spatial point pattern of *Xf* plasmid profiles

Kernel density estimation







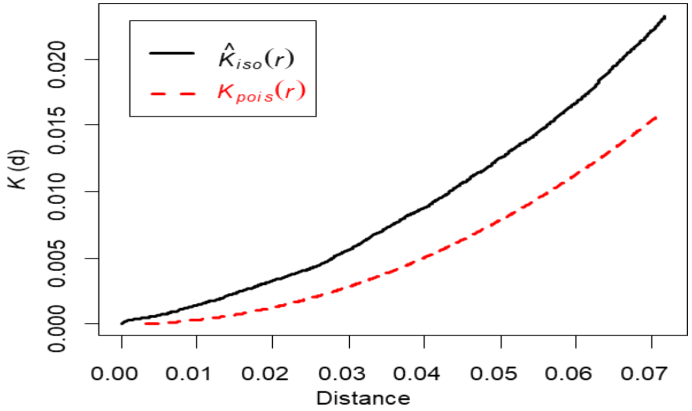


- The Kernel density plots shows that there are some areas with a higher density (clustering) for each plasmid profiles, with some overlapping geographical areas among them

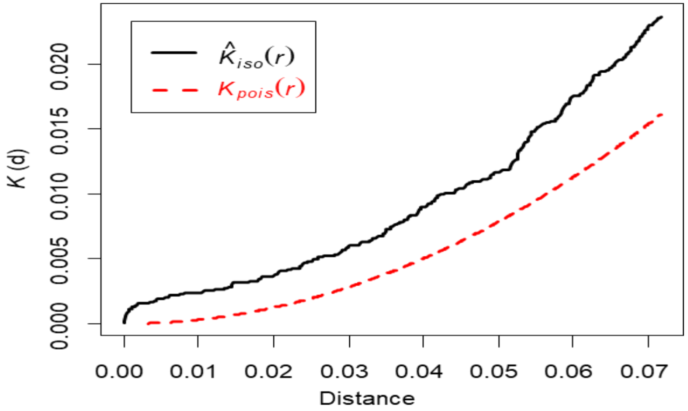
Results: Spatial point pattern analysis of *Xf* plasmid profiles

Ripley's *K* function

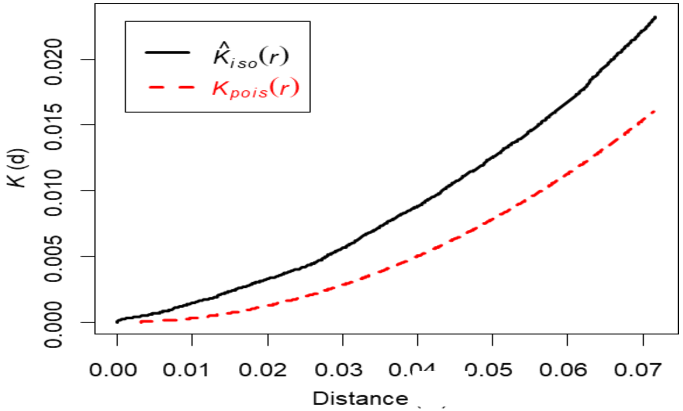
n=481



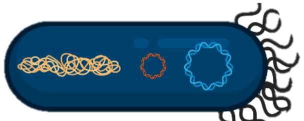
n=75

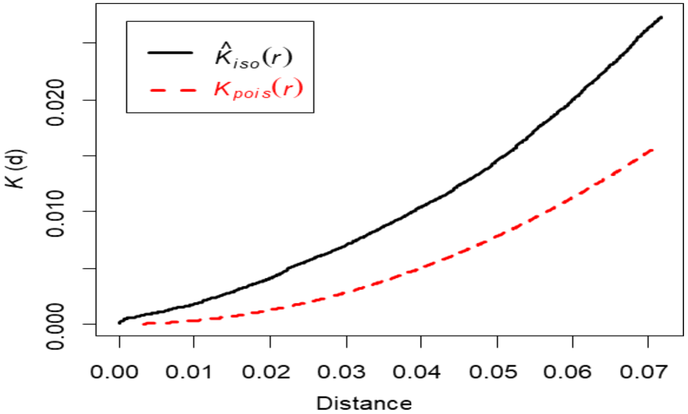


n=262



n=369

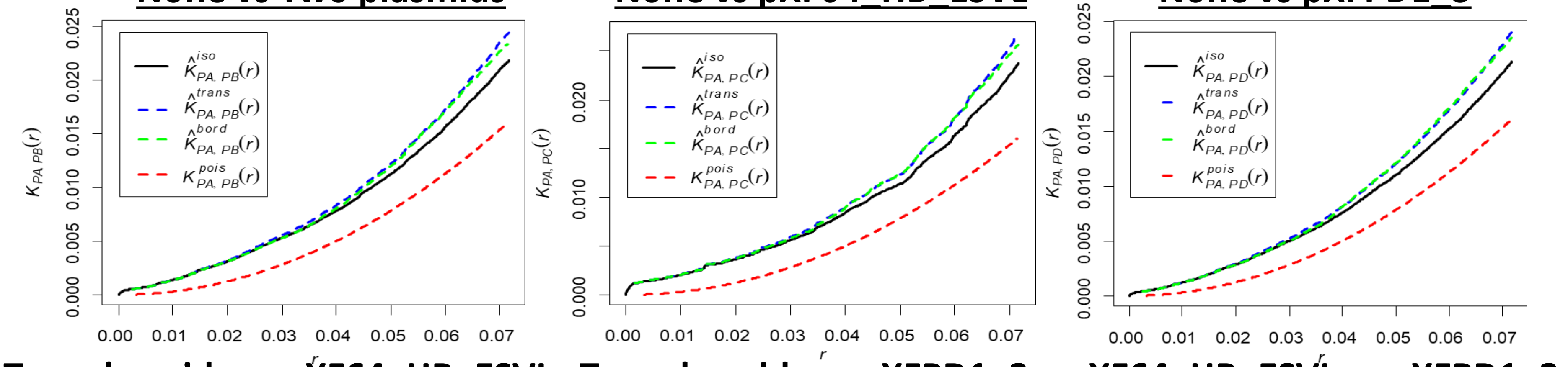




- The Ripley's *K* function plots show that at all tested distances the actual observed value of *K*-hat (**black line**) is greater than the theoretically derived expected value for a Completely Spatial Randomness (red line) -> clustering of infected samples for all plasmid profiles

Results: Spatial point pattern of *Xf* plasmid profiles

Bivariate Ripley's *K* function



None vs Two plasmids

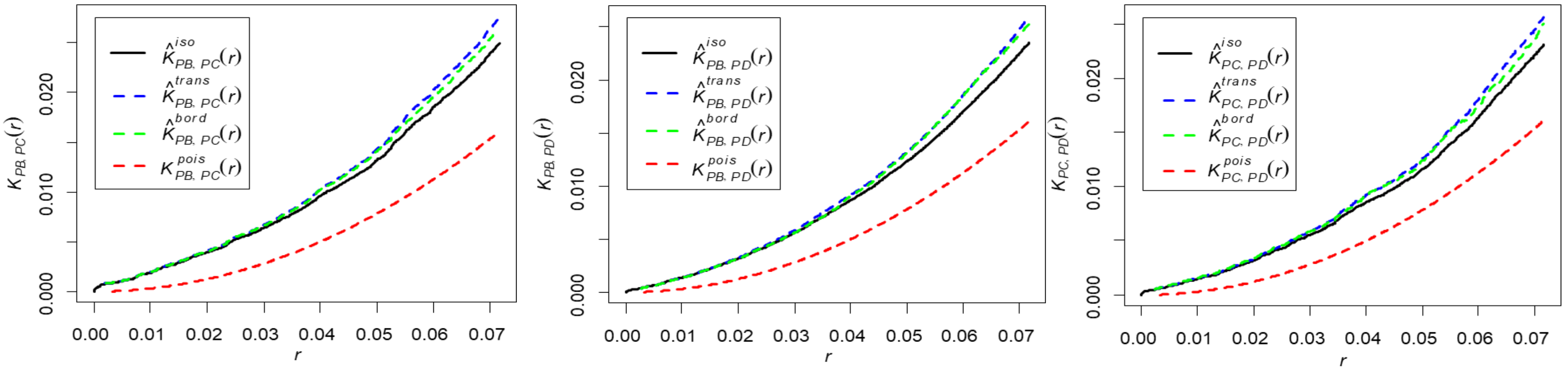
None vs pXF64_HB_ESVL

None vs pXFPD1_3

Two plasmids vs pXF64_HB_ESVL

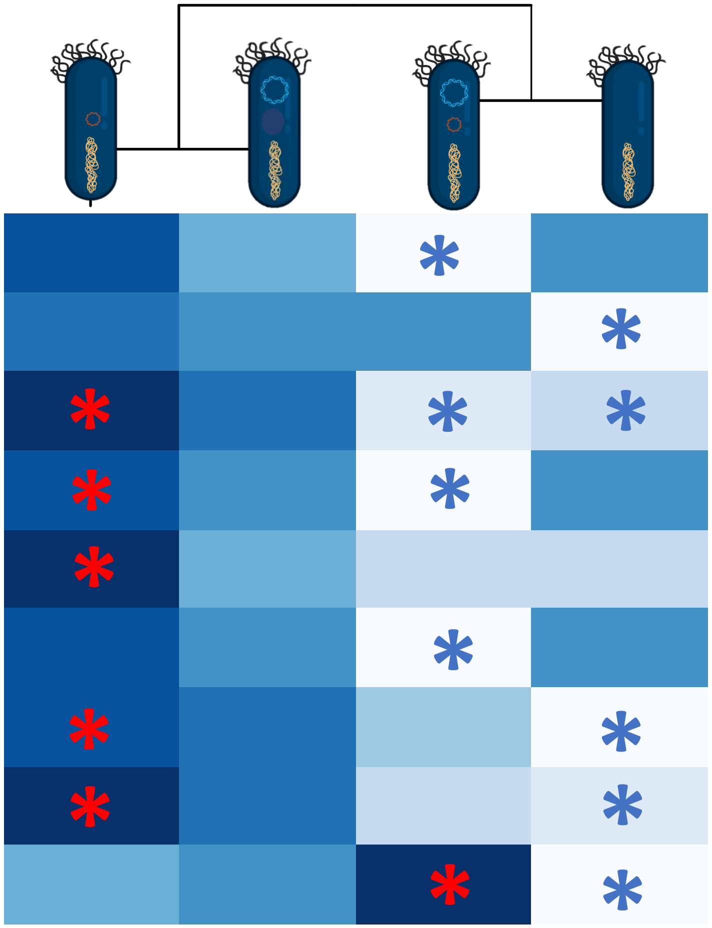
Two plasmids vs pXFPD1_3

pXF64_HB_ESVL vs pXFPD1_3



- At all tested distances the actual observed value of *K*-hat (**black line**) is above the reference line (**red line**), suggesting that the two types of points are more frequently found closer to each other than would be expected under a completely random distribution **-> clustering**

Results: Association of *Xf* plasmid profiles with environment



		pXFPD1_3	pXF64_HB_ESVL	Two Plasmids	None
Geographic location	longitude			*	
	latitude				*
	Xf suitability index	*		*	*
Temperature related bioclimatic variables	Average annual T	*		*	
	Maximum T warmest month	*			
	Minimum T coldest month			*	
Precipitation related bioclimatic variables	Annual Precipitation	*			*
	Precp. wettest month	*			*
	Precp. of driest month			*	*

General Conclusions

- The PCR-based plasmid-typing approach revealed the presence of four different plasmid profiles in the *X. fastidiosa* populations present in the DZ of Alicante, two of them being new profiles not described up to date.

- Spatial point pattern analysis suggest a significant clustering of the distribution of each plasmid profile, with some differences in their geographical distribution but some overlapping regions (No exclusion).

- Some of the plasmid profiles seem to be more adapted to specific areas and/or climatic conditions within the DA of Alicante.

- Further analyses are needed to determine whether this geographical distribution observed at present correspond to different introductions events for each plasmid profile and a subsequent dispersion of them, or the introduction of a single genotype with subsequent loss of those plasmids.

Acknowledgements

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