

➤ Multi-scale spatial genetic structure
of the vector-borne pathogen
'*Candidatus Phytoplasma prunorum*'
in orchards and in wild habitats

V. Marie-Jeanne, F. Bonnot, G. Thébaud,
J. Peccoud, G. Labonne, N. Sauvion

➤ Scales of pathogen dispersal across compartments

Why does it matter?

- Types of compartments

- wild hosts
- domestic hosts
- vectors

➡ How does the epidemiological system works?

➡ What control measures?

- Scales of dispersal

- neighboring hosts
- neighboring host groups
- within a region
- across regions

➡ What is the appropriate control scale?

➡ What should be controlled?

➤ Scales of pathogen dispersal across compartments

How to study these processes?

- Molecular epidemiology

- genetic diversity
- spatial structure
- informative patterns
- genericity

➡ From patterns to processes

- Specifying null hypotheses

- genetics is independent of the region
- genetics is independent of the compartment
- genetic distance is independent of spatial distance

➤ European stone fruit yellows (ESFY) pathosystem

- Pathogen: '*Candidatus* Phytoplasma prunorum' (intracellular bacteria)
- Infected plants: cultivated (apricot, plum) & wild (blackthorn) *Prunus*
- Symptoms: physiological disorder, yellowing, leaf curling, death



➤ European stone fruit yellows (ESFY) pathosystem

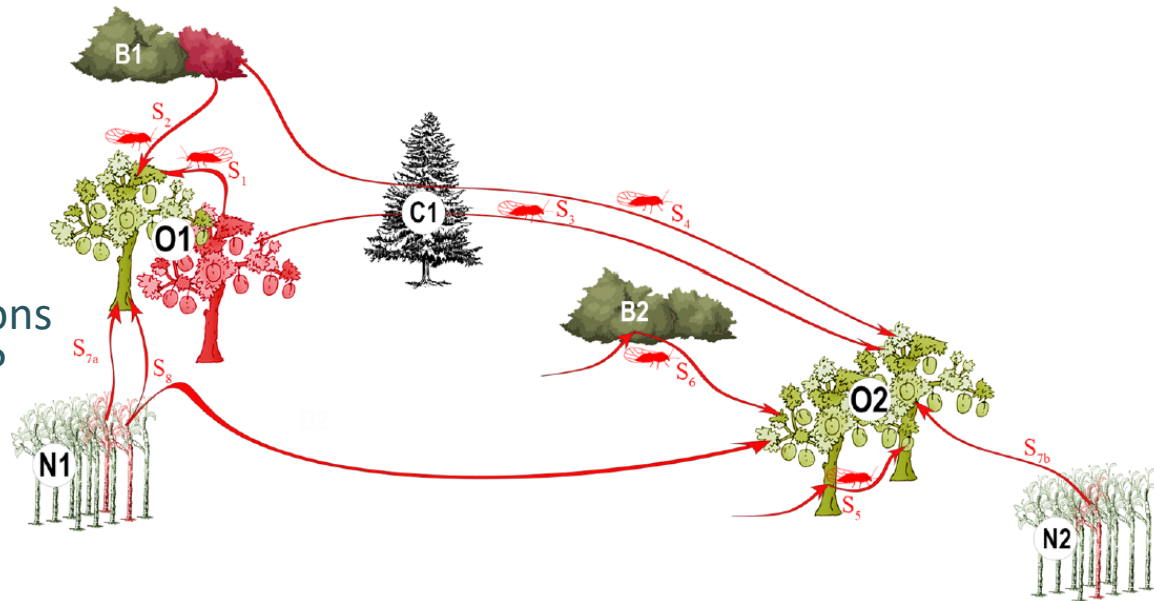
- **Pathogen:** '*Candidatus* Phytoplasma prunorum' (intracellular bacteria)
- **Infected plants:** cultivated (apricot, plum) & wild (blackthorn) *Prunus*
- **Symptoms:** physiological disorder, yellowing, leaf curling, death
- **Transmission:** infected planting material & vector-borne
- **Vector:** *Cacopsylla pruni*, a psyllid of *Prunus* (blackthorn, plum)



➤ European stone fruit yellows (ESFY) pathosystem

- **Pathogen:** '*Candidatus* Phytoplasma prunorum' (intracellular bacteria)
- **Infected plants:** cultivated (apricot, plum) & wild (blackthorn) *Prunus*
- **Symptoms:** physiological disorder, yellowing, leaf curling, death
- **Transmission:** infected planting material & vector-borne
- **Vector:** *Cacopsylla pruni*, a psyllid of *Prunus* (blackthorn, plum)

- ➡ Are there local acquisition-transmission cycles?
- ➡ What is the spatial scale of transmissions between years?
- ➡ Are there successive transmissions within or across compartments?
- ➡ Do nurseries contribute to the regional or national spread?



➤ Methods

- Sampling

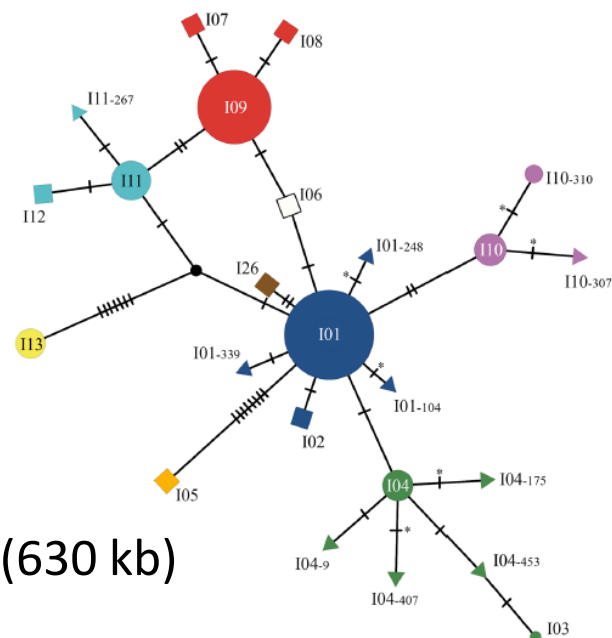
- 3 regions
- 3 compartments

- Genetics

- 'Ca. P. Prunorum' : clonal, small genome (630 kb)
- sequences from the ~500 kb *imp* locus
- frequency of 6 major haplotypes

- Statistics

- correspondence analysis of haplotypes and compartment × region
 - multinomial logistic regression ➡ $P(\text{compartment} \times \text{region}) = 6 \times 10^{-7}$
 - Fisher's exact tests on multinomial counts
 - spatial analysis (independence tests)
- } Within-region

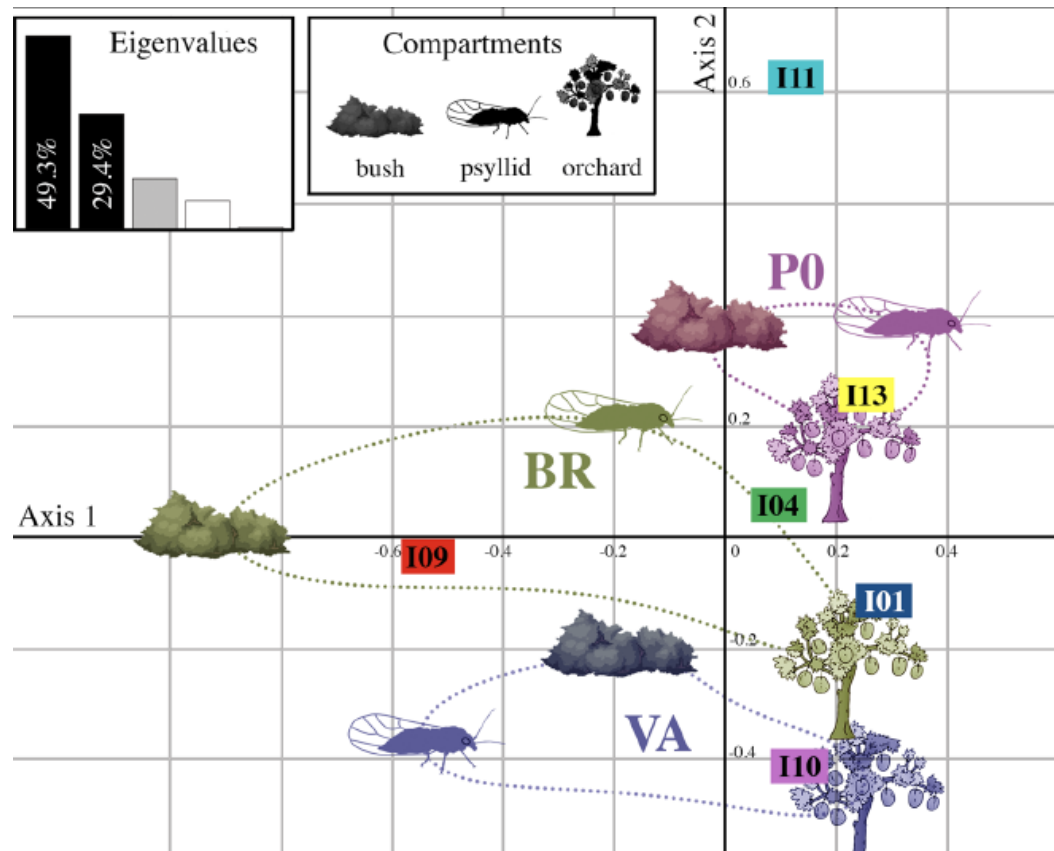


Haplotype	PO			BR			VA			Σ
	Bush	Psyllid	Orchard	Bush	Psyllid	Orchard	Bush	Psyllid	Orchard	
I01	46	28	128	8	5	82	61	6	74	438
I04	5	4	17	6	1	13	2	1	5	54
I09	37	8	55	71	7	34	48	12	25	297
I10	6	3	8	5	0	15	1	3	15	56
I11	25	12	32	6	1	3	3	0	0	82
I13	4	3	19	3	4	14	0	0	1	48
Σ	123	58	259	99	18	161	115	22	120	975

➤ Results (correspondence analysis)

Proximity of haplotype frequencies across regions and compartments

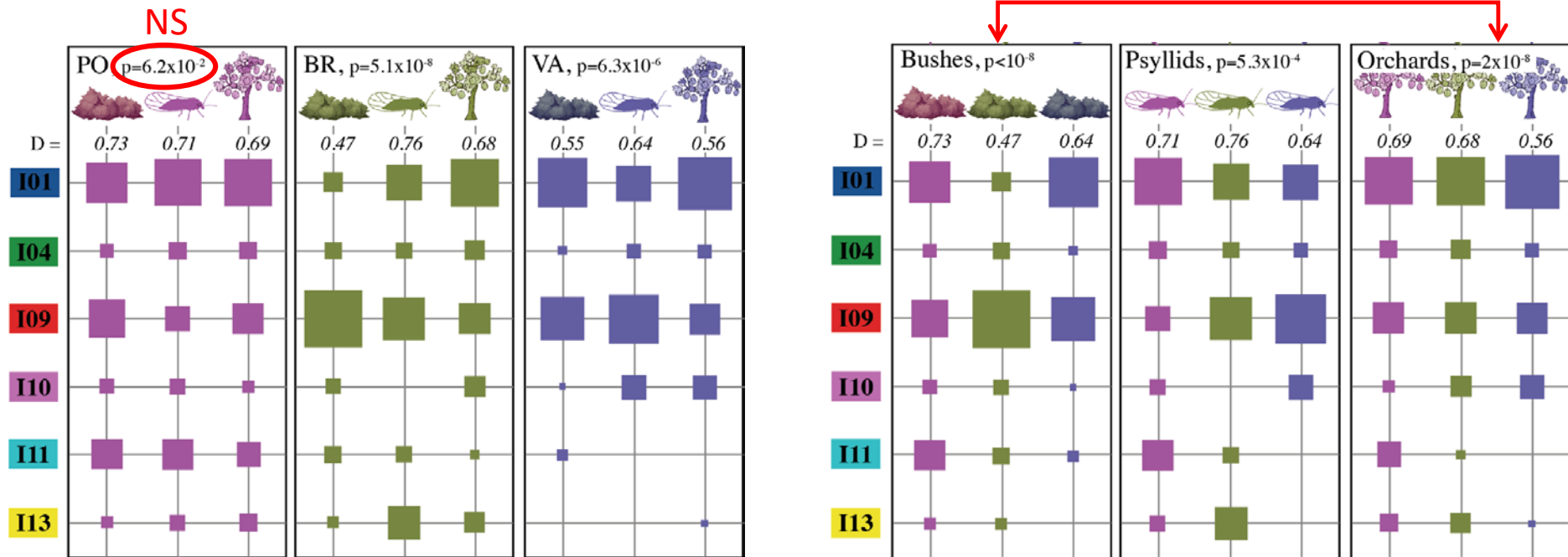
- **Regions:** Genetic homogeneity in Pyrénées-Orientales (and Valence)
- **Compartments:** Haplotype frequencies are more similar in cultivated trees than in the wild



➤ Results (Fisher's exact tests)

Proximity of haplotype frequencies across regions and compartments

- **Regions:** Haplotype frequencies non significantly different only in PO
- **Compartments:** Haplotype frequencies differ more significantly in bushes than in orchards



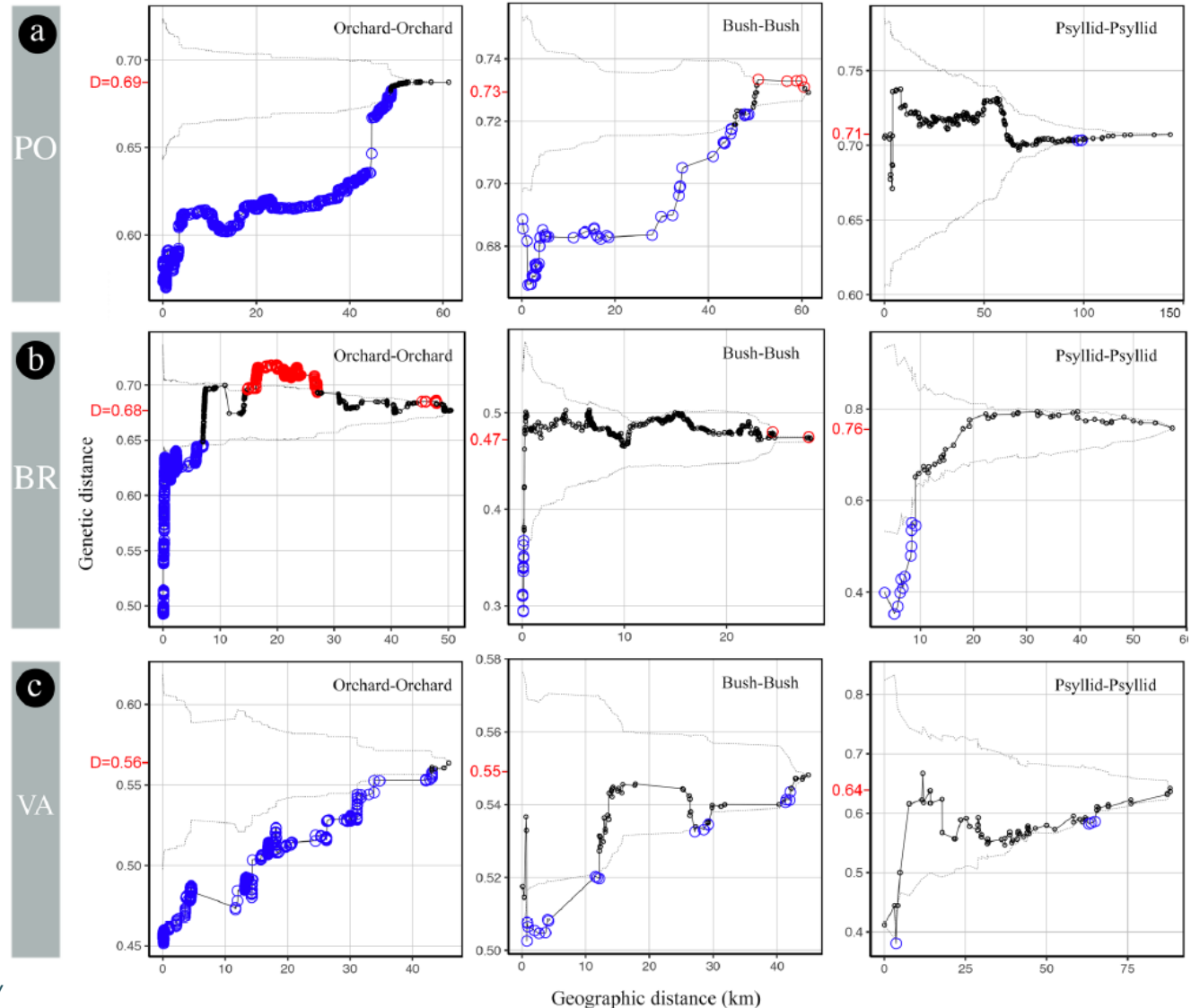
➤ Spatial analysis within compartment (method)

Join counts within an increasing radius

- Each region is treated separately
- H_0 hypothesis: Haplotypes occur at random among sampled points
- Test statistic: For each radius d , proportion of pairs of points with different haplotypes
- Test procedure: Random labelling
 $N=10,000$ permutations of haplotypes among sampled points
- P-value: For each distance class, rank of observed test statistic among the N computed statistics
- Confidence envelope: between $N\alpha/2$ and $N(1 - \alpha/2)$

➤ Spatial analysis within compartment (results)

Join counts within an increasing radius



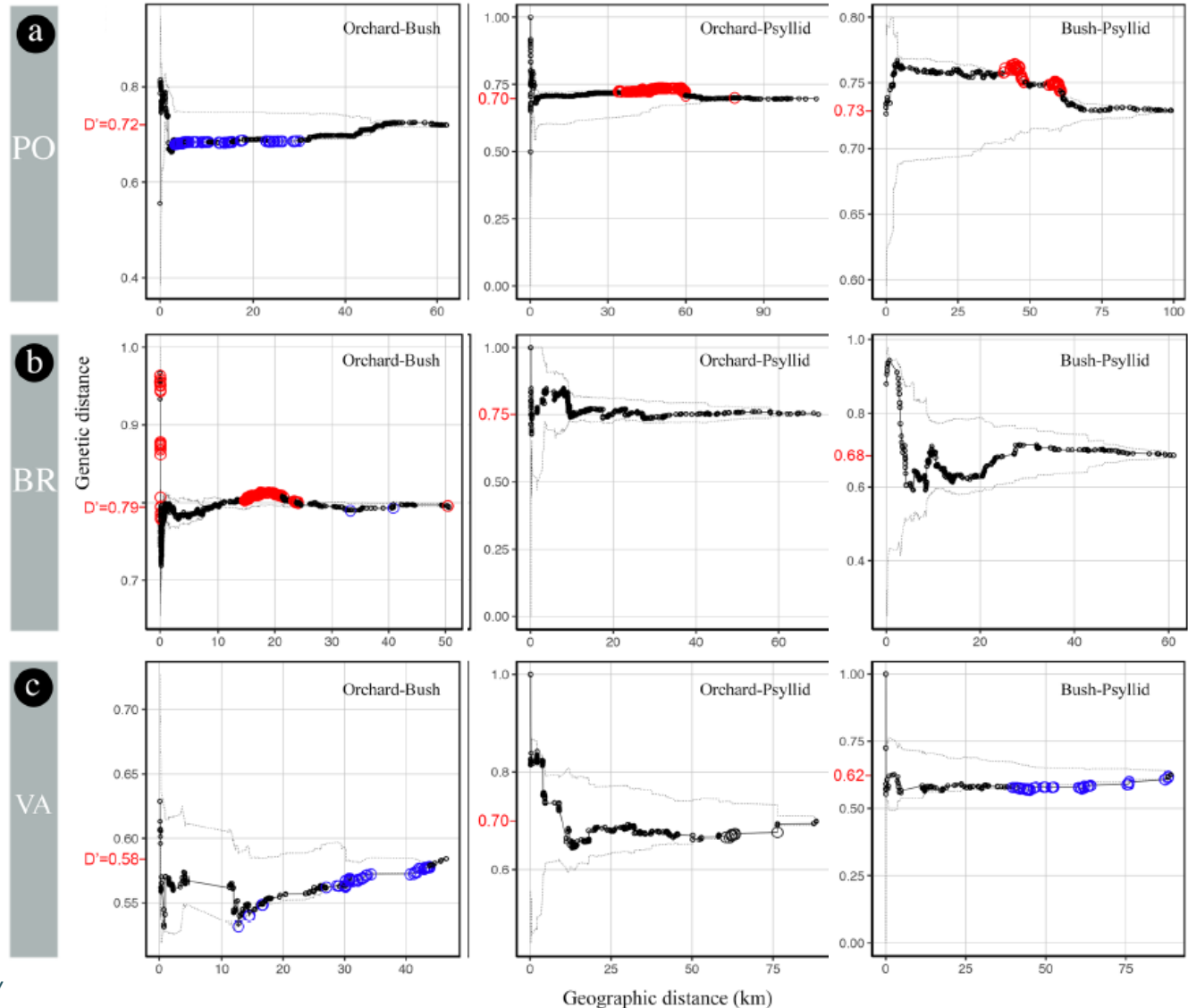
➤ Spatial analysis between compartments (method)

Join counts within an increasing radius

- Each region is treated separately
- **H₀ hypothesis:** Haplotypes of the lesser structured compartment are spatially independent of haplotypes of the other compartment
- **Test statistic:** For each radius d , proportion of pairs of points belonging to different compartment with different haplotypes
- **Test procedure:** Random labels in lesser structured compartment $N=10,000$ permutations of haplotypes among sampled points
- **P-value:** For each distance class, rank of observed test statistic among the N computed statistics
- **Confidence envelope:** between $N\alpha/2$ and $N(1 - \alpha/2)$

➤ Spatial analysis between compartments (results)

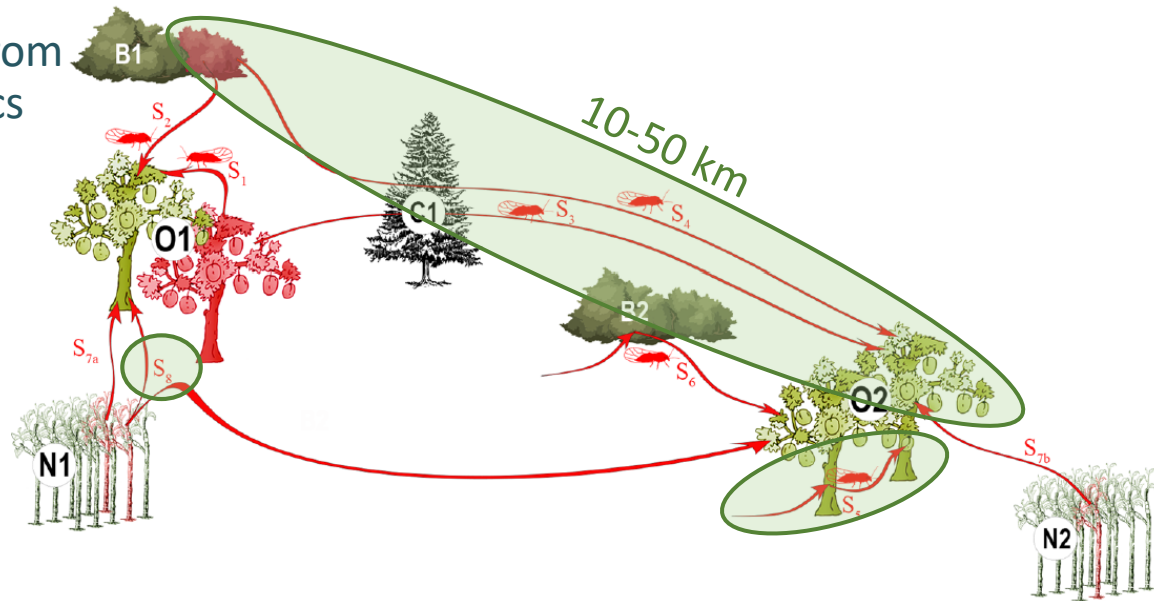
Join counts within an increasing radius



➤ Take-home messages

- Statistical tests of spatial independence
 - no need to define population units
 - considers all scales within the same analysis
 - flexible framework: enables different hypotheses and test statistics
 - easy to implement
- Conclusions on ESFY epidemiology:

- ➡ Transmissions between years (from bushes) drive the ESFY epidemics
- ➡ Epidemiological unit: 10-50 km
- ➡ Successive transmissions occur within compartments
- ➡ Nurseries contribute to the national spread



➤ Thanks for your attention

Further information in:

**SCIENTIFIC
REPORTS**

natureresearch

SCIENTIFIC REPORTS | (2020) 10:5002 | <https://doi.org/10.1038/s41598-020-61908-0>

OPEN

Multi-scale spatial genetic structure of the vector-borne pathogen '*Candidatus* Phytoplasma prunorum' in orchards and in wild habitats

Véronique Marie-Jeanne^{1,3}, François Bonnot¹, Gaël Thébaud¹, Jean Peccoud^{1,2},
Gérard Labonne¹ & Nicolas Sauvion^{1,3*}