

# PhD School on Agriculture, Environment and Bioenergy

([http://sites.unimi.it/dottorato\\_aab/](http://sites.unimi.it/dottorato_aab/))

(XXXIX cycle, 2023-26)

## Project draft

### 1. Field of interest

*AGR/12 – Plant Pathology*

### 2. Project title

Genomics and epidemiology of phytoplasmas associated with plant diseases

### 3. Tutor: Fabio Quaglino

**Co-tutor:** Piero Attilio Bianco

**4. Relevance of the topic and state of the art:** Phytoplasmas are phloem-limited, obligate parasitic bacteria of plants naturally transmitted by insect vectors. They are associated with numerous economically significant plant diseases worldwide. Many phytoplasma diseases affecting important crops, such as the grapevine bois noir (BN) and flavescence dorée (FD), are already epidemic in Italy and represent a key threat in some regions. Some others, such as the almond witches'-broom (AlmWB) epidemic in the Middle East and recently reported in South Italy, are worrying for their possible impact on the EC countries [1-3]. 'Candidatus Phytoplasma solani' (BNp), 16SrV-phytoplasma (FDp) and 'Ca. P. phoenicium', associated with BN, FD and AlmWB, respectively, have an extremely complex ecology including multiple insect vectors and host plants [4-6]. Briefly, 'Ca. P. solani' is associated with diseases of solanaceous plants, maize and e lavender; 'Ca. P. phoenicium' is associated with important peach and apricot diseases. Recently, draft genomes of 'Ca. P. solani' and 'Ca. P. phoenicium' have been sequenced opening new possibilities in finding potential virulence factors, effectors, and other mechanisms by which phytoplasmas achieve their pathogenicity and successfully manipulate plant hosts and insects [6-8]. To acquire information useful as bases for developing new efficient and sustainable control strategies, it is necessary to (i) investigate accurately BN, FD and AlmWB epidemiology through a multidisciplinary approach, (ii) study differences in the virulence of phytoplasma strains and in the susceptibility in crop varieties, (iii) identify new molecular markers and the effector pools of 'Ca. P. solani', 16SrV-phytoplasmas and 'Ca. P. phoenicium' by comparative genomics.

### 5. Layout of the project (draft)

#### 5.1. Materials & Methods:

**WP1 (Molecular epidemiology).** FDp, BNp, and 'Ca. P. phoenicium' strains, identified in host plants and insects in different agroecosystems, will be characterized through a multilocus sequence typing approach, based on known (*stamp*, *map*, *tuf*, *secY*) and newly proposed genes. Genes putatively involved in the phytoplasma-host interaction identified in WP2, will be analyzed within phytoplasma strain populations. Nucleotide sequence analyses and phylogenetic relationships will unveil intra-species diversity among phytoplasma strains, allowing to clarify the complex epidemiological patterns of FDp, BNp and 'Ca. P. phoenicium'.

**WP2 (Phytoplasma strain virulence).** Sequence Variable Mosaics (SVMs), containing putative virulence factors, will be identified within genomes of FDp, ‘*Ca. P. solani*’, and ‘*Ca. P. phoenicium*’ available in NCBI GenBank. Specific amplification of genes included in SVMs will be obtained by newly designed primers. In the case of FDp (no genome available), genes in SVMs will be amplified by primer pairs designed on draft genomes of closely related phytoplasma (‘*Ca. P. ziziphi*’) (first year) and on genomes obtained in WP3 (second year). Genes within SVMs will be amplified, sequenced, and analyzed from FDp, BNp and ‘*Ca. P. phoenicium*’ strains significantly associated with different symptom severity in infected plants. Thus, molecular markers in SVM genes distinguishing phytoplasma strains associated with distinct symptom severity should be related with different phytoplasma virulence.

**WP3 (Comparative genomics).** Phytoplasma strains identified in the WP1 and WP2, representative of distinct epidemiological patterns and/or virulence, will be selected for genome sequencing by Illumina MiSeq platform. High quality reads will be assembled and annotated using specific software packages. Genomes obtained from distinct phytoplasma strains, along with those available in GenBank, will be included in comparative genomic analysis.

### 5.2. Schedule and major steps (3 years):

Temporal development of the project (GANTT diagram)														
WP	Step	Activity	1st year				2nd year				3rd year			
			Months				Months				Months			
			1-3	4-6	7-9	10-12	13-15	16-18	19-21	22-24	25-27	28-30	31-33	34-36
WP1	1.1	Field survey and sample collection in different agroecosystems	[Timeline bar from month 1 to 24]											
	1.2	Phytoplasma molecular typing	[Timeline bar from month 4 to 30]											
	1.3	Definition of epidemiological patterns	[Timeline bar from month 13 to 36]											
WP2	2.1	Characterization of genes within sequence variable mosaic genomic regions	[Timeline bar from month 1 to 18]											
	2.2	Characterization of phytoplasma strain populations	[Timeline bar from month 7 to 24]											
	2.3	Definition of strain virulence	[Timeline bar from month 13 to 36]											
WP3	3.1	Phytoplasma strain selection and genome sequencing	[Timeline bar from month 13 to 36]											
	3.2	Comparative genomics	[Timeline bar from month 19 to 36]											

### 6. Available funds

- **Source:** Scientific collaboration agreement for the project "Grapevine yellows in Franciacorta: containment and epidemiological investigations" (Funded by: Consorzio per la Tutela del Franciacorta; Years: 2023-25); **Amount:** 30.000,00€
- **Source:** Project PRIN 2022 [Exploring the genomic variability and the molecular mechanisms underlying the pathogenesis of ‘*Candidatus Phytoplasma solani*’ (MEPASOL)] (Funded by: MUR; Years: 2023-2025); **Amount:** 99.000,00€

## 7. Literature:

- [1] Angelini E, Constable F, Duduk B et al. (2018) Grapevine phytoplasmas. In: Rao GP, Bertaccini A, Fiore N, Liefting LW (Ed.) Characterisation and Epidemiology of Phytoplasma - Associated Diseases. Phytoplasmas: Plant Pathogenic Bacteria–I. Springer Nature, Singapore, 123-52.
- [2] Fiore N, Bertaccini A, Bianco PA et al. (2018) Fruit crop phytoplasmas. In: Rao GP, Bertaccini A, Fiore N, Liefting LW (Ed.) Characterisation and Epidemiology of Phytoplasma - Associated Diseases. Phytoplasmas: Plant Pathogenic Bacteria–I. Springer Nature, Singapore, 153-90.
- [3] Bertaccini A, Duduk B, Paltrinieri S et al. (2014) Phytoplasmas and phytoplasma diseases: a severe threat to agriculture. *Am J Plant Sci* 5: 1763-88.
- [4] Pierro R, Panattoni A, Passera A et al. (2020) Proposal of a new Bois Noir epidemiological pattern related to '*Candidatus* Phytoplasma solani' strains characterized by a possible moderate virulence in Tuscany. *Pathogens* 9: 268.
- [5] Davis RE, Dally EL, Zhao Y et al. (2018) Genotyping points to divergent evolution of '*Candidatus* Phytoplasma asteris' causing North America Grapevine Yellows and strains causing Aster Yellows. *Plant Dis* 102: 1696-702.
- [6] Quaglino F, Kube M, Jawhari M et al. (2015) '*Candidatus* Phytoplasma phoenicium' associated with almond witches'-broom disease: from draft genome to genetic diversity among strain populations. *BMC Microbiol* 15: 148.
- [7] Seruga Music M, Samarzija I, Hogenhout SA et al. (2019) The genome of '*Candidatus* Phytoplasma solani' strain SA-1 is highly dynamic and prone to adopting foreign sequences. *Syst Appl Microbiol* 42: 117-27.
- [8] Mitrovic J, Siewert C, Duduk B et al. (2014) Generation and analysis of draft sequences of 'stolbur' phytoplasma from multiple displacement amplification templates. *J Mol Microbiol Biotech* 24: 1-11.