Phytoplasmas associated with grapevine yellows diseases: an overview

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PHYTOPLASMAS: MAIN FEATURES

- Cell-wall less bacteria of the class *Mollicutes*
- Reduced genome
- Obligate intracellular parasites
- Trans-kingdom lifestyle: plants and insects

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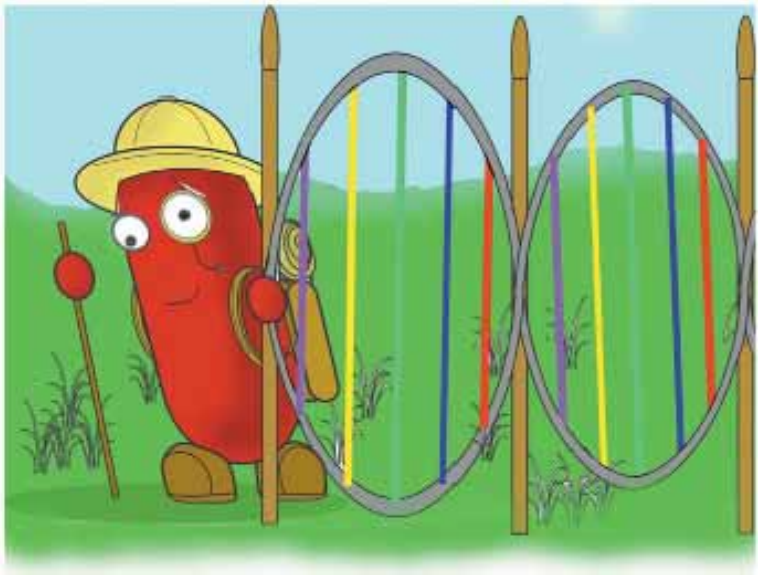
Microbial Genomics

Exploring the boundaries of life

Claire M. Fraser



MOLECULAR PLANT PATHOLOGY (2008) 9(4), 403–423

Pathogen profile

Phytoplasmas: bacteria that manipulate plants and insects

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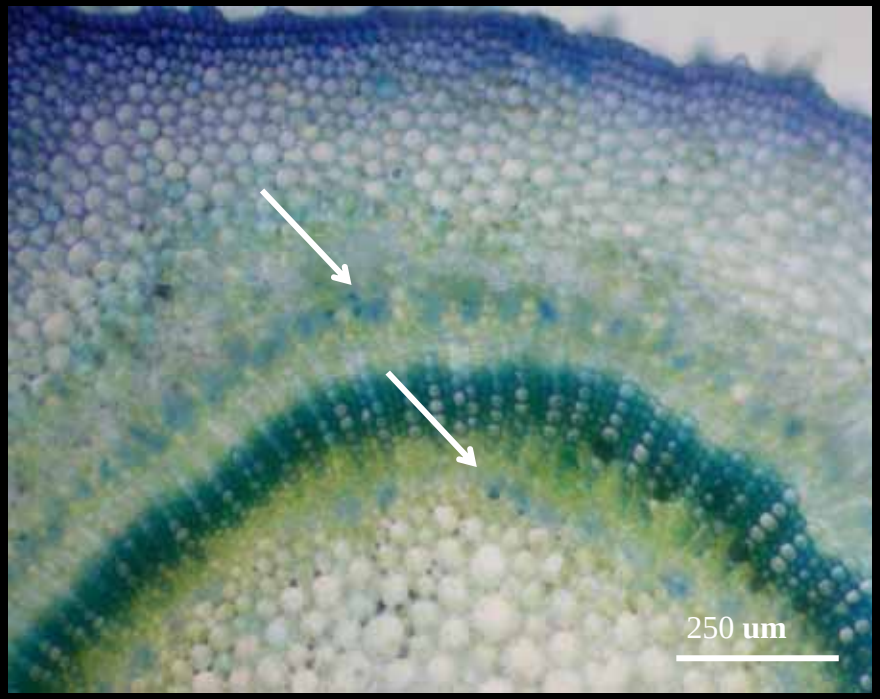
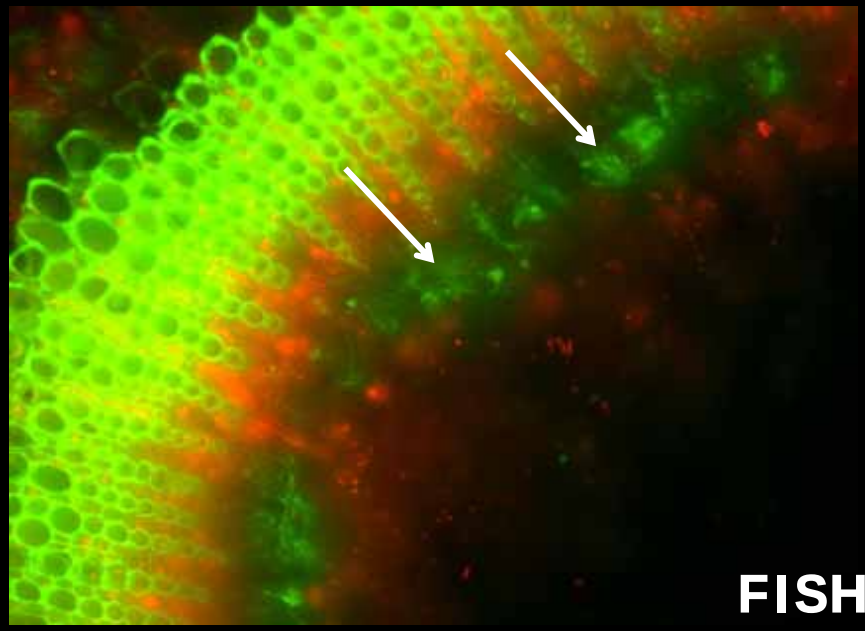
Restricted to the phloem sieve elements of the infected plants



M.O. DIENES



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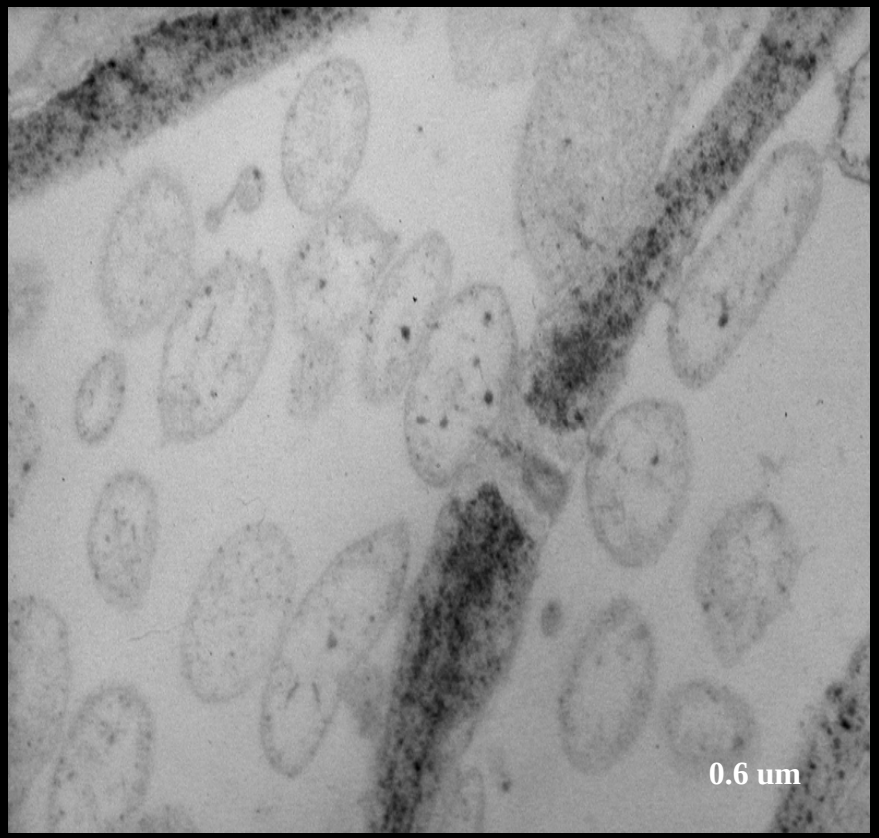


TEM

Phytoplasma Taxonomy and Classification

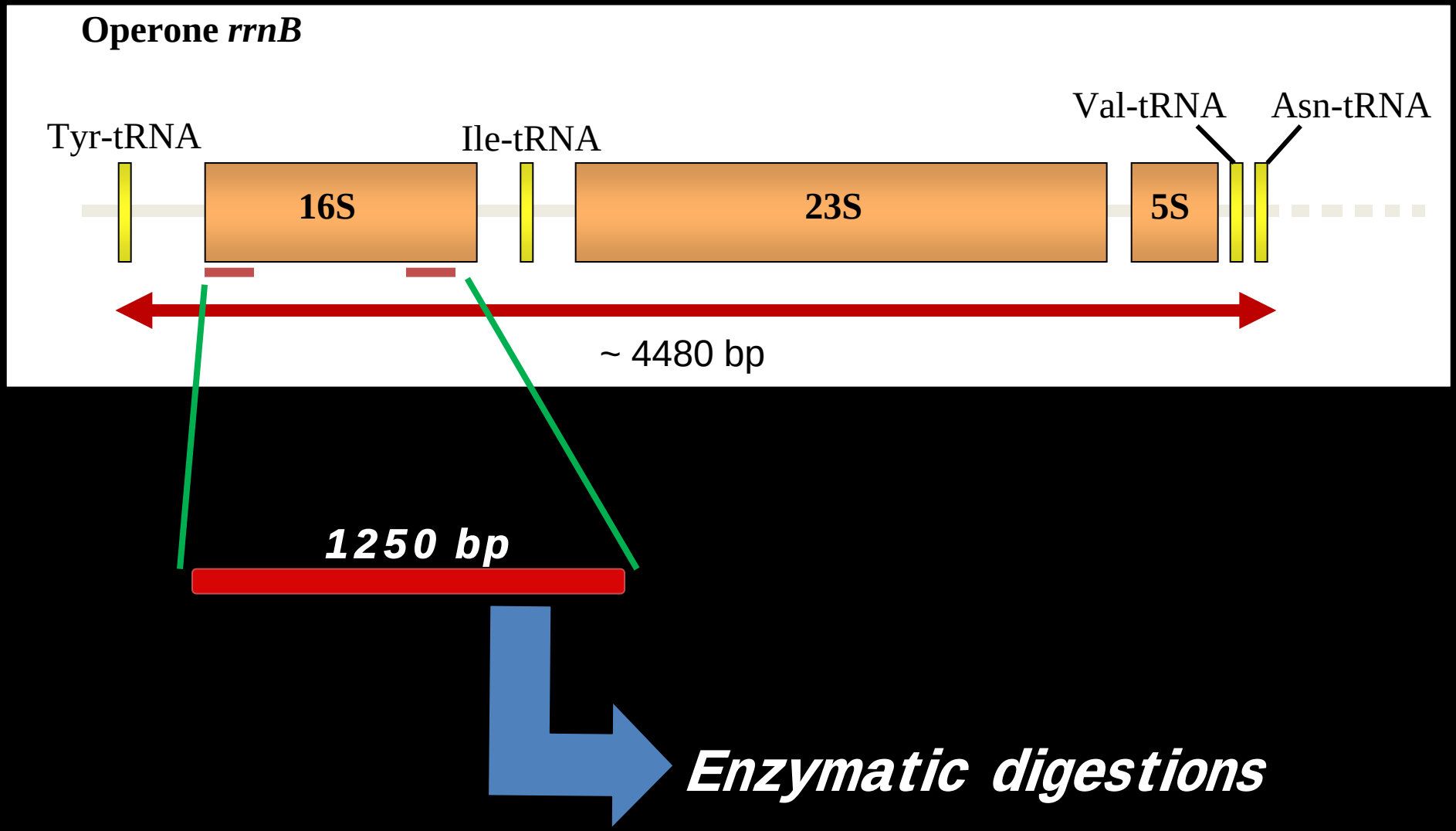
Phytoplasmas have been characterized and distinguished by molecular analyses of house-keeping genes (16S rRNA gene)

Two classification schemes

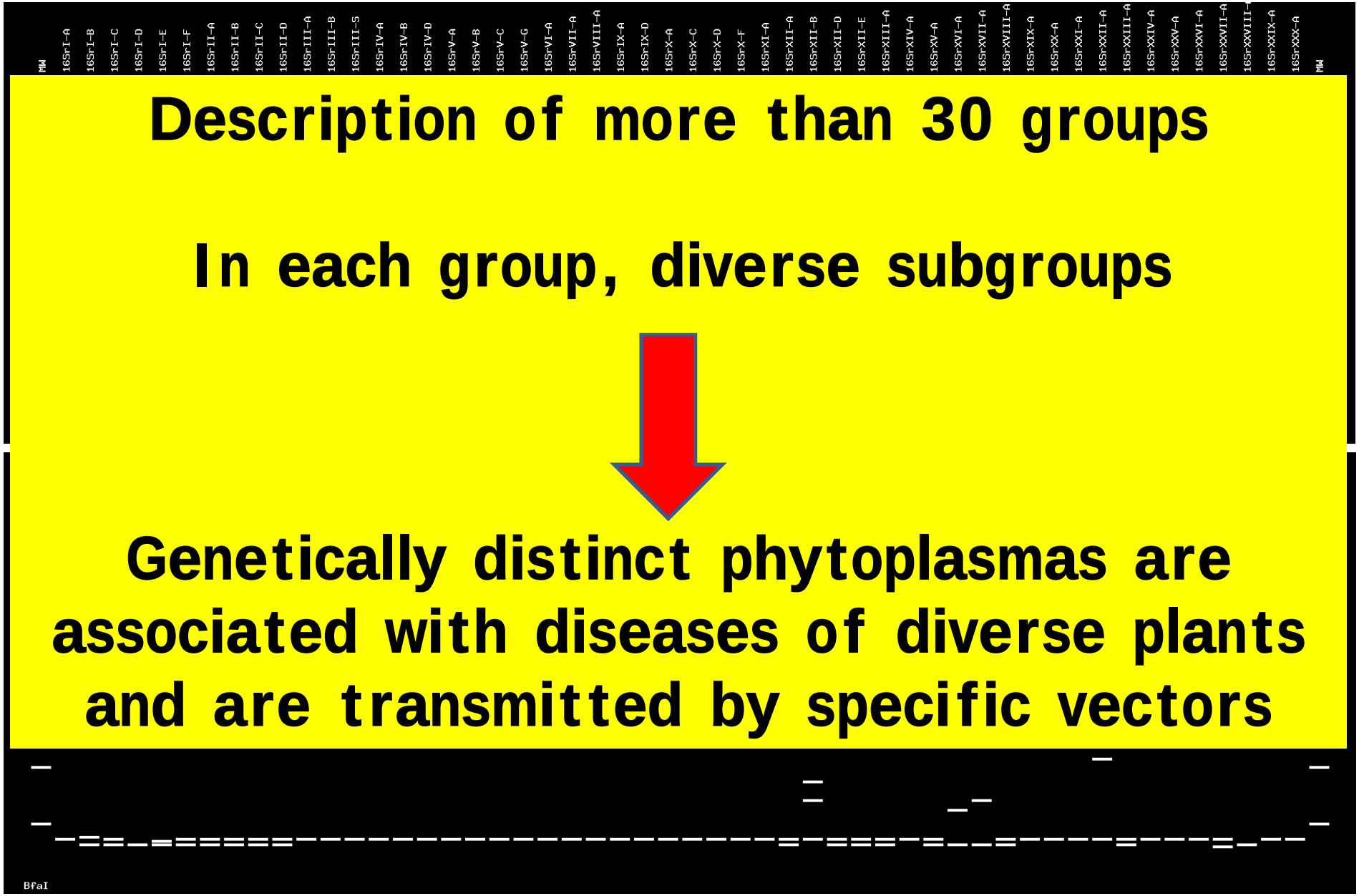
- '*Candidatus* Phytoplasma' species: identity scores of 16S rDNA nucleotide sequences (97.5%) and biological properties

- RFLP analysis-based classification scheme (classification in 16Sr groups and subgroups): similarity coefficient based on restriction patterns

PCR-RFLP analyses: phytoplasma groups



Phytoplasma Groups



Description of more than 30 groups

In each group, diverse subgroups

Genetically distinct phytoplasmas are associated with diseases of diverse plants and are transmitted by specific vectors

Wei *et al.*, 2007

Phytoplasmas are associated with diseases affecting hundreds of plant species

ECONOMICAL INTEREST

Grapevine yellows: a disease complex associated with distinct phytoplasmas

In Europe

Disease	Phytoplasma	Vector
Flavescence dorée	16SrV-C / -D	*Scaphoideus titanus*
Bois noir	16SrXII-A	*Hyalesthes obsoletus*
Palatinate Grapevine Yellows	16SrV-C	*Oncopsis alni*
Other grapevine yellows	16SrI-B /-C	Undetermined
	16SrIII	Undetermined
	16SrV-A	Undetermined
	16SrX-B	Undetermined

Grapevine yellows: symptoms

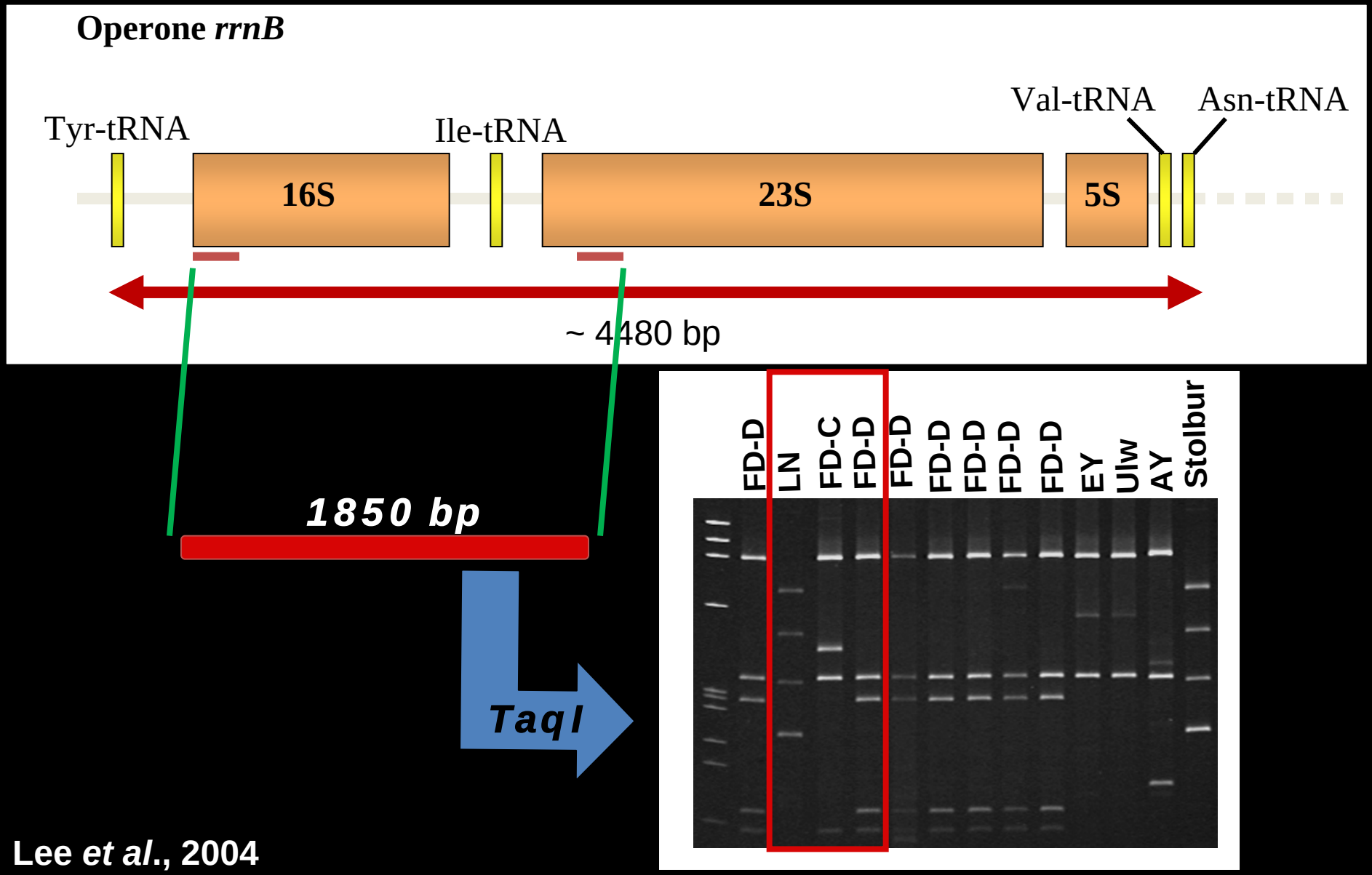
Rolling of leaf laminas

Color alterations of leaf veins

Desiccation of inflorescences

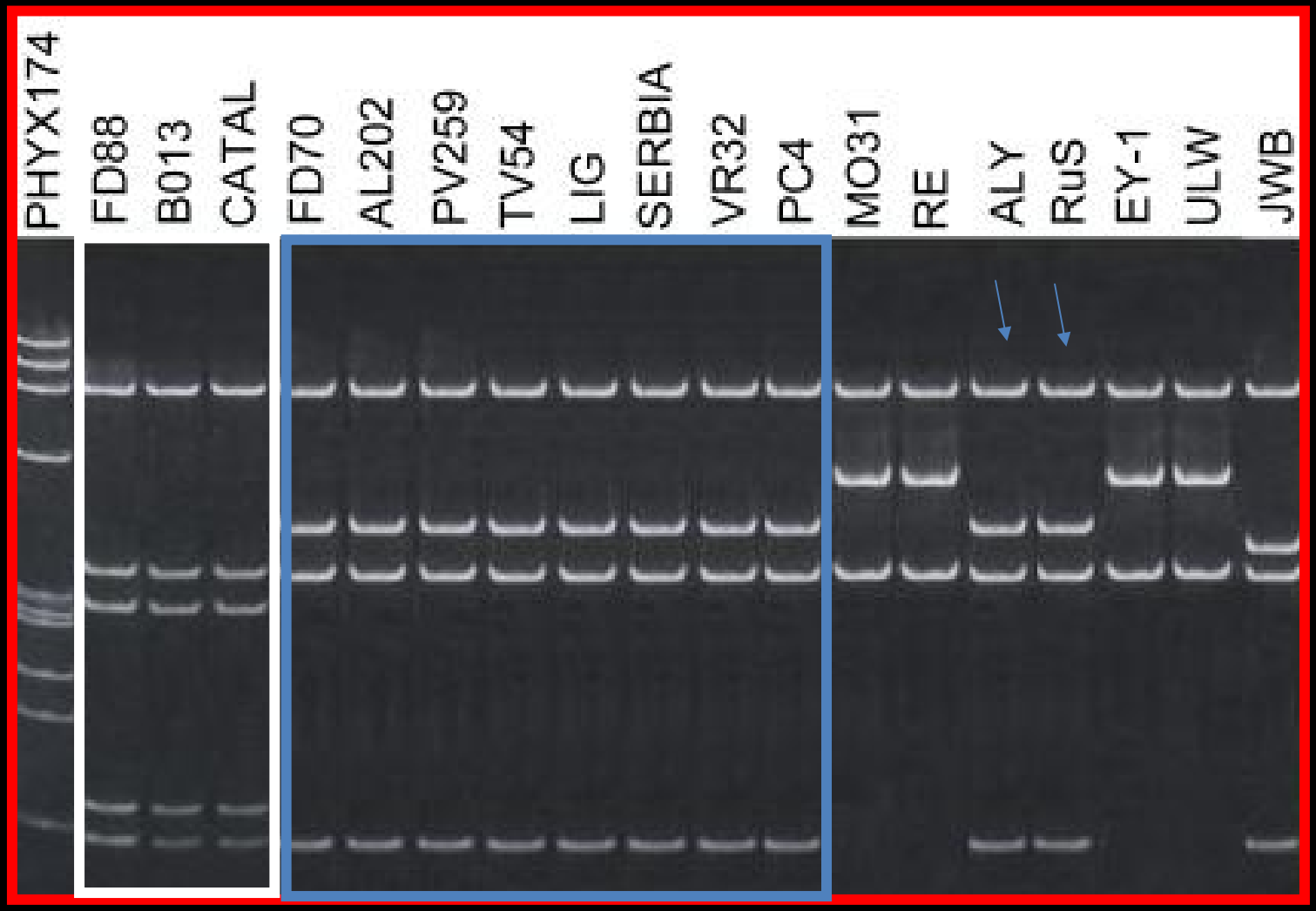
Irregular maturation of wood

PCR-RFLP analyses: GYs phytoplasmas



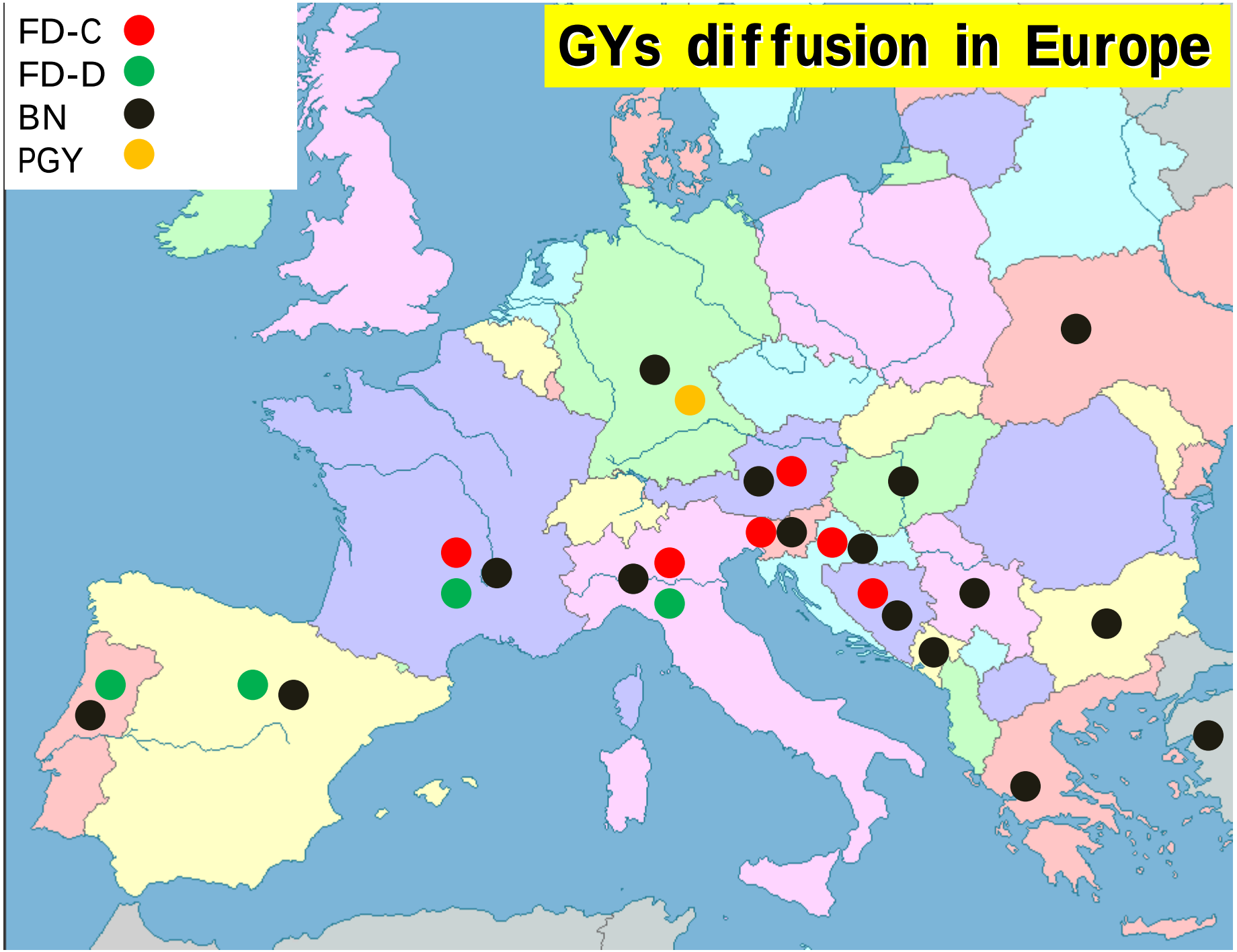
Lee *et al*., 2004

Genetically distinct FD phytoplasmas



RFLP with *Taq*I on 16Sr DNA amplicons

Martini *et al.*, 1999, 2002


FD-C
FD-D
BN
PGY
GYs diffusion in Europe

Flavescence dorée: ecology in vineyards

Orientus ishidae

Alnus glutinosa

Ailanthus altissima

Dictyophara europea

Clematis vitalba

Scaphoideus titanus

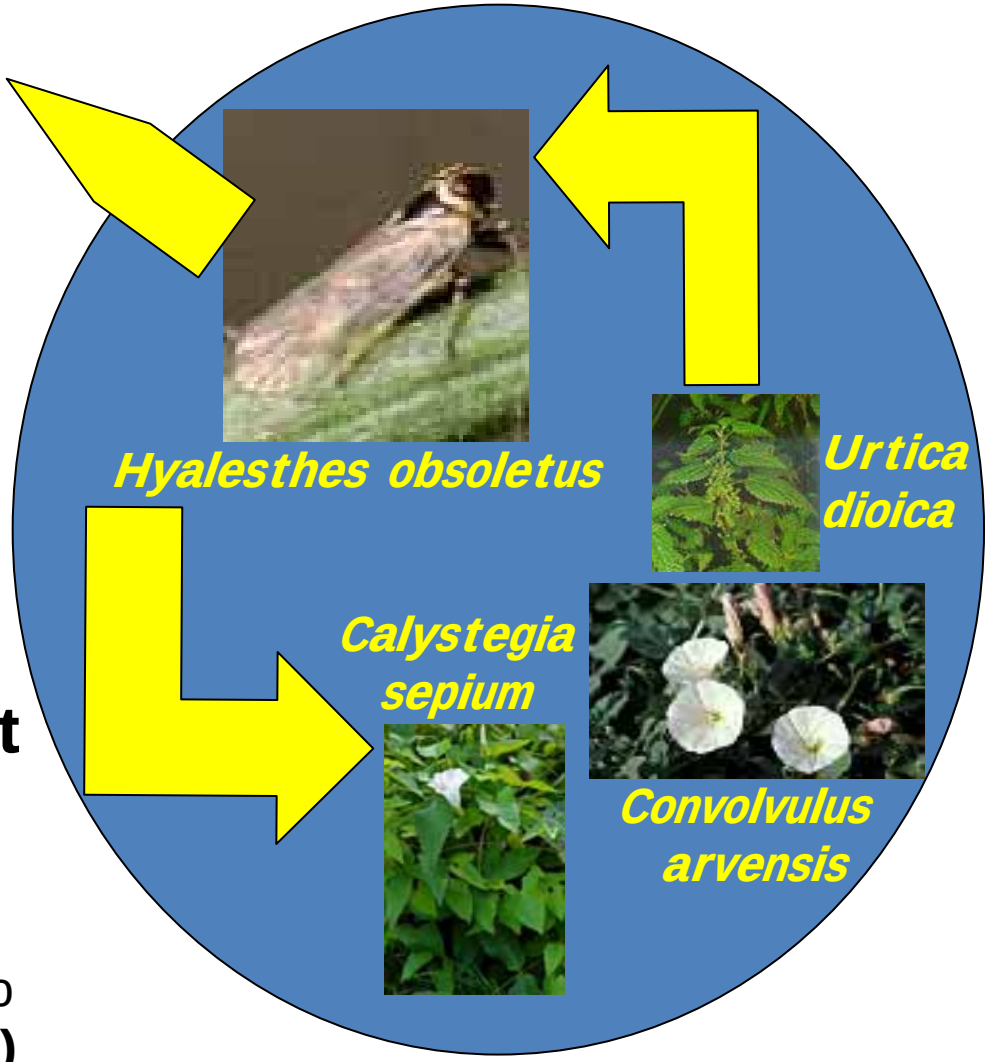
Vitis vinifera

Filippin *et al.*, 2009; Gaffuri *et al.*, 2011

Bois noir: ecology in vineyards

Vitis vinifera

Life cycle of BN phytoplasma



Other possible insect vectors and natural host plants

(Batlle *et al.*, 2000; Boudon-Padieu, 2000; Gatineau *et al.*, 2001; Garau *et al.*, 2004; Palermo *et al.*, 2004; Pinzauti *et al.*, 2008)

Bois noir: prevalence in Italian vineyards

(Botti & Bertaccini, 2007)

Region	Total of samples tested	Samples positive to ribosomal group 16SrV phytoplasmas	Samples positive to ribosomal subgroup 16SrXII-A phytoplasmas	% of negative samples
Veneto	653	195	261	30
Emilia-Romagna	421	22	294	25
Liguria	38	12	8	47
Tuscany	312	27	85	64
Total	1424	256	648	37

28.3%
FD phytoplasma

71.7%
BN phytoplasma

Strategies for GY management

FD: treatments against insect vector populations
BN: no efficient control measures

Key point: identification of phytoplasmas associated with GY symptoms

Problems

- propagation material (mother plants and rootstocks)
- visual inspections: detection of latent infections?

- irregular distribution of phytoplasmas in the plants
- low phytoplasma titer in symptomless plants
- fluctuation in phytoplasma concentration

- additional plant hosts and insect vectors

Develop and employment of molecular techniques for phytoplasma diagnostics

Phytoplasma identification: sensitive molecular approaches

(i) Real Time PCR and reverse transcription – Real Time PCR for the detection of phytoplasmas associated with FD and BN (Bianco et al., 2004; Galetto et al., 2005; Angelini et al., 2007; Margaria et al., 2007; Durante et al., 2009)

(ii) Nanobiotransducer for detecting FD phytoplasmas (Firrao et al., 2006);

(iii) Multiplex nested PCR for simultaneous identification of FD and BN phytoplasmas (Clair et al., 2003);

(iv) Ligase Detection Reaction (LDR) DNA microarray to detect and distinguish FD and BN phytoplasmas (Frosini et al., 2002)

Phytoplasma identification: improved diagnostic tools

- FD and BN phytoplasmas identified in grapevine rootstocks (Borgo et al., 2009)
- BN phytoplasmas can be transmitted by rootstocks to healthy plants (Zorloni et al., 2011)
- Identification of additional host plants
- Higher sensitivity for monitoring phytoplasma infections in grapevine mother plants

Phytoplasma ecologies:
finer molecular characterization

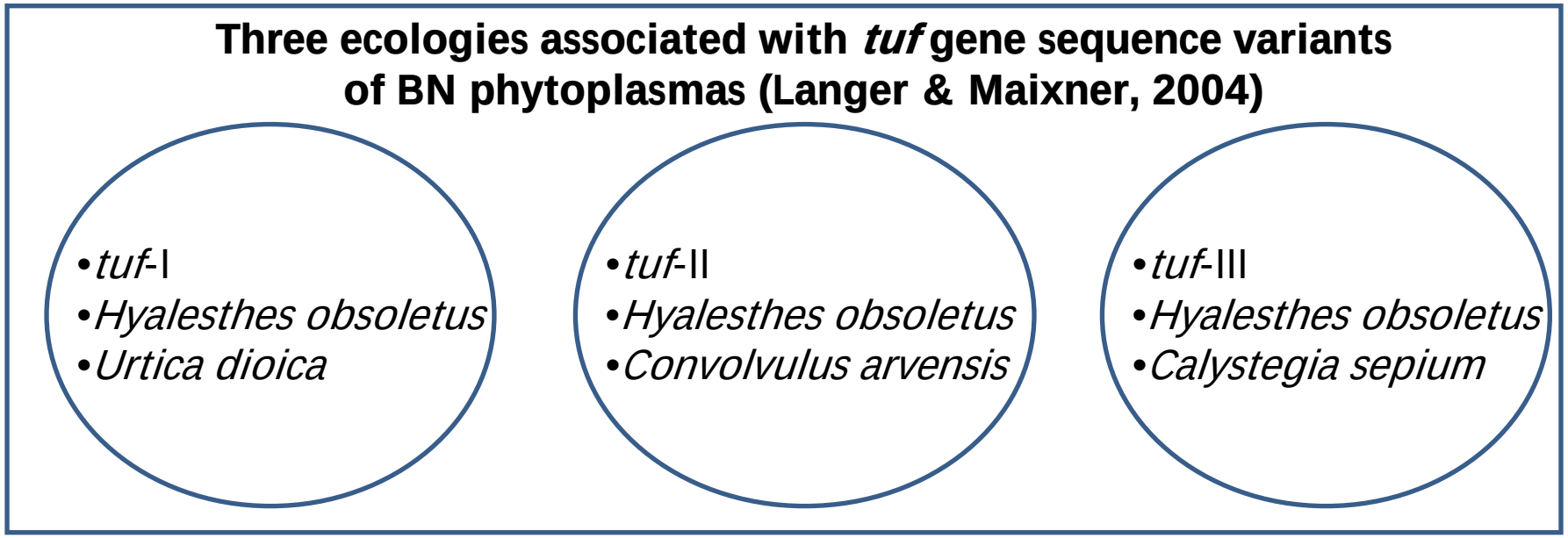
- Multiple gene sequence analyses (Multi Locus Sequence Typing, MLST) on ribosomal (*rplV-rpsC*) and non ribosomal (*secY*, *map*, *uvrB*, *degV*, *hlyC*, *vmp*, and *tuf*) genes

- Unexpected genetic heterogeneity among both FD and BN phytoplasma populations

- Identification of different FD and BN phytoplasma strains that can be associated with specific ecological niches (plant hosts, insect vectors, geographic origin)

- Improved the chance to associate phytoplasma-specific molecular markers with biological features

Genetic diversity among Bois noir phytoplasma strains: *tuf* gene



CONCLUSIONS (I)

- Grapevine yellows are associated with genetically different phytoplasmas

- In Europe, FD and BN are the prevalent GY diseases

- Management strategies are different because the diverse lifecycles of the etiological agents

- **Key point**: specific identification of phytoplasmas

- PCR-based techniques (standard and innovative)

CONCLUSIONS (II)

- Finer molecular characterization of phytoplasmas

- Improved knowledge of pathogen lifecycle and disease epidemiology

- Molecular markers for determining geographic origin of the pathogens