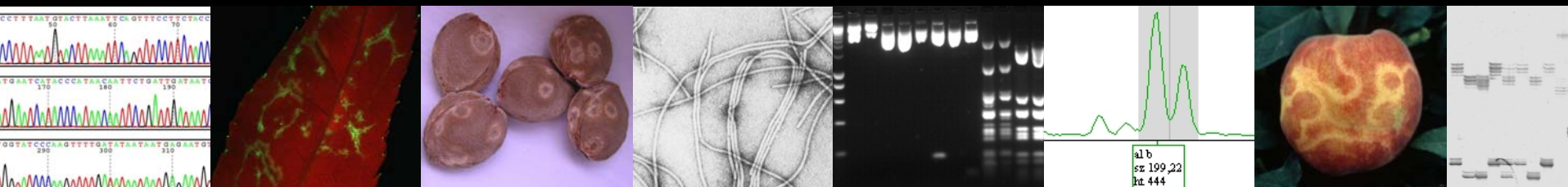
UMR INRA Université Bordeaux 2 Génomique Diversité Pouvoir Pathogène

Nouvelles approches biotechnologiques de lutte contre la sharka

Dr Véronique DECROOCQ

09 Octobre 2009



The Sharka disease in Stone fruit trees

P. domestica / plum	*P. persica* / peach	*P. armeniaca* / Apricot
		
		

Un-marketable fruits

Cost of the disease in the last 3à years to EU : 30 billions €

Its causal agent, the *Plum pox virus*, is classified as quarantine pathogen

The *Plum pox virus*, a virus of the *potyviridae* family

Family	*Potyviridae*
Group	*Potyvirus*
Genomic form	RNA sb + ~10 kb

Natural hosts

Stone fruit trees of the *Prunus* species

- Apricot, peach plum, cherry, almond
- Ornementals and rootstock *Prunus* trees

Some herbaceous plants

Transmission

- Aphids
- Grafting

The *Plum pox virus*

6 viral strains described to date

D: Dideron

M: Marcus

Rec: Recombinant

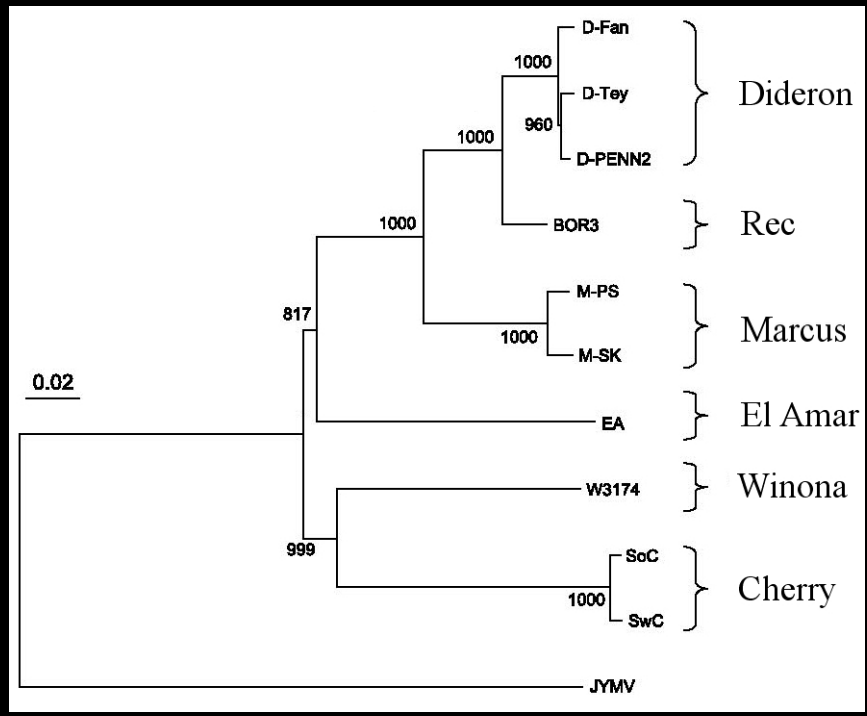
EA: El Amar

W: Winona

C: Cherry

Epidemiologic behaviour

differing from one strain to another



Arbre N-J (séquences nucléotidiques complètes), Myrta *et al.* (2006)

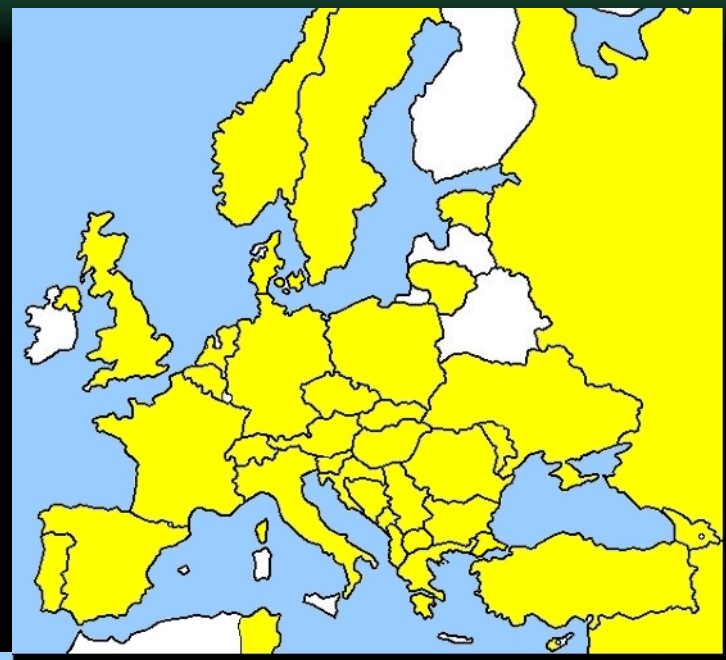
Origin of the disease and current situation

Described for the first time in 1917 in Bulgaria

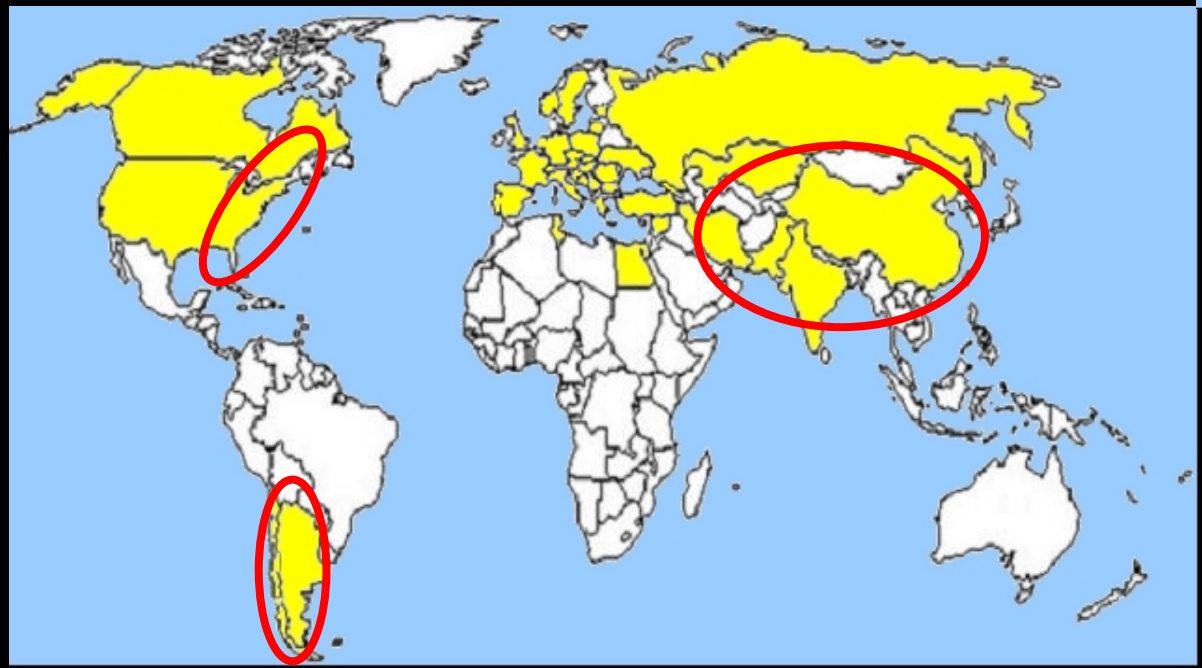
Dissemination around the Mediterannee and in Eastern Europe

In the last 10 years:

Outbreaks of the disease in America and Asia



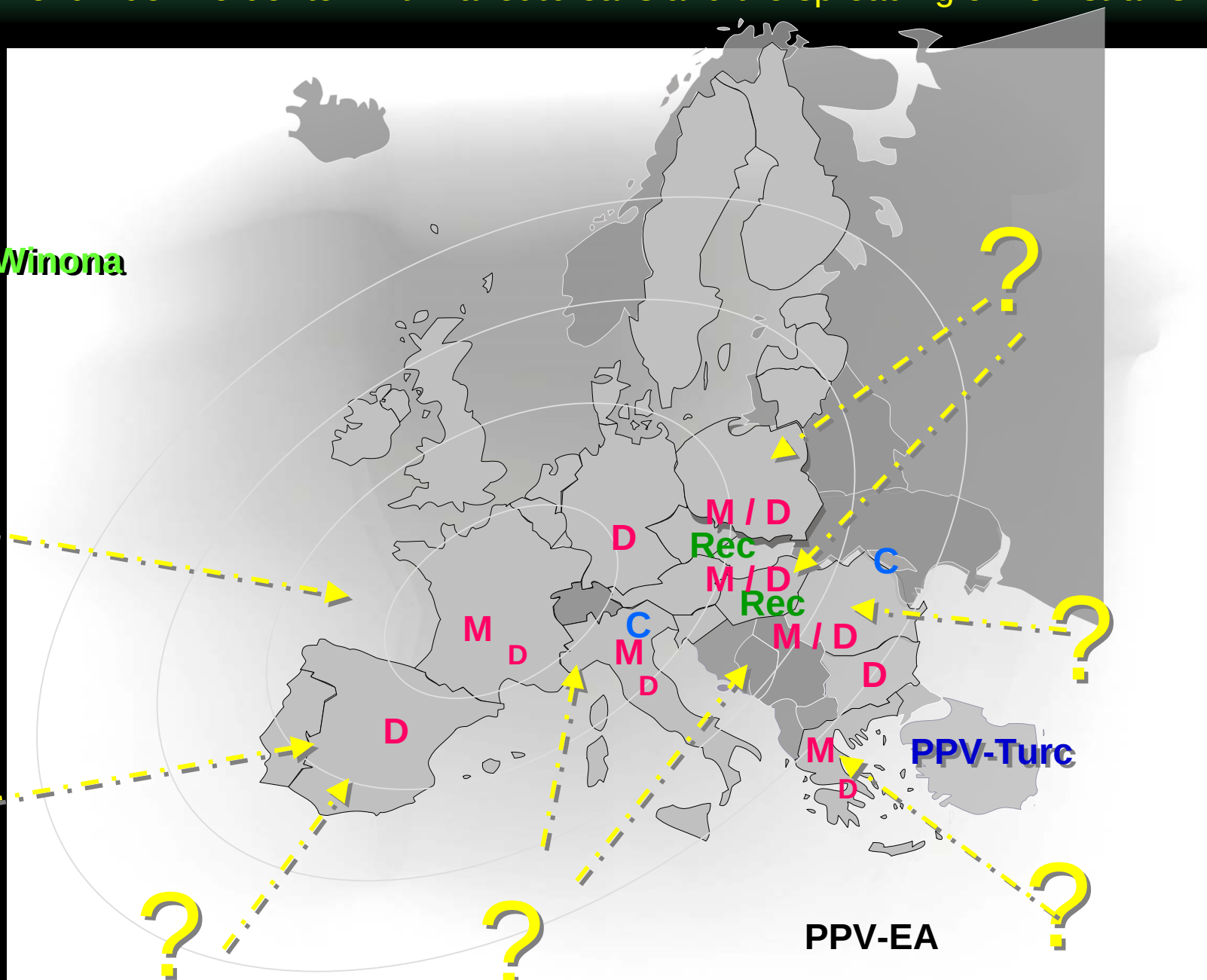
Garcia et Cambra (2007)

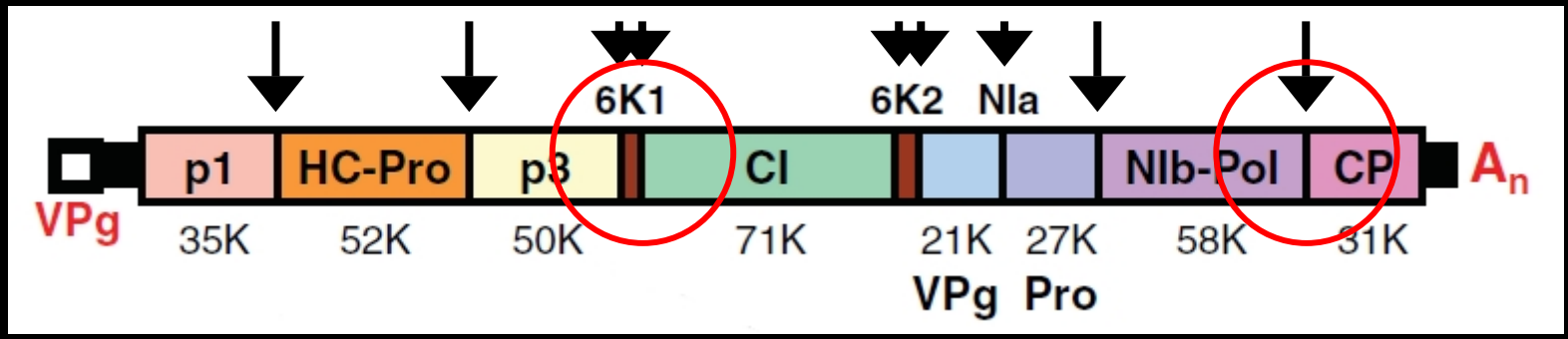


Country were the virus has been detected

To better know the enemy .....

PPV diversity worldwide in order to limit viral outbreaks and the spreading of new strains

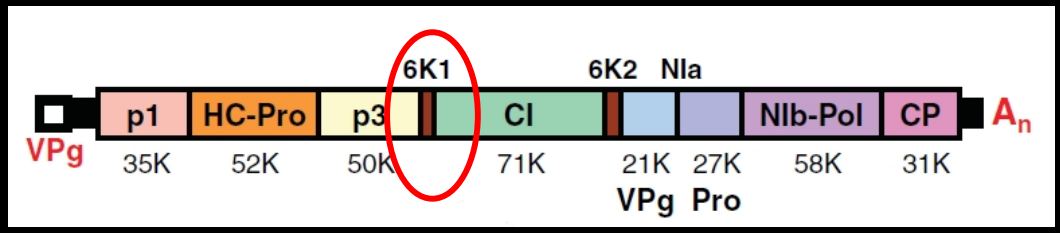




- Two main viral genomic regions used to evaluate the diversity of the virus worldwide (among over 1,000 isolates)

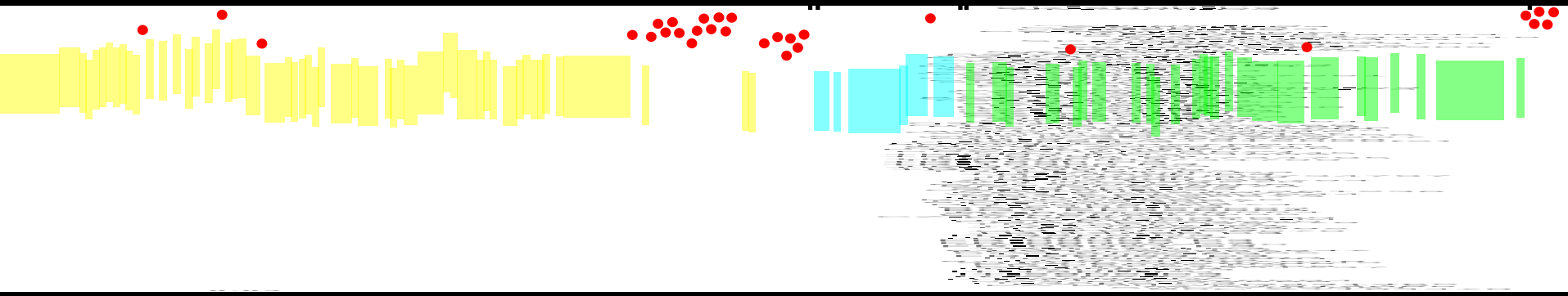
-Full genome sequencing of the 50 most representative/peculiar isolates.

•Establishement of a live core-collection of PPV isolates, maintained in France and Italie.



P3-6K1-CI viral sequence

D + Rec Tk M

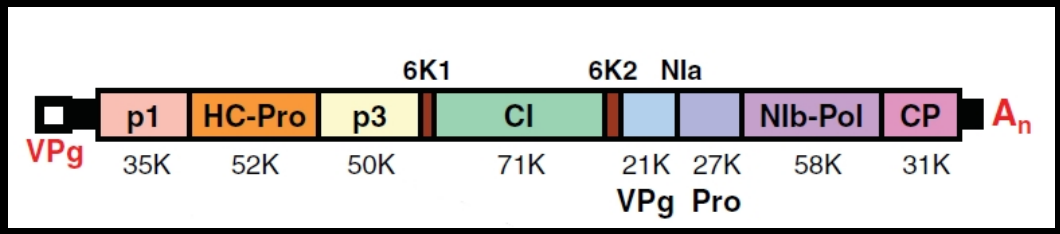


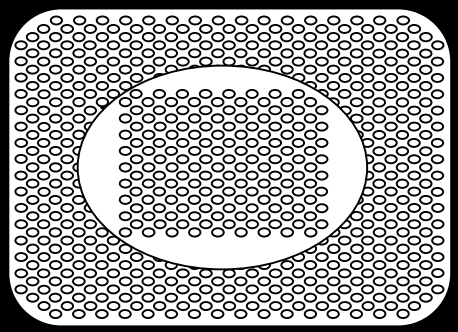
- Unexpected higher diversity of the European PPV strains, especially PPV-D

-Turkish PPV strains are grouping aside of the European M and D strains. They were not detected yet in EU.

•Occurrence of the Rec strain only in countries where both PPV-M and PPV-D are present.

Predicting and limiting new PPV outbreaks





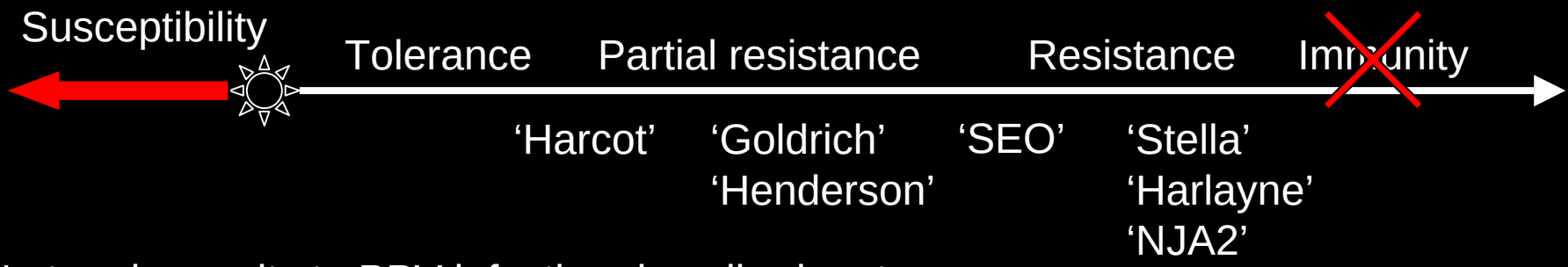
Micro-arrays or SNPlex technology

based on 1,000 viral sequences, representing the maxi of PPV diversity, and generating thousands of probes covering the full viral genome

Use at a larger scale (EU) by the **Plant Protection Services** to detect earlier new PPV outbreaks and new strains (i.e PPV-T)

To fight against the virus ……

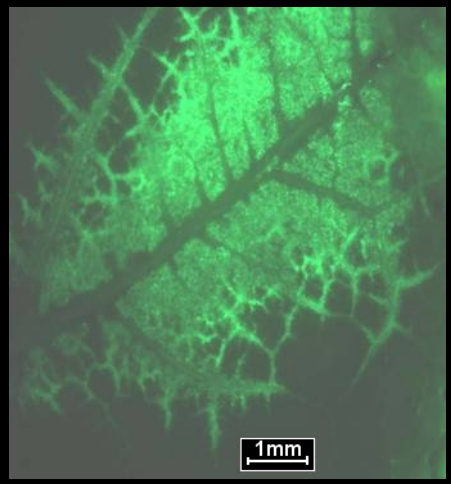
Genetic resistance to PPV in Apricot



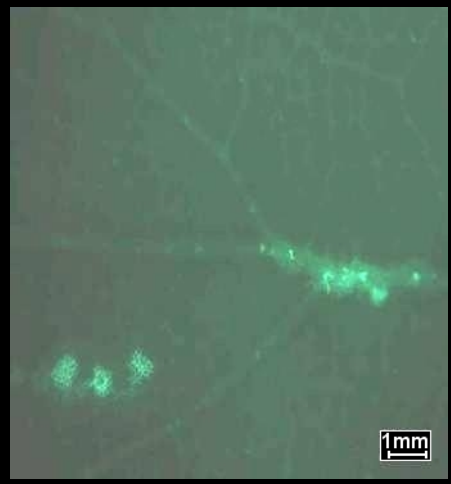
No true immunity to PPV infection described up to now

Few North-American cultivars are resistant

Susceptible apricot
Moniqui



Resistant apricot
Harlayne, SEO



Genetic determinism of the resistance still under discussion (1, 2
were mapped. However, **Importance of the Chromosome 1**

Résistances génétiques au PPV, chez l'abricotier et *Prunus davidiana*

Sensibilité — Tolérance — Résistance partielle — Résistance — Immunité

'Harcot' 'Goldrich' 'Henderson' 'SEO' 'Stella' 'Harlayne' 'NJA2'

GL1

UDP96018
CFCha
FG83A
AG109
GC308
CC18
AG102
CFFaa
PC78
pchcms4
PC102
CFFm
CFOj
PC30
AG29
UDP96005
FG9
PC35
CFLhd
pchgms3
AG107
AG47
AG44
FG79
CFFad
AC18
CFFaj1
CC28
AG11a

P. Davidiana P1908 (Decroocq *et al.* 2005)

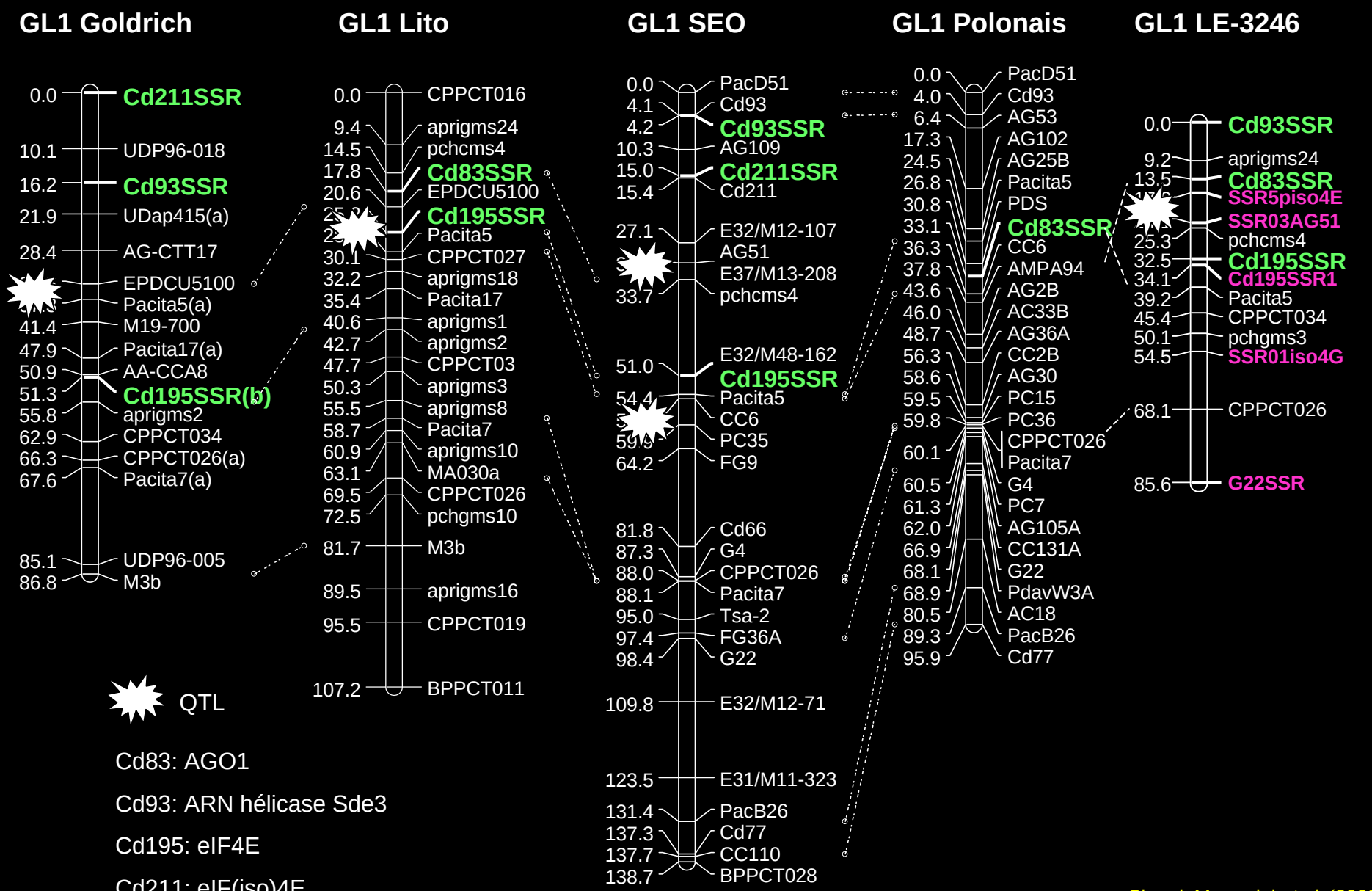
'Goldrich' (Soriano *et al.* 2002)

'Lito' (Dirlewanger *et al.* 2006) (descendant F1 de 'SEO')

'Stark Early Orange' (Lambert *et al.* 2007)

'LE-3246' (Lalli *et al.* 2008) (descendant F1 de 'SEO')

Développement ciblé de marqueurs moléculaires

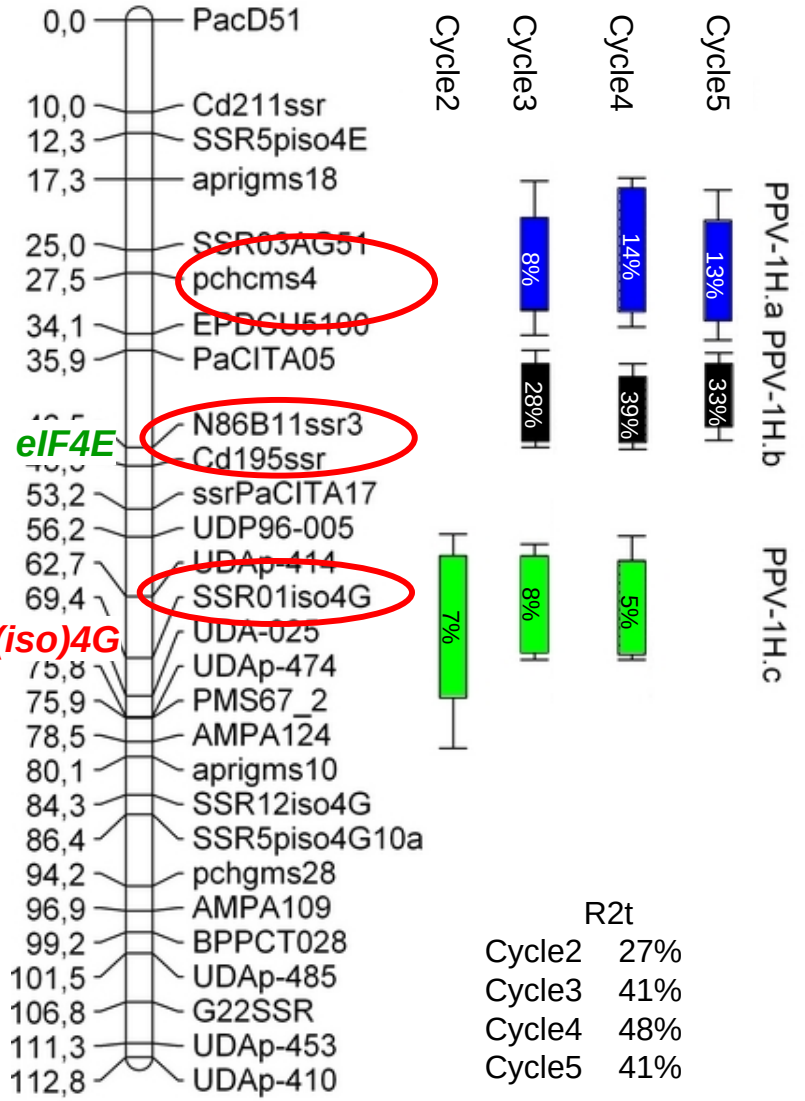


Sicard, Marandel *et al.* (2008)

Partie II

Resistance to PPV in Apricot

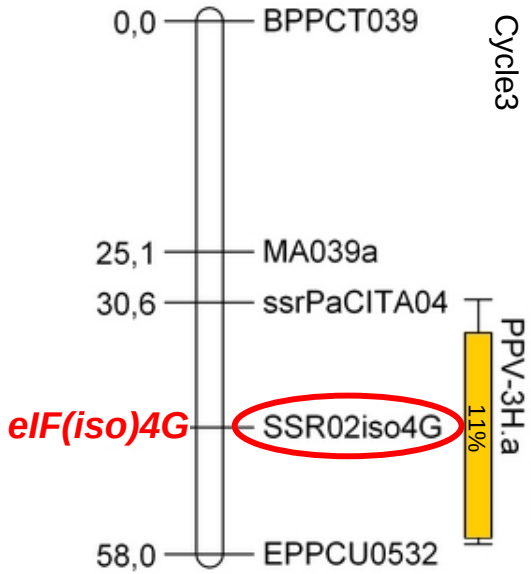
Harlayne GL1



R2t

Cycle2	27%
Cycle3	41%
Cycle4	48%
Cycle5	41%

Harlayne GL3



Marandel *et al.* (soumis)

In summary

Relation between the number of QTL and the level of resistance observed

	MetaPPV-1.a	MetaPPV-1.b	MetaPPV-1.c	MetaPPV-3
Goldrich	✓	✗	✗	✗
Stark Early Orange	✓	✗	✓	✓
Harlayne	✓	✓	✓	✓

Origin of the resistance

Harlayne (Sunglo et Reliable), Goldrich (Sunglo), Stark Early Orange (?)

Interest to add to the study the PPV resistant gentors: Stella and NJA2

(however, problem of adaptation of those genitors to climate changes and global warming, requesting long cold period in Winter))

In summary

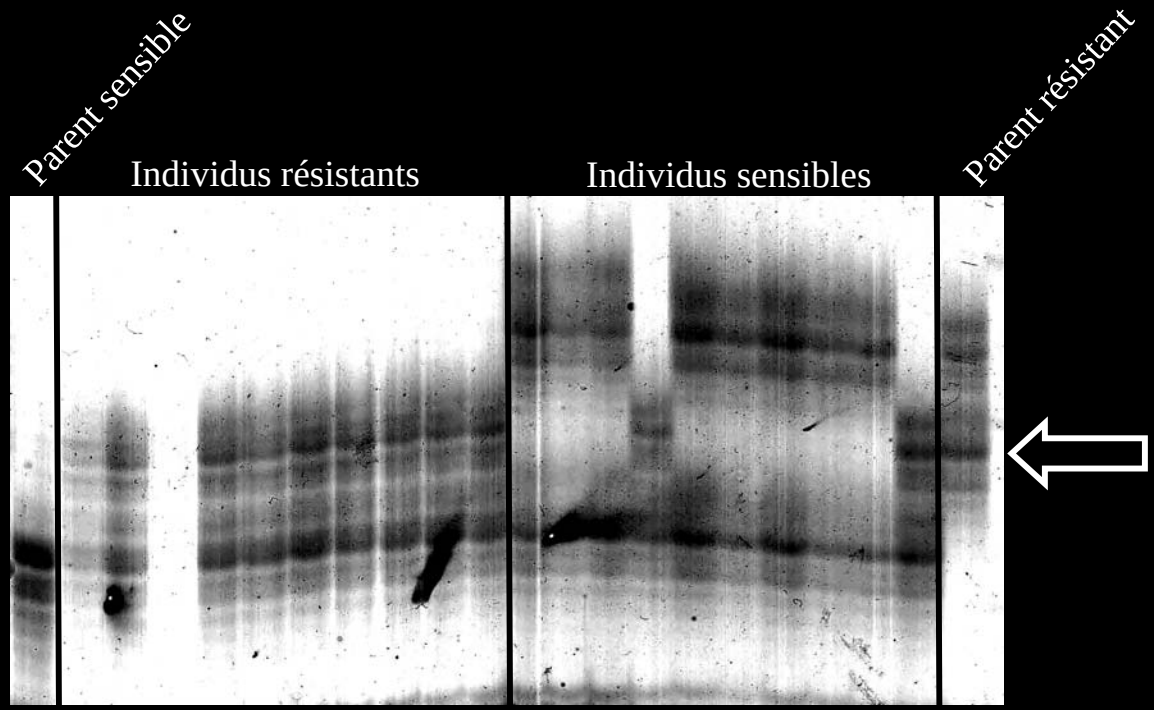
A set of molecular markers available for Marker Assisted Selection

Tools for

➔ Study of co-localisation QTL/candidate gene for the resistance

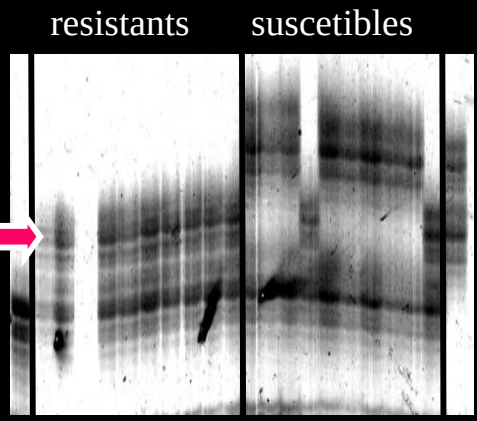
➔ Compare the resistance between cultivars (alignement of maps)

➔ **Use for and Validation in Marker Assisted Selection**



Partie II

Marker Assisted Selection for resistance to PPV



Early selection of fruit trees bearing the favourable allele of resistance to PPV

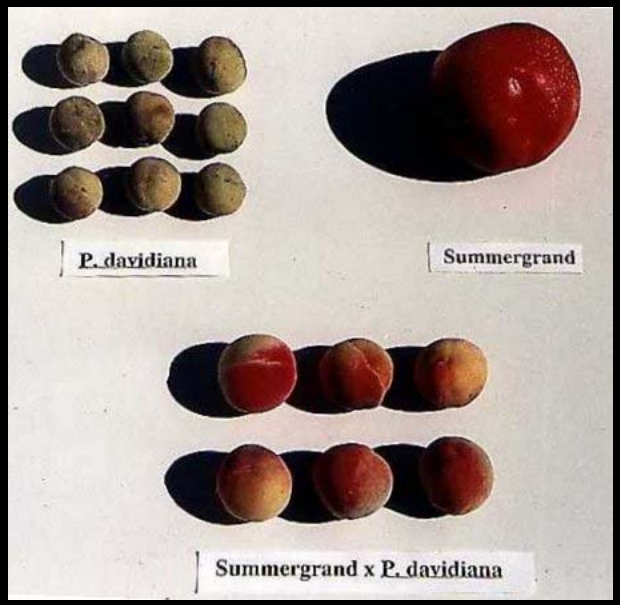
Speeding up of the EU breeding programmes for resistance to PPV

Genetic resistance to PPV in Peach

No *true* resistance to PPV described in Peach

Wild species related to peach, *P. davidiana* : one clone

P1908 is resistant



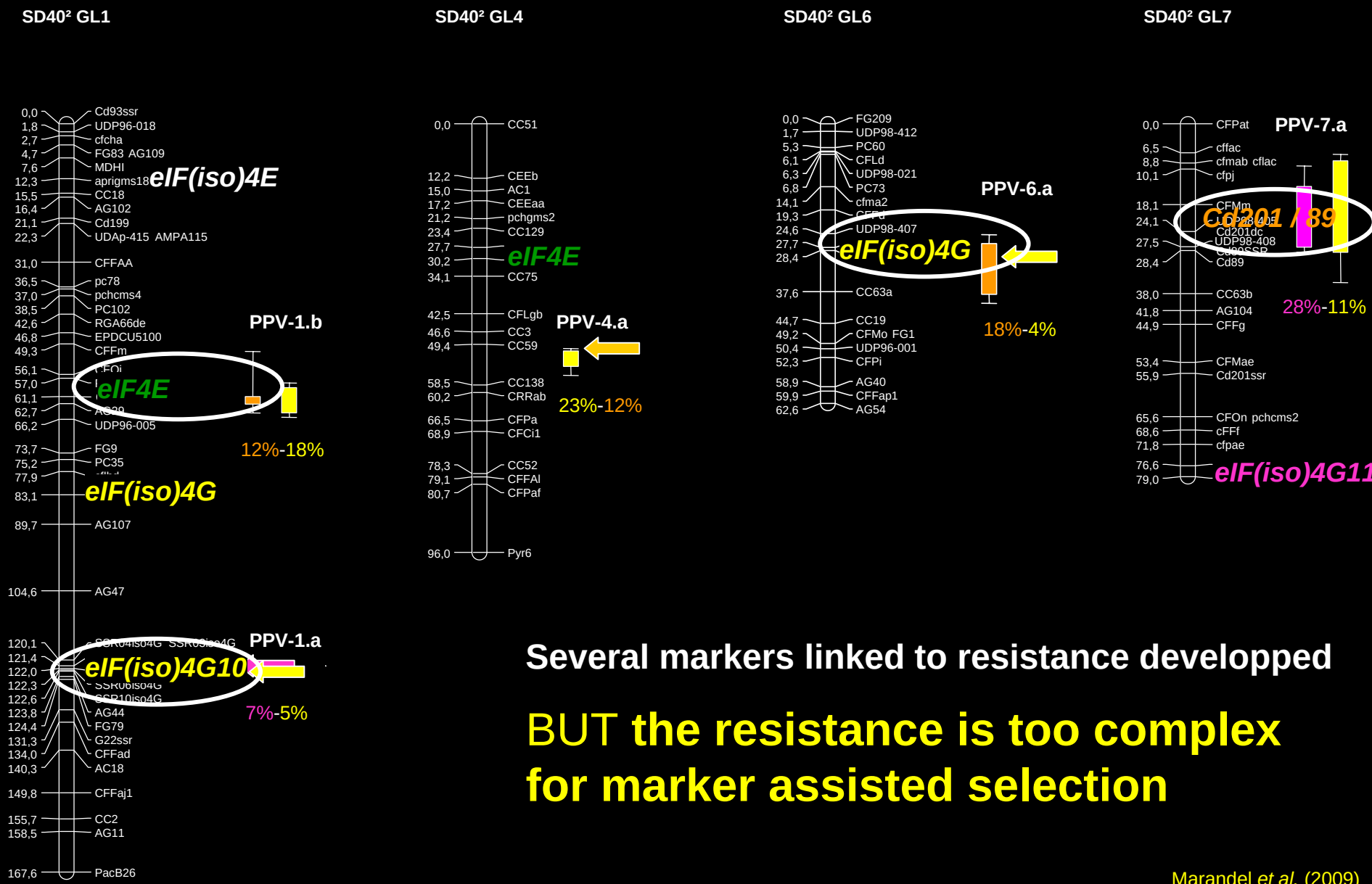
P. persica cv. 'Summergrand' x *P. davidiana* P1908

Populations F1 and F2 studied for resistance to PPV

(Decroocq et al., 2005; Marandel et al, 2009)

BUT **the resistance is complex**

Quantitative resistance in *P. davidiana* P1908 to PPV



Several markers linked to resistance developped

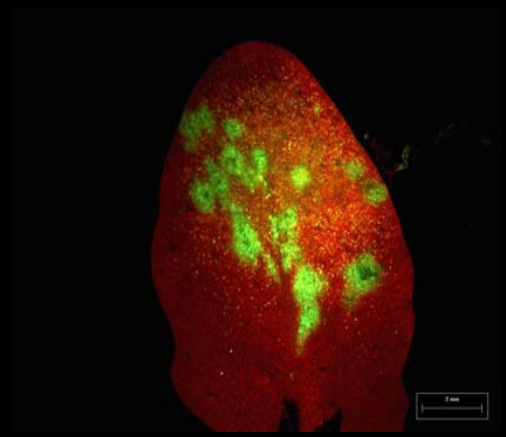
BUT **the resistance is too complex for marker assisted selection**

Marandel *et al.* (2009)

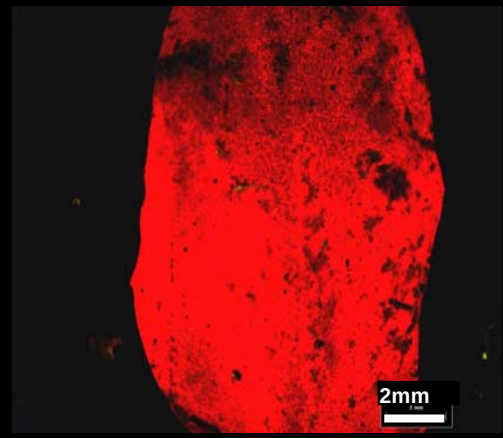
Looking for new sources of resistance to PPV

Genre	Virus	Plante hôte	Locus/mutant	Gène	Référence
Potyvirus	BYMV	*P. sativum*	*sbm1*	*eIF4E*	Bruun-Rasmussen *et al.* (2007)
	CIYVV	*A. thaliana*	*cum1*	*eIF4E1*	Sato *et al.* (2005)
			-	*eIF4G*	Nicaise *et al.* (2007)
	LMV	*A. thaliana*	E6	*eIF(iso)4E*	Duprat *et al.* (2002)
			-	*eIF(iso)4G1*	Nicaise *et al.* (2007)
		Lactuca spp.	*mo1*	*eIF4E*	Nicaise *et al.* (2003)
	PPV	*A. thaliana*	E6	*eIF(iso)4E*	Decroocq *et al.* (2006)
			-	*eIF(iso)4G1*	Nicaise *et al.* (2007)
	PVY	*Capsicum spp.*	*pvr2*	*eIF4E*	Ruffel *et al.* (2002), Charron *et al.* (2008)
		Lycopersicon spp.	*pot1*	*eIF4E*	Ruffel *et al.* (2005)
	TuMV	*A. thaliana*	*lsp1*, E6	*eIF(iso)4E*	Lellis *et al.* (2002), Duprat *et al.* (2002)
			-	*eIF(iso)4G1*	Nicaise *et al.* (2007)
			-	*eIF(iso)4G2*	Nicaise *et al.* (2007)
	TEV	*A. thaliana*	*lsp1*	*eIF(iso)4E*	Lellis *et al.* (2002)
		Capsicum spp.	*pvr2*	*eIF4E*	Ruffel *et al.* (2002), Charron *et al.* (2008)
		Lycopersicon spp.	*pot1*	*eIF4E*	Ruffel *et al.* (2005)
	PSbMV	*P. sativum*	*sbm1*	*eIF4E*	Gao *et al.* (2004b)
	PVMV	*Capsicum spp.*	*pvr6*	*eIF(iso)4E*	Ruffel *et al.* (2006)
			pvr2	*eIF4E*	Ruffel *et al.* (2006)
Cucumovirus	CMV	*A. thaliana*	*cum1*	*eIF4E*	Yoshii *et al.* (2004)
			cum2	*eIF4G*	Yoshii *et al.* (2004)
Carmovirus	MNSV	*C. melo*	*nsv*	*eIF4E*	Nieto *et al.* (2006)
	TCV	*A. thaliana*	*cum2*	*eIF4G*	Yoshii *et al.* (2004)
Bymovirus	BaYMV,BaMMV	*H. vulgare*	*rym4/5*	*eIF4E*	Stein *et al.* (2005)
			rym4	*eIF4E*	Kanuyka *et al.* (2005)
Sobemovirus	RYMV	*O. sativa*	*rymv1*	*eIF(iso)4G*	Albar *et al.* (2006)

From *Arabidopsis thaliana* to fruit trees: New sources of resistance to PPV



Wildtype Arabidopsis inoculated with PPV-GFP



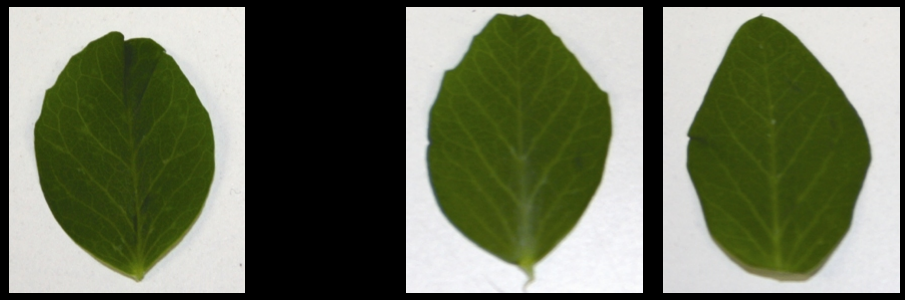
Mutant *eIFiso4E* inoculated with PPV

Decroocq et al., 2006

… And identification of variants in *Prunus* species that might correspond to resistance genes

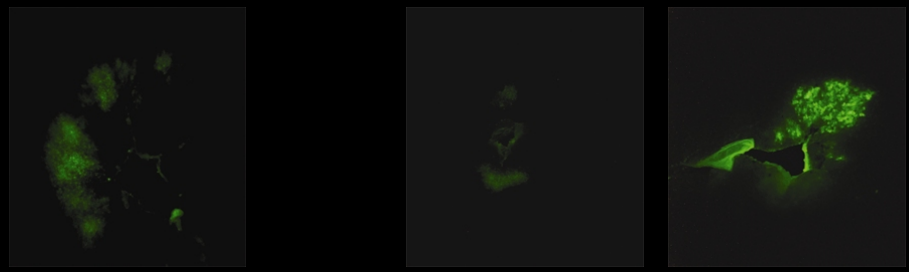
Mostly originating from old peach cultivars and mid-Asian *Prunus* trees

In pea, mutations in the *same* gene compromise the infection by PPV

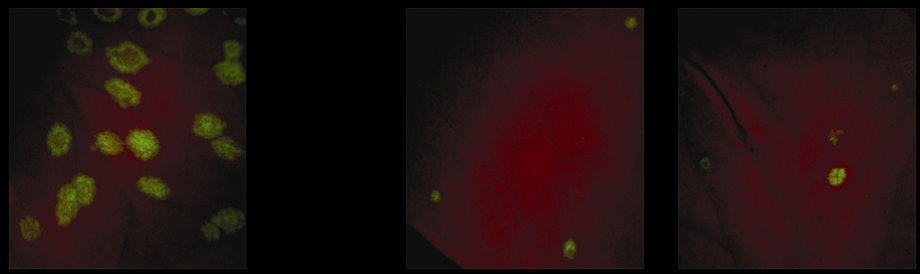


Caméor m652 m2587

GFP tagged PPV 10 dpi (inoculated leaf)

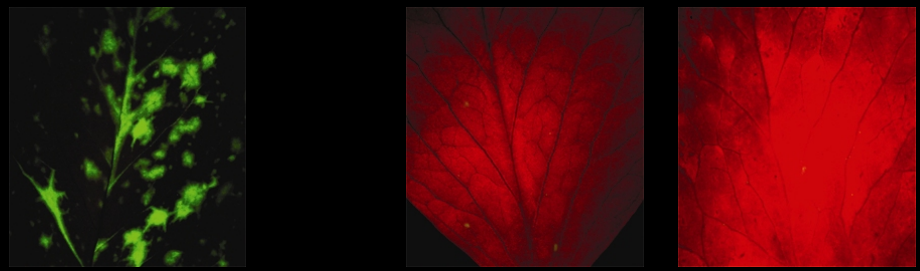


GFP tagged PPV 17 dpi (non-inoculated leaf)



Same mutation detected in an apricot cultivar, ‘NJA2’, under test for resistance to PPV

GFP tagged PPV 25 dpi (non-inoculated leaf)



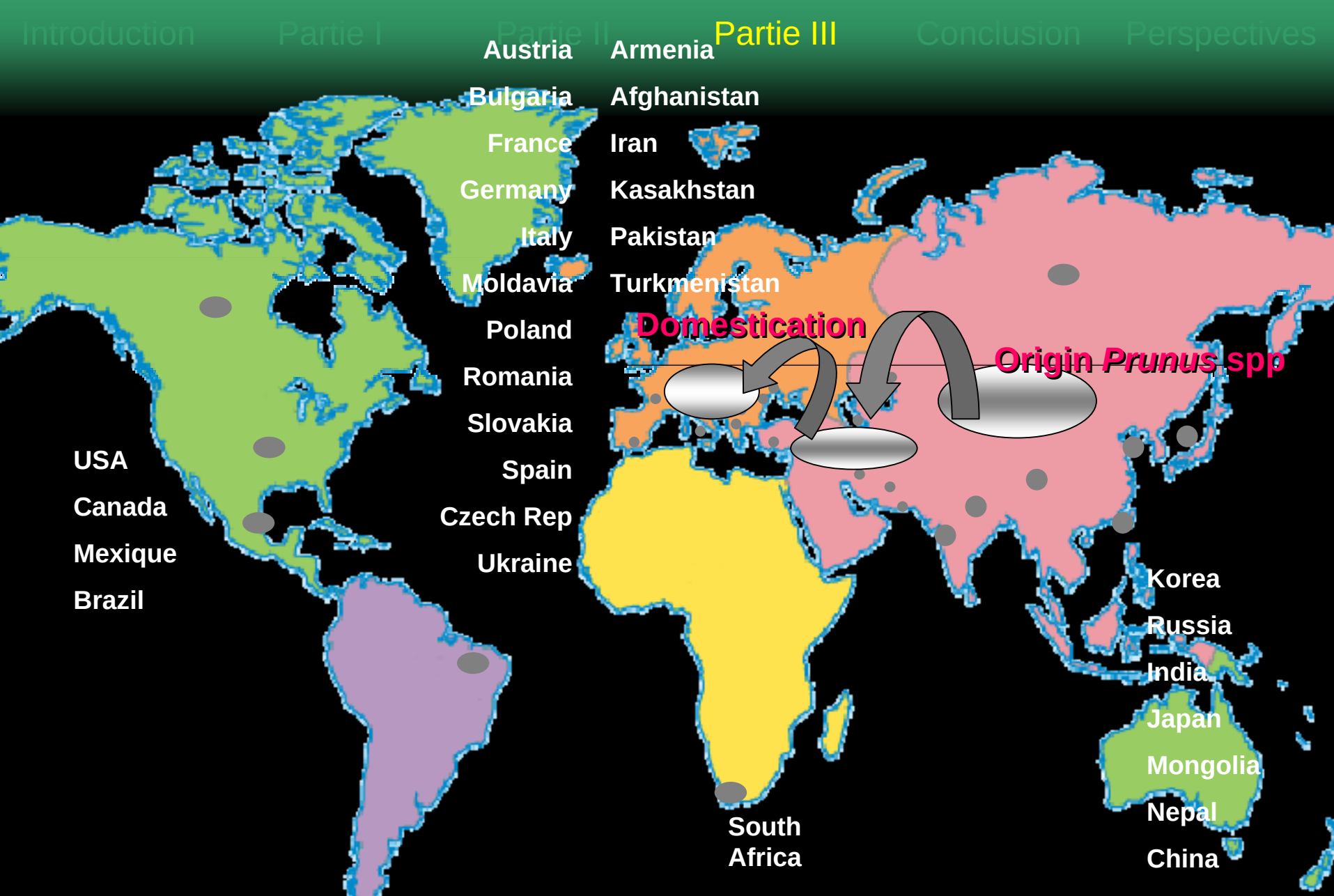
Over 500 *Prunus* individuals screened, originating from all over the world (Germplasm collections)

```
418 EEVDEADEIWGVGASVRNMLDKLDLCTRKAGHDVAWM-------ESKEMINVIINSHCYYHE 582
    E+ DEADEI GV ASVR DKL L T+ A ++ A M +KE+I+ V +H+
122 EQFDEADEICGVVASVRQRQ DKLSLWTKNAANEAAQ MSIGRKWKEIIDVTDKITYNFHD 180
```

Resistance tests currently going on in the greenhouse in Bordeaux for:

13 peach genotypes
13 Apricot genotypes
11 Almond genotypes

grafted on 'GF305' PPV infected rootstocks in 2008-2009, in 5 replicates, currently in the first cycle of phenotyping observation



Next step : Sampling extra material in Caucasian and Mid-Asian regions (IRSES European project, STONE)

Perspectives

Use of biotechnology to develop molecular tools to contain the Sharka disease

*** High-through put tools for early detection of new PPV outbreaks**

*** Implementation of Marker Assisted Selection for resistance to PPV in EU**

STONE

*** Identification of new genitors for pyramiding in breeding programmes and a durable resistance to PPV**

Collaborations

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