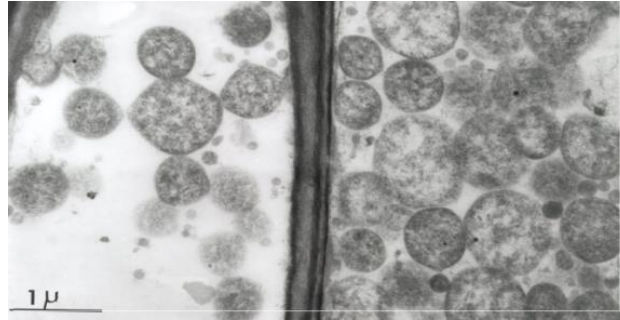


Contrasting susceptibilities to Flavescence dorée in wild *Vitis* species, *Vitis vinifera* cultivars and progenies suggest segregation of genetic traits involved in disease response

S. Eveillard, C. Jollard, F. Labroussaa, D. Khalil, M. Perrin, D. Desqué, P. Salar, F. Razan, C. Hévin, L. Bordenave, X. Foissac, J. E. Masson, S. Malembic-Maher



Grapevine Flavescence dorée (FD)



Grapevine

Phytoplasma

Scaphoideus titanus



Grapevine susceptibility to Flavescence dorée (FD) in vineyards

Difference in susceptibility to FD is well described in vineyards

- percentage of infected stocks
- symptom severity
- recovery

Susceptibility/ recovery	<i>Vitis vinifera</i>
Less susceptible/ good recovery	Cot, Merlot , Syrah
Susceptible/ recovery	Cabernet Franc, Carignan, Cinsault, Colombard, Gamay, Mourverdre, Muscat, Pinot Noir
Highly susceptible	Alicante bouschet, Aramont, Baco 22A, Carbernet Sauvignon , Chardonnay, Grenache B, Grenache N, Sauvignon B, Ugni B

Symptom severity	Rootstocks (<i>Vitis sp. hybrids</i>)
No symptom	5BB , 41B , 161.49C, 5C
Low index	101.14MG, 125AA, SO4, 110R, 140Ru
Both indexes	3309C, Fercal, 420 A

Boudon-Padieu 1996

Are these differences observed in vineyards related to phytoplasma titers?

Grapevine susceptibility to FD in vineyards

Two *V. vinifera* cultivars emblematic of Bordeaux



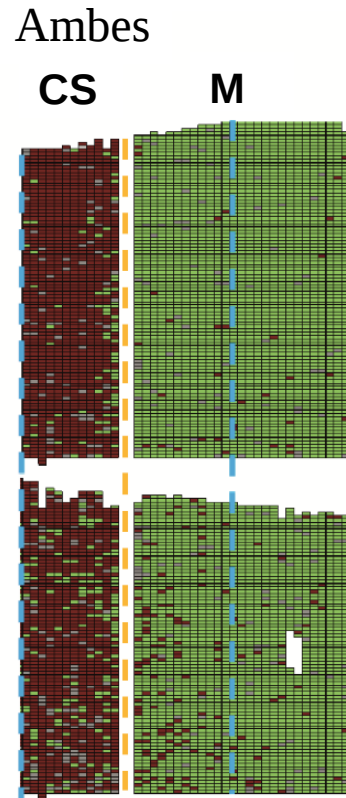
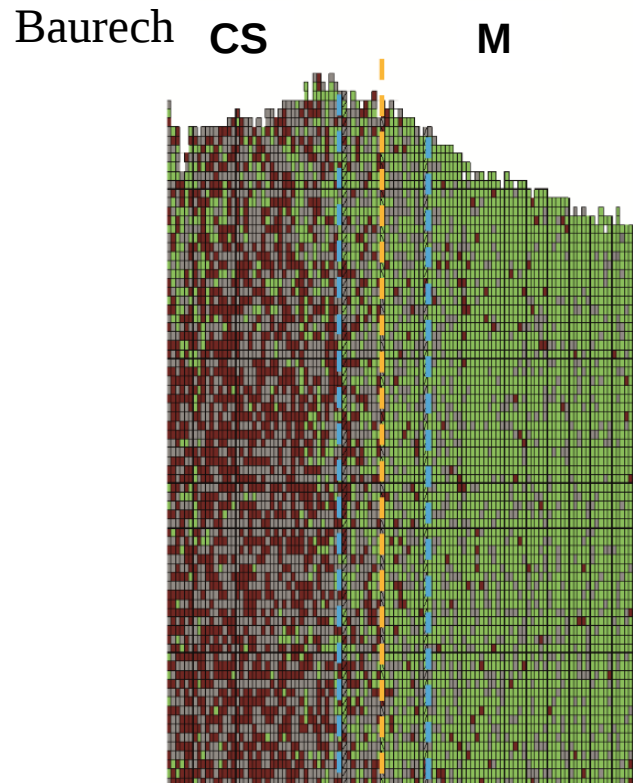
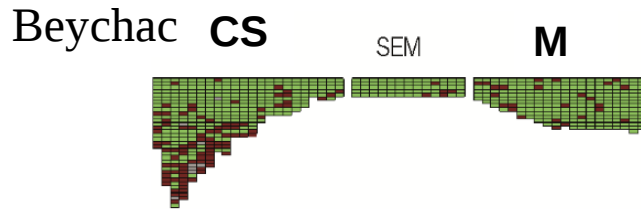
Cabernet Sauvignon
susceptibility +++



Merlot
susceptibility +

Comparison of the susceptibility to FD of Cabernet Sauvignon (CS) and Merlot (M) in vineyard

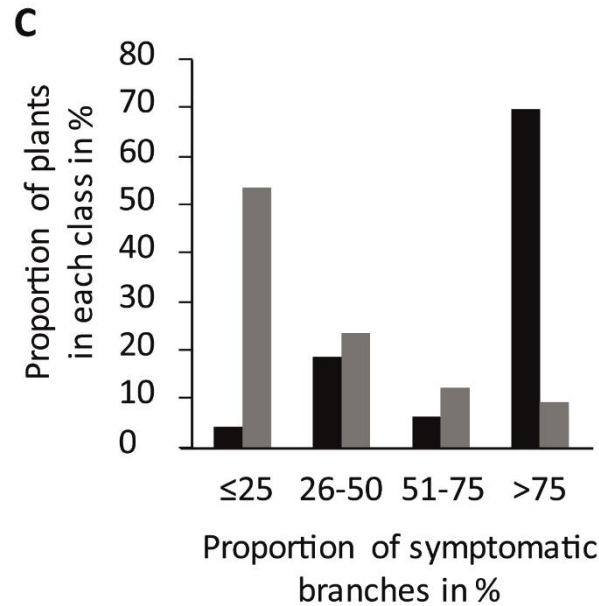
3 vineyard plots from Bordeaux area with CS and M growing side by side and with a FD outbreak



-  Vine with symptoms (38% of CS and 3% of M)
-  Vine without symptoms
-  Missing vine

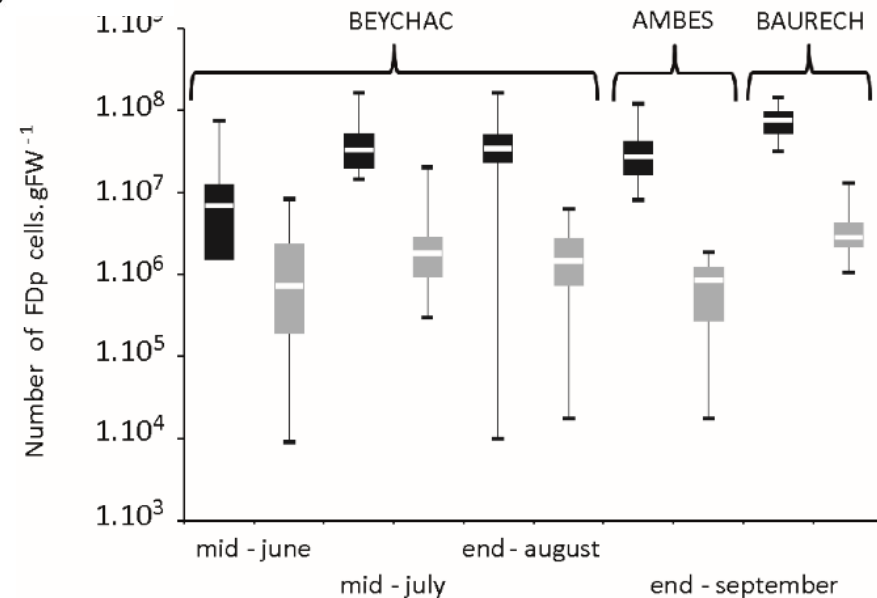
M showed less symptomatic stocks

Comparison of the susceptibility to FD of Cabernet Sauvignon (CS) and Merlot (M) in vineyard

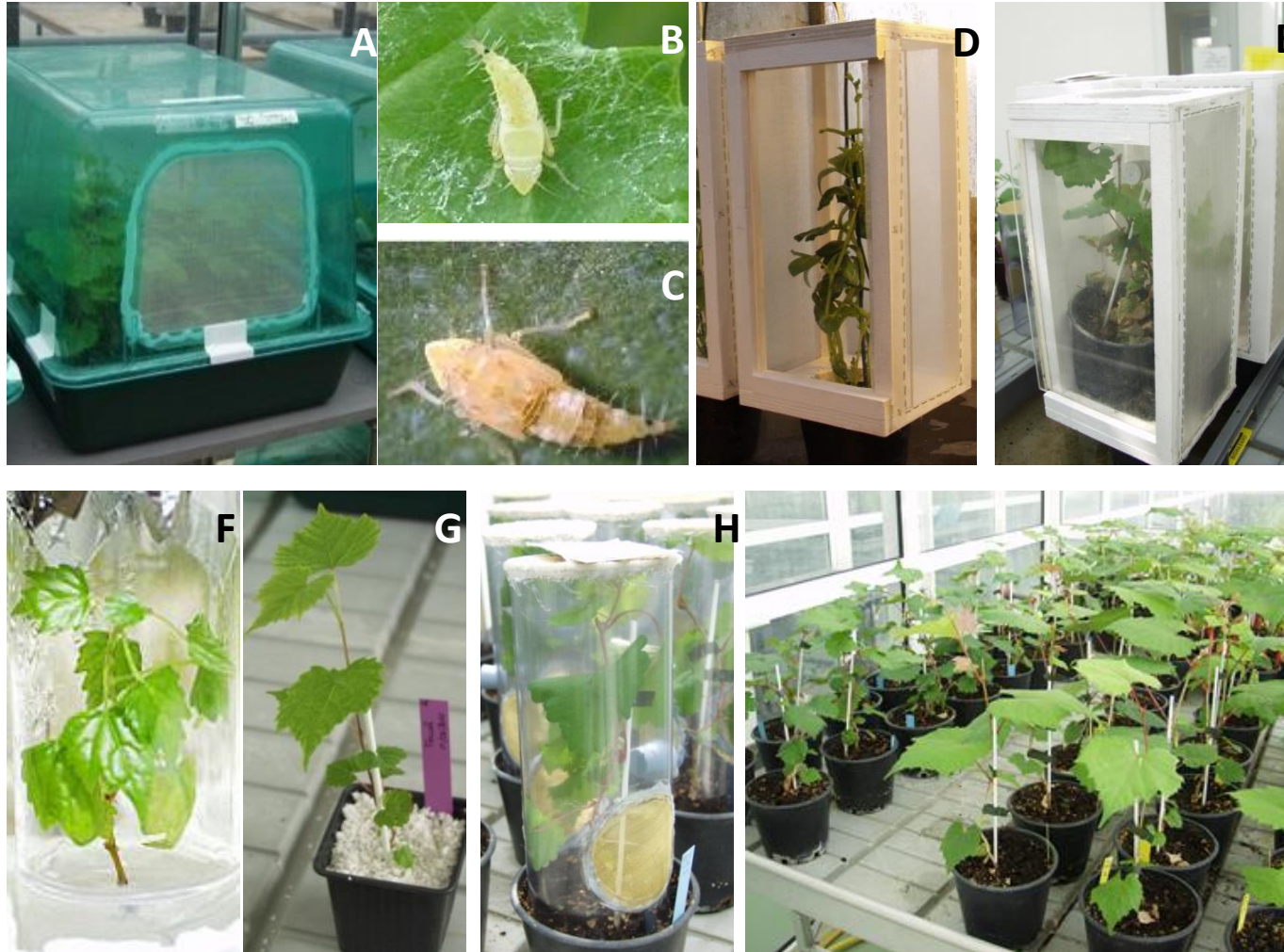


M showed less symptomatic branches

M showed lower FD phytoplasma (FDp) titers



Innovative protocol of controlled FD transmission by *S. titanus* in confined greenhouse allowed the characterization of the response to FD in *Vitis*



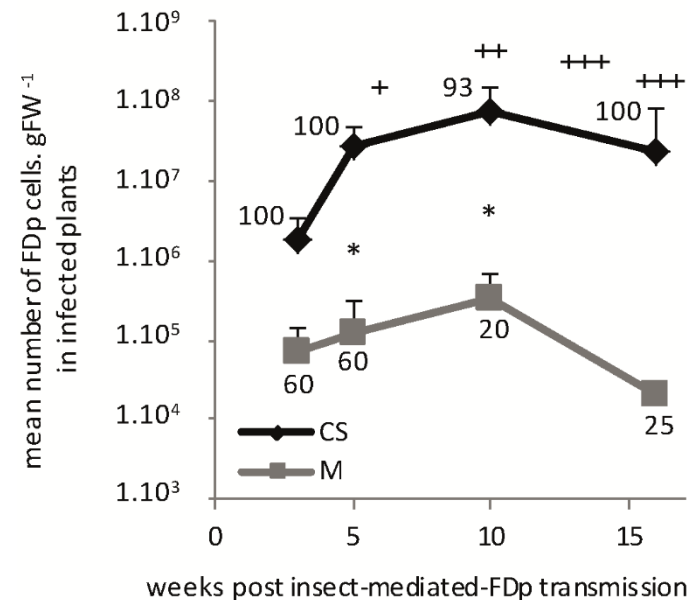
24–32-week protocol

A–C: hatching of *S. titanus* from grapevine canes collected in the vineyard, 6–8 weeks.
D: FDp acquisition by L3–L5 *S. titanus* larvae on FDp-infected broad bean, 1 week.
E: latency period on grapevine, 3–4 weeks.

F–G: acclimatisation of grapevine *in vitro* plantlets, 8 weeks.
H: FDp transmission to grape plants by infectious *S. titanus*, 1 week.
I: incubation of plants, 5–10 weeks, until sampling for FDp quantification and symptoms evaluation.

Comparison of the susceptibility to FD of Cabernet Sauvignon (CS) and Merlot (M) in greenhouse

Time course : determination of FDp titers in M and CS

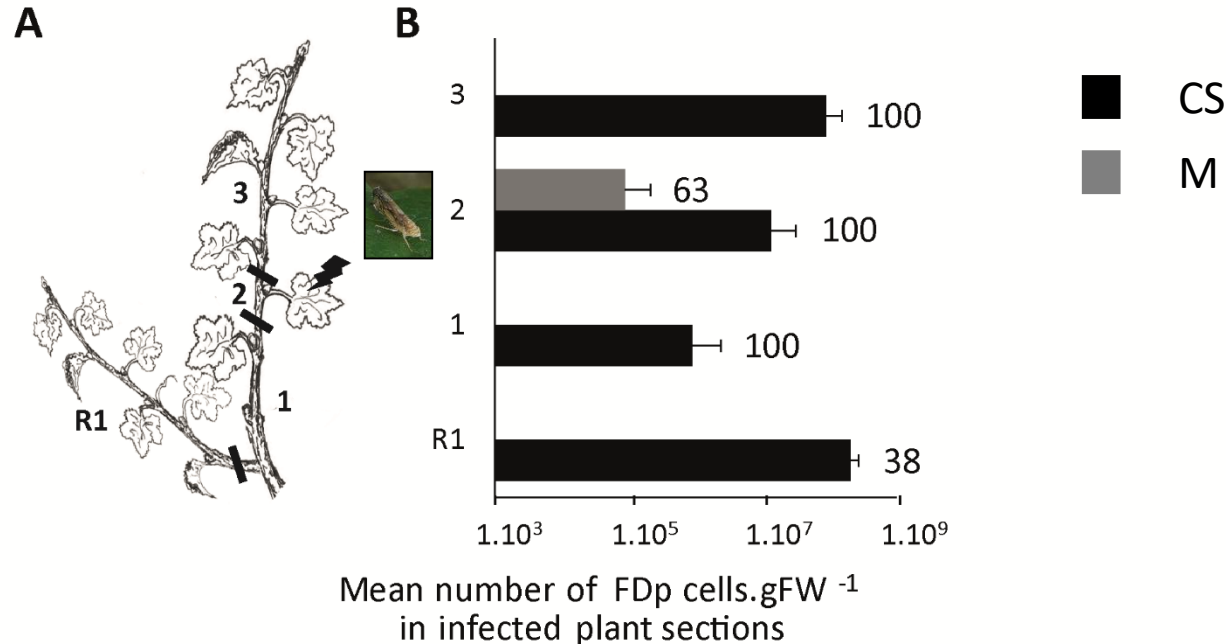


In greenhouse, FDp titers were lower for M (resembling data obtained in vineyards)

Comparison of the susceptibility to FD of Cabernet Sauvignon (CS) and Merlot (M) in greenhouse

Diffusion: inoculation on one leaf

- All CS sections were infected
- in M only the section including the leaf that received the infectious *S. titanus* was positive with low mean FDp titers

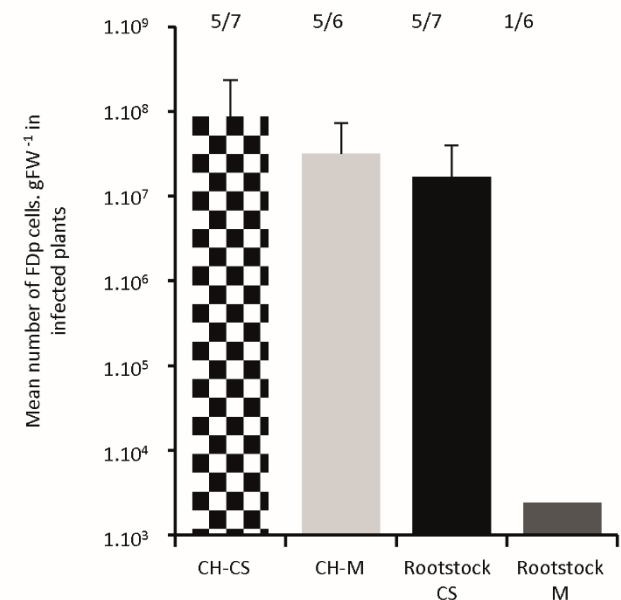
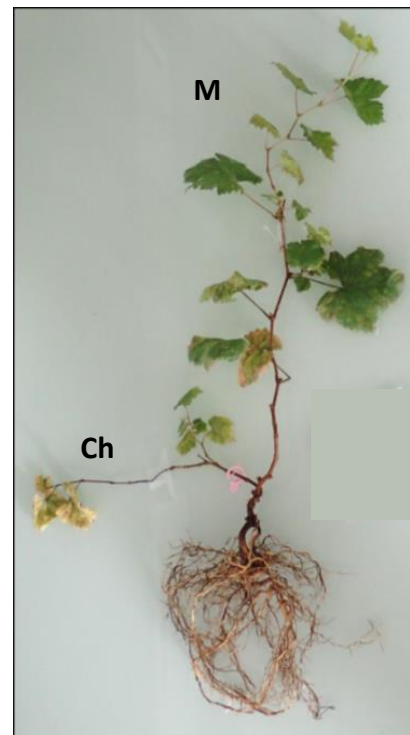
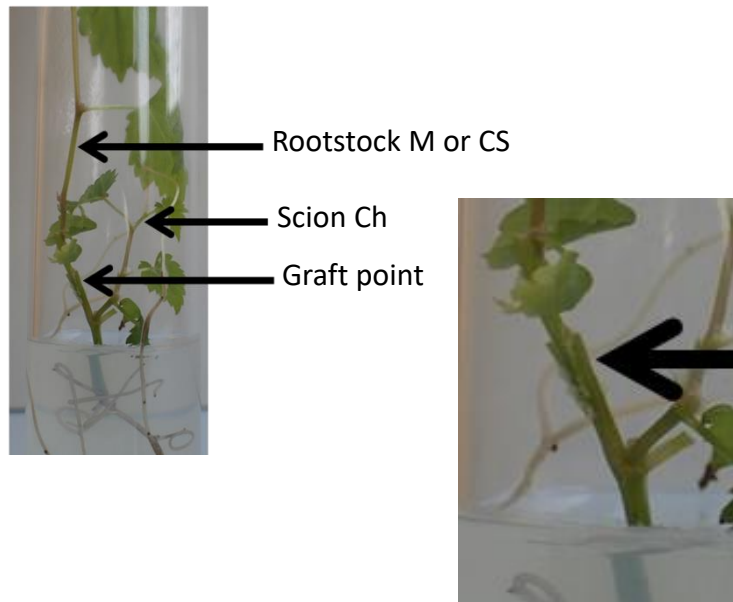


Diffusion of FDp is limited to the site of infection in M

Comparison of the susceptibility to FD of Cabernet Sauvignon (CS) and Merlot (M) in greenhouse

Response results from plant-insect or FDp-plant interactions?

- *In vitro* grafting of a scion of Chardonnay (Ch) on CS and M
- Insect were put exclusively on scion
- All CS rootstocks were infected with high FDp titers, M remained FDp free or occasionally with a very low FDp titer



M resistance to FD can be explained by a plant-FDp specific response

Experimental infections of different cultivars with FD

Plant inoculation
with FD-infected *S. titanus*



Incubation of plants
(25° C, 10 weeks)



Size of vines: 20-30 cm

9 cultivars (*Vitis vinifera*)

7 hybrid rootstocks

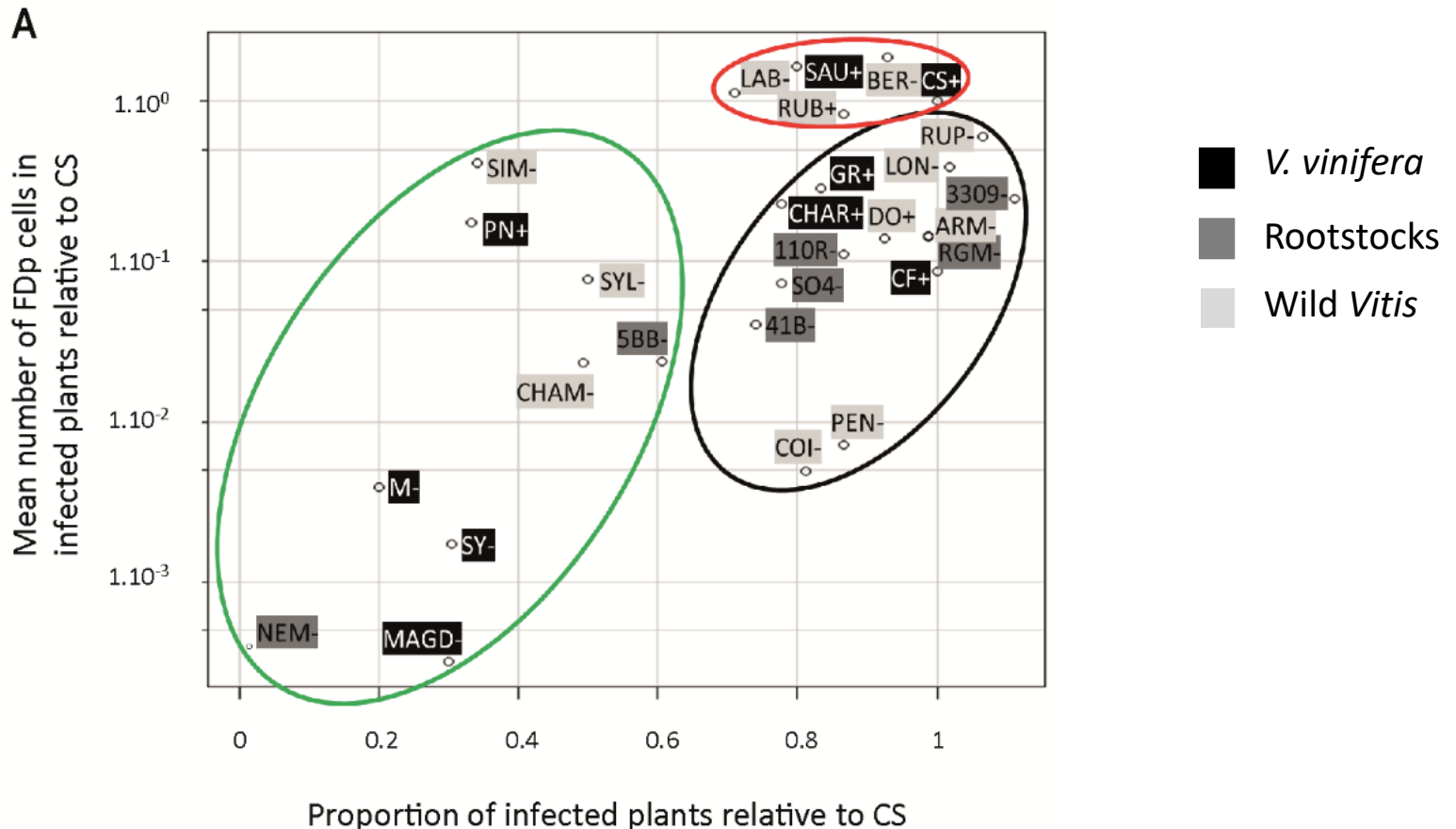
12 *Vitis* sp. from America
and Asia (Wild *Vitis*)

- Cabernet Sauvignon as control
- Symptoms evaluation
- Phytoplasma quantification (QPCR)

Responses to FD infection in a collection of *Vitis*

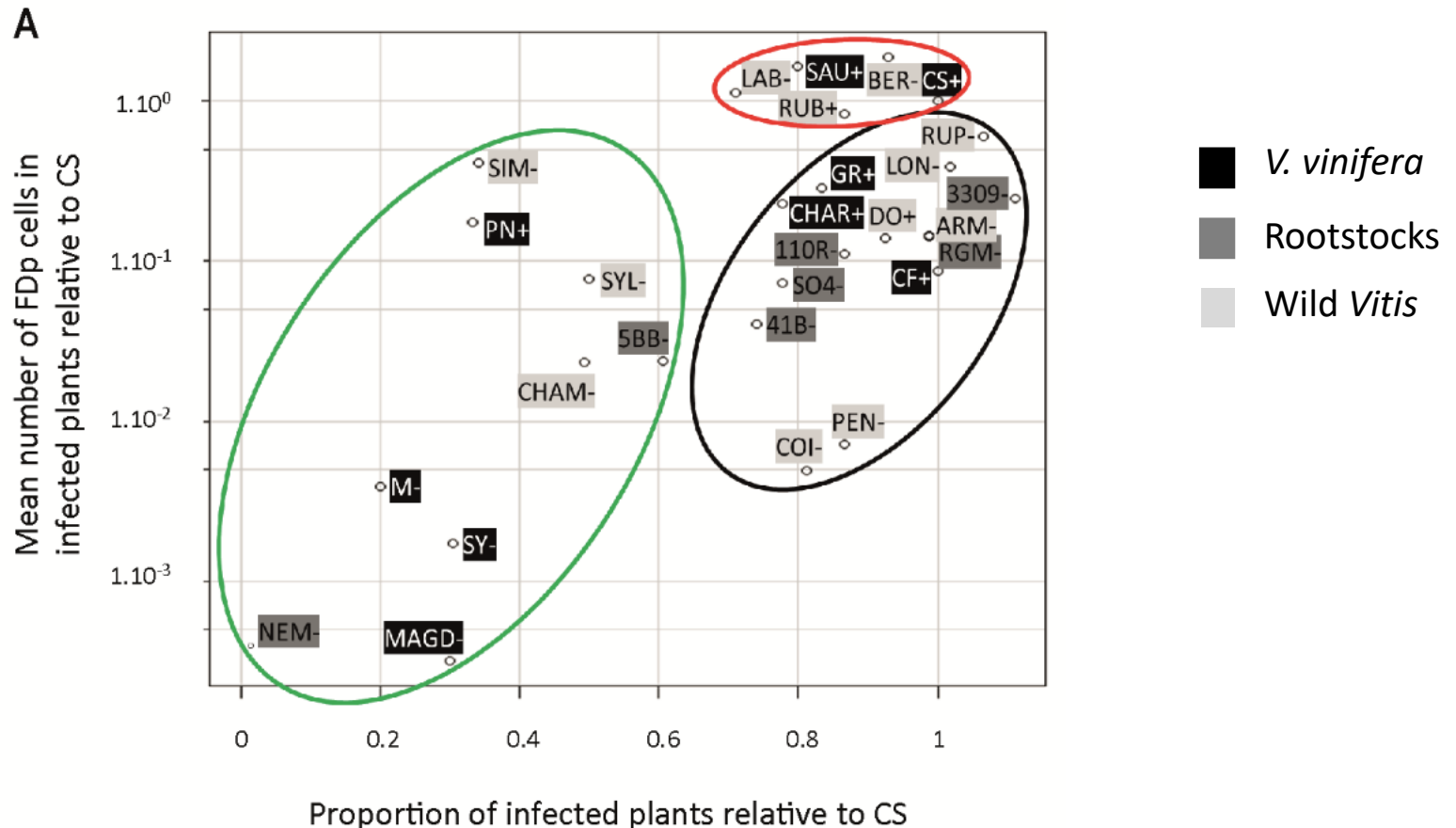
Collection of 28 *Vitis* was phenotyped

- organized in 3 categories:
 - high FDP titers and high proportion of infected plants (red)
 - intermediate FDP titers and high proportion of infected plants (black)
 - intermediate to low FDP titers and low proportion of infected plants (green)



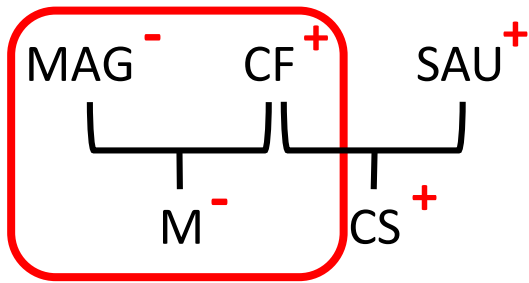
Responses to FD infection in a collection of *Vitis*

- **Rootstocks** : symptomless with intermediate to high FDp titers: warning, silent reservoir
- ***Vitis vinifera*** in the 3 categories and correspond to the field observations
- No **Wild *Vitis*** showed any resistance to FD

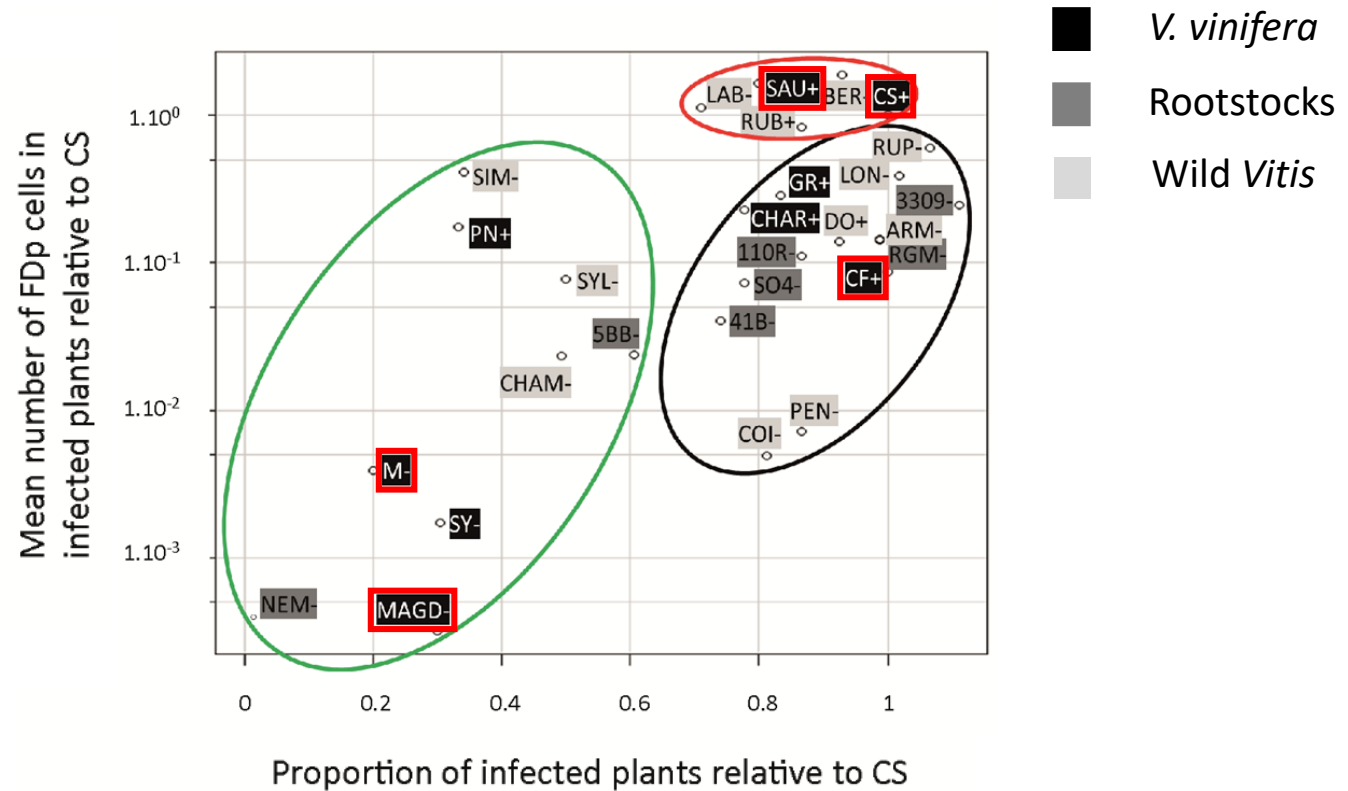


Experimental infections of different cultivars with FD

- Parents of M and CS are Magdeleine Noire des Charentes (Mag), Cabernet Franc (CF) and Sauvignon (Sau) have different susceptibility to FDP



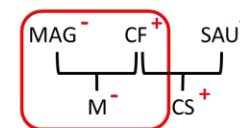
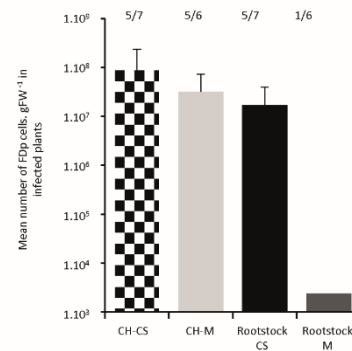
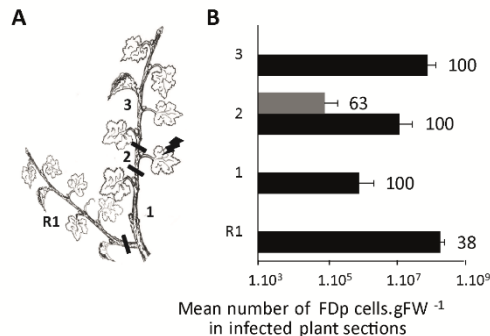
Boursiquot et al., 2009



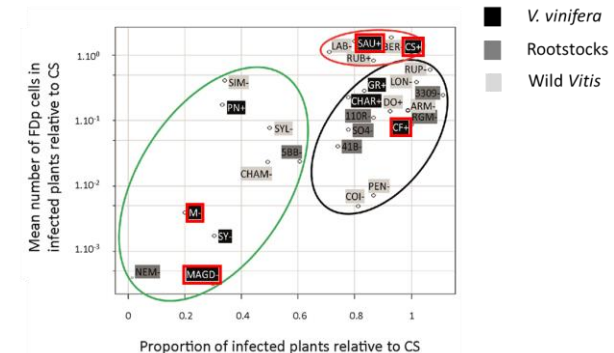
Experimental infections of different cultivars with FD

Distribution of different genetic traits within the *Vitis* genus involved either in

- insect-mediated phytoplasma transmission,
- multiplication,
- circulation,
- symptom development.



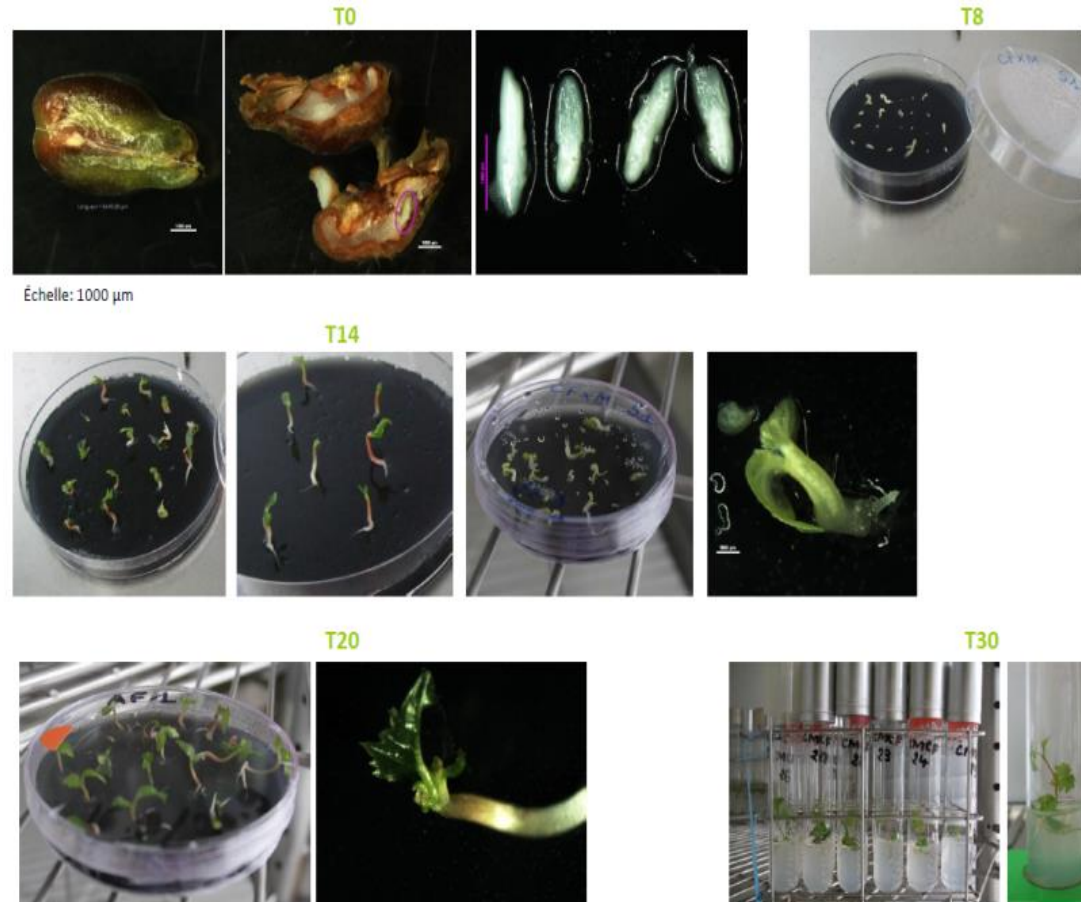
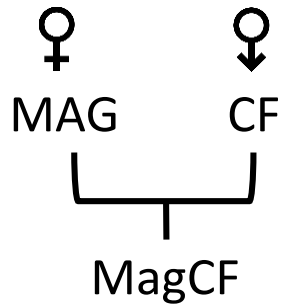
Boursiquot et al., 2009



-> cross

Cross between M's parents: Mag and CF

- Crosses were made in Bordeaux, Montpellier and Cognac
- Embryo rescue allowed to obtain seedlings from seeds in only 30 days



30 days from seeds to seedlings with the embryo rescue
(M. Perrin and J. Masson, INRA Colmar)

Phenotyping of M's brothers for FDp susceptibility

- Preliminary data after progeny phenotyping show
 - Progeny is distributed in the 3 groups, highly susceptible, poorly susceptible and intermediate
 - Identification of genotypes with resistance equivalent to Mag or M.

This suggests a segregation in the genetic traits concerning FDp resistance and a quantitative determinism

- **Next:**

- Phenotyping of CFxMag progeny (maternal heredity)
- Phenotyping of MagxMag progeny

- **In the long-term:**

- Increase our phenotyping capacity (by doing less repetition)

Thank to

- All the authors
- My colleagues from the mollicutes team
- The conservatoire du vignoble charentais for providing the cultivar Magdeleine Noire des Charentes
- Conseil Interprofessionnel du Vin de Bordeaux, France Agrimer, the regional council of Aquitaine and INRA which funded this research



Thank you for your attention