RÉPUBLIQUE FRANÇAISE
Liberté
Égalité
Fraternité

INRAE

FRANCE22
FRENCH PRESIDENCY OF THE COUNCIL OF THE EUROPEAN UNION

Reduction of pesticide use in vineyards by producing disease-resistant vines

French presidency of the Council of the European Union
Visit of the European Ministers of Agriculture and of the European Commissioner for Agriculture

INRAE Grand Est-Colmar

Theme of the visit: Reduction of pesticide use in vineyards by producing disease-resistant vines

Issue 1

Like all perennial crops, viticulture is particularly sensitive to pests. *Making vines naturally resistant to fungal infections, especially downy and powdery mildew, the two main vine leaf diseases, can have a strong effect on reducing the overall use of pesticides in France.*

Issue 2

Resistance can be achieved by crossing naturally resistant vines (genetic factor) with vines dedicated to wine production. Stability through the introduction of several genetic resistance factors against each targeted pathogen is a factor as important as the level of resistance (as per an OIV resolution [1]) and the organoleptic quality of the final product.

1. International Organisation of Vine and Wine, Resolution OIV-VITI 515 of 2013.

Summary: results at INRAE Grand Est-Colmar

INRA launched the RESDUR (Sustainable Resistance) programme in 2000, led by INRAE Research Director Didier Merdinoglu. The goal was to develop a range of varieties that are durably resistant to downy and powdery mildew, the two diseases responsible for the vast majority of phytosanitary treatments in viticulture.

RESDUR is based on crossing American and Asian wild *(Vitis sp.)* vines that carry resistance factors with European varieties (*Vitis vinifera*[2]).

In order to avoid resistance breakdown, each RESDUR variety carries at least two genes of resistance against each of the two fungi, downy mildew and powdery mildew.

This specific approach is based on a three-stage selection process: **early selection** by MAS (marker-assisted selection = PCR screening), **intermediate selection** based on a network of trials at INRAE experimental units, and **final selection** to assess the agronomic, technological and environmental value in collaboration with the Institut français de la vigne et du vin (IFV). The process takes a total of 15 years[3].

In 2018, RESDUR enabled the registration in the French catalogue of Artaban, Floreal, Vidoc and Voltis,[4] varieties with high resistance to downy and powdery mildew. **Artaban and Vidoc are co-owned with the Julius Kuhn Institute in Siebeldingen, Germany,** which has been an important partner, particularly for the launch of the programme.

Result: a reduction of over 90% in phytosanitary treatments for these four varieties

Tangible proof of this reduction in fungicides was made possible by OSCAR, an observatory for the implementation of resistant grape varieties created in 2017. OSCAR monitors over 30 sites around France and works with the chambers of agriculture, IFV, technical agricultural colleges and wine organisations. **INRAE Colmar hosts one of these sites.**

How do these varieties taste? Watch Jamie Good's blog on YouTube https://www.youtube.com/watch?v=ifcrapyACdY.

Having obtained the **proof of concept,** we are now working with the IFV to make our expertise available to the industry, for the creation of new varieties with regional characteristics using emblematic grape varieties specific to each region, such as Cabernet Sauvignon, Gewurztraminer or Chardonnay.

2. Only *V. vinifera* is used to make wine. Wild varieties carrying resistance genes are unsuitable for winemaking.
3. It takes three years to carry out the crosses, the MAS, the growing of selected vines in the greenhouse, and propagation by grafting before moving on to the field experiments. The two stages of experimentation in the vineyard, intermediate selection and final selection, each last six years (three years to reach fruiting + three years of data collection).
4. This means that any winegrower can already grow them, and ~550 ha are already planted.

How RESDUR selection is used to obtain disease-resistant vine varieties combining viticultural and oenological qualities

SOURCES OF RESISTANCE

- *Vitis sp.* genetic resources = parent stock with factors of resistance to different diseases
- Bred from several French and international (including German, Swiss, American) collections, produced for dozens of years

INRAE Colmar RESDUR ('durable resistance') Programme launched in 2000

- **STEP 1:** Among the SOURCES OF RESISTANCE, **choice of resistance parent stock** which initially present the best potential + **Molecular characterisation of resistance factors** for monitoring across generations
- **STEP 2:** Crossing of resistance parent stock with *Vitis vinifera* to obtain resistant varieties that produce high-quality wines
- **STEP 3:** Three-step selection process (total duration of 15 years):
  Early selection: only keep individuals with targeted resistance genes **(three years)**
  Intermediate selection: only keep plants with viticultural and oenological potential **(six years)**
  Final selection: selection of the best individuals for registration in the catalogue **(six years)**
- **STEP 4:** Registration in catalogue, distribution to wine growers (from year 15)

- **STEP 5:** Monitoring new varieties over time thanks to the OSCAR observatory, in operation throughout France, with assessments carried out by students from agricultural high schools, chambers of agriculture and winegrower groups under **the supervision of INRAE**

Step by step: the RESDUR process and the visit plan

STEP 1: **Selection of resistance parent stock + Molecular characterisation of resistance factors**
Use of international resistance gene banks created from the 19th century onwards, particularly the collection of the Julius Kuhn Institute in Germany and creation by the centre of new resistance parent stocks
~100 resistance parent stocks analysed, ~20 selected + ~15 created by the centre
> Production of resistant parent stock carrying two genes resistant to powdery mildew and two genes resistant to downy mildew

STEP 2: **Crossing resistance parent stock with *Vitis vinifera***

Father Y_n x Mother X_n — **STATION 1**
59 crosses
85,000 manual emasculations
or 15,000 emasculations / work cycle

4,000 seeds harvested / work cycle
Representative of genetic diversity
Only 2,000 will germinate

STATION 2

STEP 3 **Selection of individuals with the desired resistance + viticultural and oenological properties**

2,000 seedlings

1) Early selection
Objective: only keep seedlings carrying the four targeted resistance genes (i.e. 1/16)

STATION 2

Seedlings are analysed using MAS
(Marker-assisted selection = molecular analysis by PCR)

2) Intermediate selection
Objective: only keep plants with viticultural and oenological potential

STATION 3 + 4 (microvinification)

Individuals (~125/2000) are multiplied to obtain five vines/individual,
Planting in vineyards in at least two geographically distinct locations,
To produce bunches that will be vinified in test batches
Over the course of the programme ~700 individuals were subject to intermediate selection,

3) Final selection
Objective: select the best individuals for inclusion in the catalogue

STATION 4 (vinification)

83 individuals analysed in large batches for vinification, in collaboration with the IFV and its partners.
The Colmar platform carries out certain vinifications

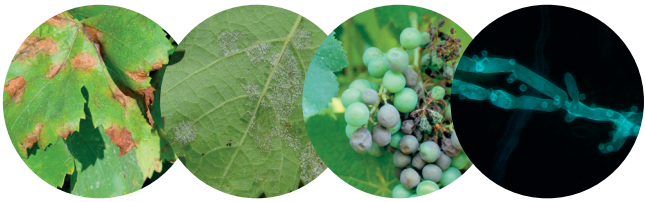
STEP 4 **Registration in the catalogue**
2018: First four varieties registered, of which two are co-owned with the Julius Kuhn Institute (Germany)
2022-2023: Five varieties registered and 2-3 forthcoming
> 2024: a dozen
In total, ~20 entries

STEP 5 **Monitoring new varieties over time thanks to the OSCAR observatory,** in operation throughout France, with assessments carried out by students from agricultural high schools, chambers of agriculture and winegrower groups under the supervision of INRAE

Symptoms caused by downy and powdery mildew, the two diseases targeted in the RESDUR programme

Downy and powdery mildew are the two vine diseases responsible for most of the phytosanitary treatments in viticulture.

Downy mildew. An oomycete fungus, **native to North America, first reported in the Bordeaux region in 1879**. Develops on all herbaceous organs of the vine.
The "oil spot" appearance of grapevine downy mildew, often observed on young leaves, is characterised by the appearance of discoloured, yellow patches on the top surface, followed by the formation of a fairly dense white down on the back of the leaf. The altered tissue turns brown and dries out.
It also attacks and dries the grapes.



Powdery mildew. Ascomycete fungus. **Powdery mildew was the first disease of American origin to be brought to Europe (1845)** and was for a long time called "the grapevine disease".
Present in all vineyards. Symptoms include oily spots (similar to those of downy mildew), and blackening of the veins (necrotic cells) on the underside. Then a greyish, dusty felting appears on the spots. The berries are initially covered with a grey, ashy dust that eventually bursts. This burst creates a gaping entry point for botrytis. Diseased bunches give off a strong mouldy smell.

Station 1
grapevine collection

Collection of American and Asian plants with resistance to downy and powdery mildew.
This plot is where the crosses are made.
If the visit had taken place in the summer, you would have seen hundreds of bagged grape bunches grown from manual crossings.



Crosses are made manually by emasculating the flowers of the female parent, i.e. removing all the male flower parts, stamens and pollen using tweezers

1 Female parent: emasculation of the inflorescence **The tips of flower buds and pollen are removed**

2 Pollen from the male parent is placed on the emasculated inflorescence of the female parent using a brush. **The inflorescence is bagged in paper to protect it from external pollen**

3 Fruit set: berries begin to grow **(six days after pollination)**

4 Unpollinated flowers fall **(15 days after pollination)**

5 Berry softening, or 'Véraison': berries soften, lignification of seeds **(69 days after pollination)**

6 Maturation: seeds are extracted from the berries and placed in cold storage **(100 days after pollination)**

Station 2
early selection

The harvested seeds are germinated to obtain seedlings.

7 Germinated seeds are transferred to stone wool blocks

8 Progeny of a cross: the selection process can begin

Each seedling is screened using **marker-assisted selection (= PCR screening).**

Objective = only keep seedlings that carry the four targeted resistance genes (two genes for resistance against powdery mildew, two genes for resistance against downy mildew).

The genetics indicate that only 1/16 of the seedlings (i.e. 1/16 of the 2,000 individuals/work cycle, i.e. ~125 seedlings) will carry the four targeted resistance genes and be included in the intermediate selection.

Station 3
preparation for intermediate selection

The seedlings chosen in the early selection process are transferred to a greenhouse allowing strong growth for the production of five vines/individuals obtained using grafting/cutting.



The branches produced will be cut in order to prepare plants intended, **after grafting**, to be planted and observed in the vineyard in intermediate selection systems.

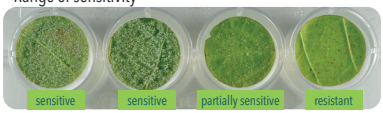
OMEGA grafting



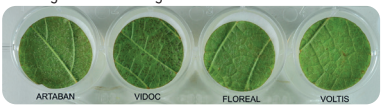
Laboratory resistance test: Resistance to downy mildew and powdery mildew can be measured first in the laboratory using simple leaf discs

Example of a downy mildew resistance test

Range of sensitivity



Testing the first four registered varieties



Station 4
intermediate selection, microvinification/final selection, vinification

In the cellar, the objective is to characterise the main oenological properties using bunches harvested from five vines for each genotype from the **intermediate selection**.

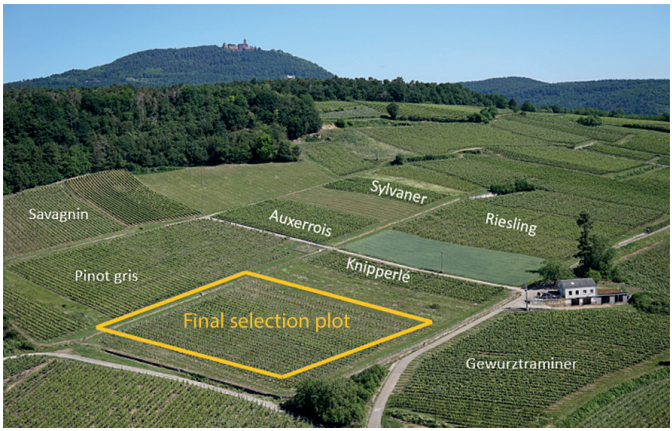
This is done four years after sowing the seeds and for three years in a row to consolidate the results.

These very small quantities of grapes are convenient for microvinification. Each year, around 200 new candidate varieties undergo this evaluation stage.

Chosen varieties go to **final selection**. For each of the varieties, 90 vines are planted in the vineyard in two geographically distinct locations, for example Alsace and Bordeaux.

This development is mainly carried out by the IFV and its partners. The wines are vinified locally and the Colmar platform takes part in the vinification of the varieties grown on site.

Process at the centre for final selection: INRAE plot in the Alsace vineyard. Plots of resistant vines are planted in the vineyard next to plots of traditional variety grapevine.



Grapevine fanleaf virus ('court-noué')
(not included in the visit due to time constraints)

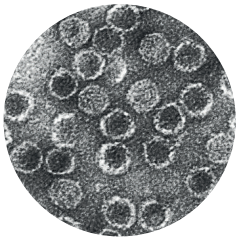
Fanleaf is a viral disease transmitted to grapevine roots by a nematode, or round worm found in soil.

This other very serious disease contributes to what is known as the 'vineyard decline' affecting all French vineyards.

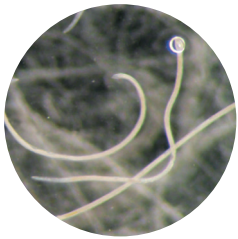
In the case of fanleaf virus, the stage of creating commercial varieties has not been reached; we are at an upstream study phase. We recently discovered in the Riesling a total resistance to fanleaf virus.

We can reasonably expect to transfer this resistance to rootstocks using the techniques developed in the RESDUR programme.

Symptoms in the vineyard of 'court-noué'
Leaves yellow and die



Grapevine fanleaf (GFLV) virus
seen under electron microscope



The vector responsible
for transmission of virus
the nematode *Xiphinema index*

We have developed a unique soil control system that lets us analyse about 900 greenhouse plants at a time.

Once installed in the boxes, we use serology to look for the virus in the leaves, similar to the antigenic tests used to detect the COVID virus.

A reliable assessment takes about three years because the transmission and progression of the virus are relatively slow.

INRAE