

Plant Health Workshop: Advances in plant disease and pest management: a holistic approach

February 28th, 2023

IRTA, Spain

➤ Breeding for resistance/tolerance in fruit crops: New trends

Bénédicte QUILOT-TURION

INRAE GAFL



➤ Major stakes for stone fruit production today



Need for low input cultivation

Treatment frequency [15-30]
High dependence to irrigation and fertilization

Varieties with low adaptation to agro-ecological transition:

Few resilient varieties
No incentive in registration procedure

Perennial crops

Health management over years
~20 years to create a new variety

Major solutions:

→ Genetic diagnostics
→ Screening plant material faster and more accurately

Potential for agro-ecology

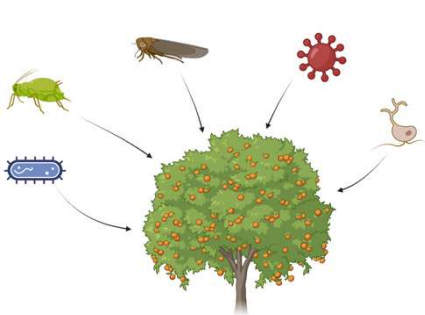
Large diversity within stone fruit
Development of agroforestry
Carbon storage in soil
Demand for organic fruits
New « eco-labels » on market



INRAE

Breeding for resistance/tolerance in fruit crops: New trends
2023/02/28 Bénédicte QUILOT-TURION

➤ The use of pesticides: a real problem in fruit production



Stone fruit trees are attacked by multiple pests and diseases



Few varieties bear resistance or tolerance genes



Phytosanitary products remain the most effective means of control



Environmental and health problems caused by pesticides

We need to find alternative solutions!

Treatment Frequency Index	
Cereals	3,9
Apple	35,9
Grape Vine	12,7
Peach	24,2

➤ Makes genetic an efficient lever for agroecology

➔ **Combining genetics and agrotechnical levers to develop agroecological, sustainable systems**

1. Mobilize the arsenal of **modern tools**
 - Sequencing, genotyping, genetic engineering
 - Digital phenotyping, NIRS
2. Ensure the **durability** of the resistance genes
3. Enlarge genetic diversity <- use **wild relatives**
4. Target new traits : eg **resilience**

Methodologies

Marker Assisted Selection
Marker Assisted Introgression
Genomic prediction
Gene editing

Phenotype characterization
Phenomic prediction



INRAE

Breeding for resistance/tolerance in fruit crops: New trends
2023/02/28 Bénédicte QUILOT-TURION

➤ Makes genetic an efficient lever for agroecology

**Climate change
Abiotic stresses**

Icons include: a water droplet, a snowflake, a thermometer with a blue liquid level, a sun, a thermometer with a red liquid level, a molecular model of NaCl, a periodic table entry for Potassium (K), and a coin with the letter N.

X

Biotic environnement

Icons include: seeds, a microscopic view of plant tissue, a green insect, and a bee on a pink flower.

Pathogens

Auxiliaries
Pollination

Genetic lever

Collections

Wild species

Icons include: a DNA double helix and various types of fruits and vegetables.

Sustainable systems

Resistance
Resilience

Icons include: a large tree with fruit, a smaller tree, and a cross-section of soil with roots and microorganisms.

**Cultural practices
Diversification**

Innovative technical itineraries

Sanitazing and companion plants

Plant defense inducers

Biocontrol

Substances naturelles, Moléculaires chimiques, Micro-organismes

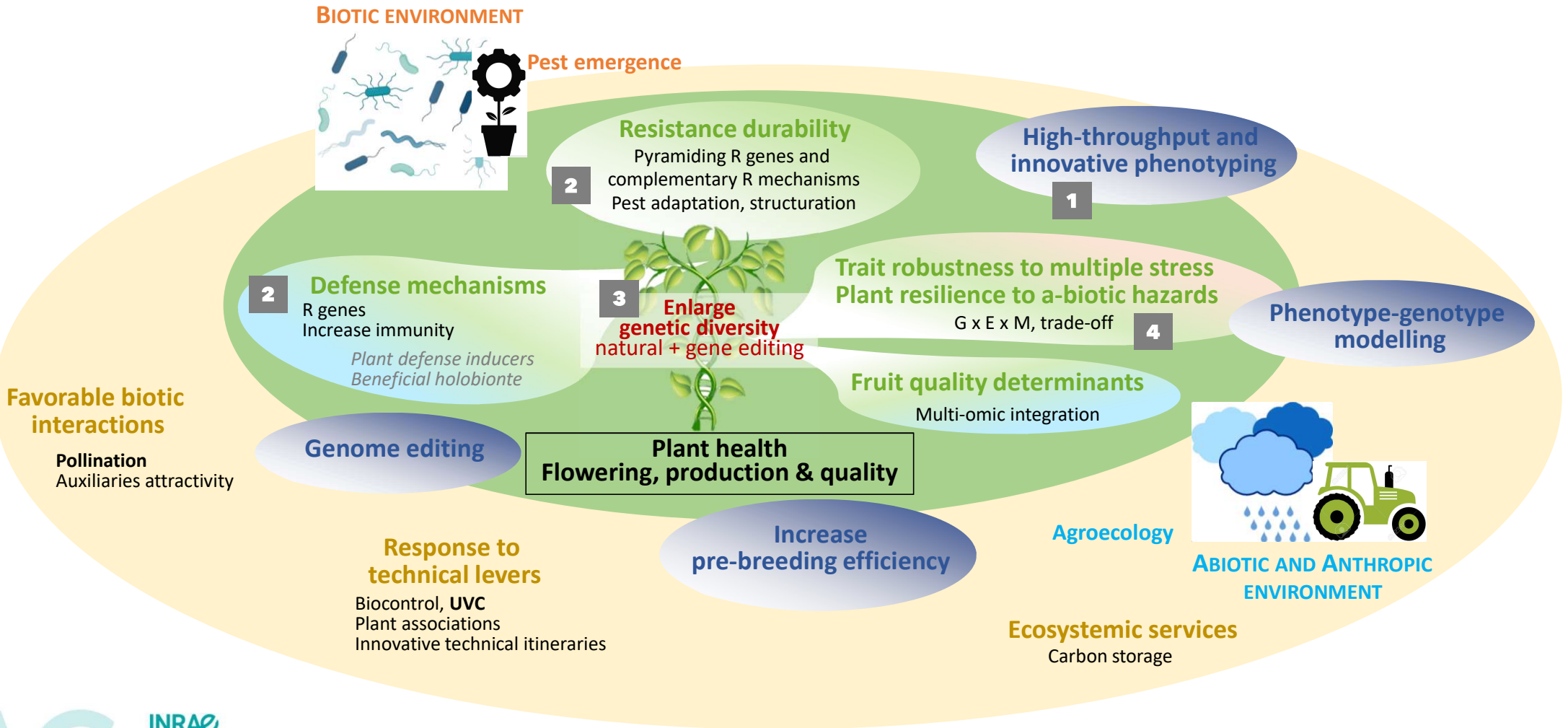
Icons include: a lavender field, a field of colorful flowers, a cartoon corn character with weights, and icons for natural substances, chemical molecules, and microorganisms.



INRAE

Breeding for resistance/tolerance in fruit crops: New trends
2023/02/28 Bénédicte QUILOT-TURION

➤ Makes genetic an efficient lever for agroecology



INRAE

Breeding for resistance/tolerance in fruit crops: New trends
2023/02/28 Bénédicte QUILOT-TURION

➤ Summary

1. Mobilize the arsenal of **modern tools**
 - Sequencing, genotyping, genetic engineering
 - Digital phenotyping, NIRS
2. Ensure the **durability** of the resistance genes
3. Enlarge genetic diversity <- use **wild relatives**
4. Target new traits : eg **resilience**



The genetician pipeline

Genetic lever

Collections

Wild species

Collections, populations

Enlarge diversity

Interspecific crosses

Sequencing Genotyping

Hundreds of thousands of identical probes per feature

Phenotyping

Traits
Methods
Tools

Genetic determinism

QTL, GWAS, coGWAS

Multi Omics data

Integration

Gene discovery

Fine mapping

Gene validation

Cloning

Genome editing

Loci & gene discovery

Ideotyping

New traits for new challenges

Trait associations

GxExM

CWR introgressions

Movement of genes from one species to another by recurrent backcrossing of hybrid to a parent

Marker-assisted selection

Marker-Assisted Selection (MAS)

Genomic selection

Genomic Prediction

Enhanced GxE modeling

Phenomic selection

Phenotyping of the target trait

Selection of type of HETs from the model

Calibration of the model

Prediction of the target trait

Advanced selection

Genome editing

Plant improvement

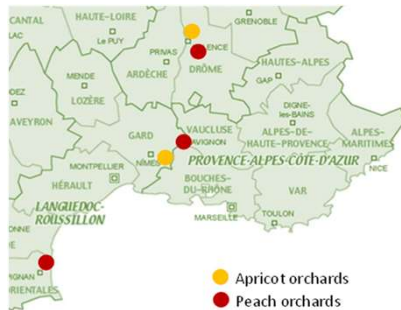
➤ Phenotyping

Controlled experiments



Emphasis platform network

Field multisite



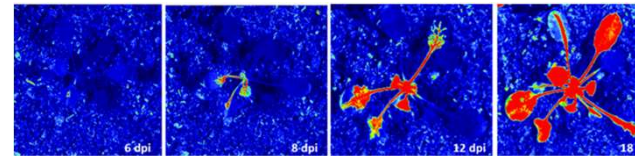
Phenotyping tools

Highthroughput phenotyping

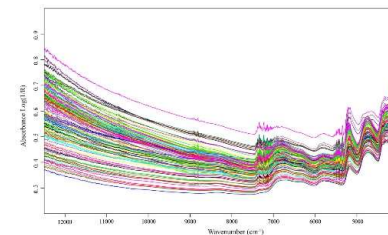


Captors for ecophysiological traits

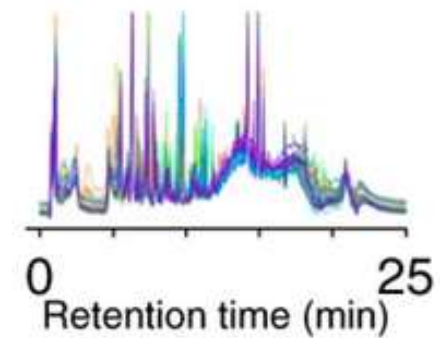
Images for symptoms / architecture



NIRS for phenomics



Metabolomics



INRAE

Breeding for resistance/tolerance in fruit crops: New trends
2023/02/28 Bénédicte QUILOT-TURION

➤ Summary

1. Mobilize the arsenal of modern tools
 - Sequencing, genotyping, genetic engineering
 - Digital phenotyping, NIRS
2. Ensure the **durability** of the resistance genes
3. Enlarge genetic diversity <- use **wild relatives**
4. Target new traits : eg **resilience**



➤ Durable management of resistance

- Resistance genes are a limited resource
- Resistance gene breakdowns have already been observed
- A breeding program is a long-term and costly process
- The question of the management of resistance durability is crucial, particularly for perennial species

Quantitative resistance (QR) : the foundation of breeding for disease resistance in crops to achieve durable resistance

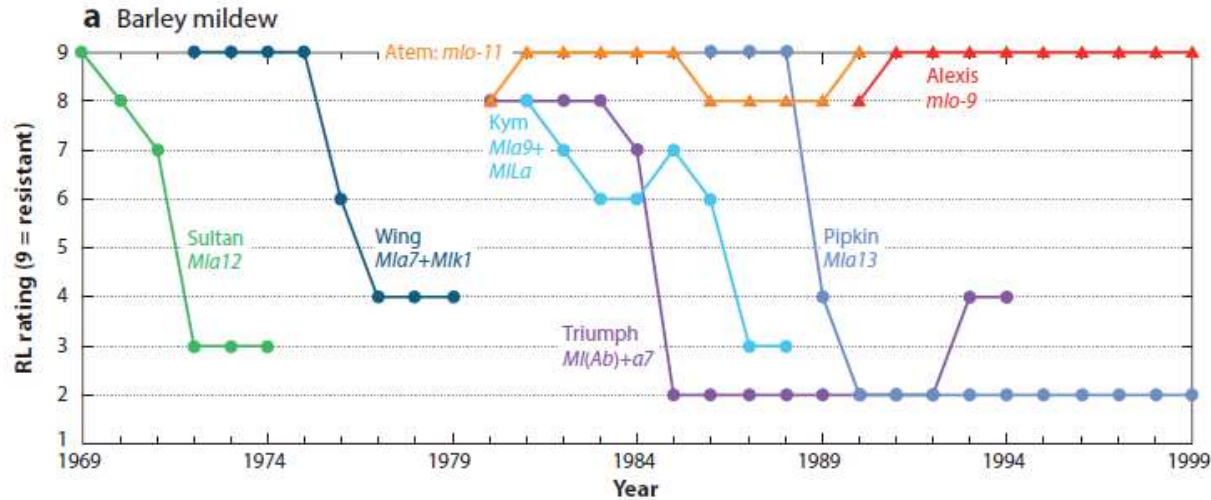
-> it remains poorly understood in comparison to the well studied gene-for-gene recognition process

Pyramiding resistance genes is an efficient strategy to increase resistance durability

Appropriate cultural practices and monitoring of variety deployment also contribute to the durability of resistance



➤ Durable management of resistance



2022

Annual Review of Phytopathology

Durability of Quantitative Resistance in Crops: Greater Than We Know?

Christina Cowger¹ and James K.M. Brown²

Durable Resistance of Crops to Disease: A Darwinian Perspective

James K.M. Brown

Annual Review of Phytopathology 2015 53:1, 513-539

Durable resistance is defined as resistance that has been effective for a relatively long time over a relatively large area where conditions are generally conducive to the disease



INRAE

Breeding for resistance/tolerance in fruit crops: New trends
2023/02/28 Bénédicte QUILOT-TURION

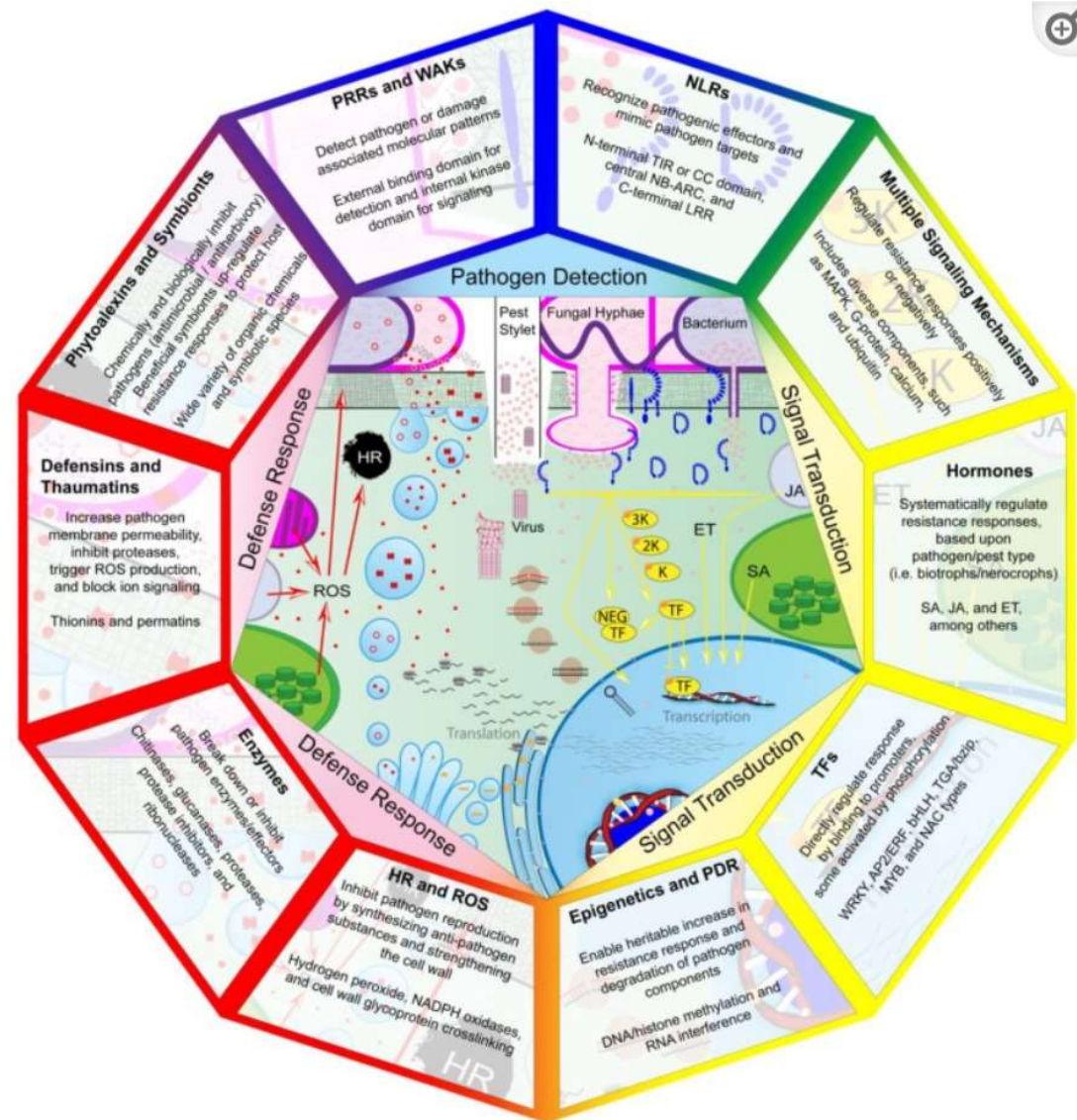
➤ Plant defense mechanisms



Review

Disease Resistance Mechanisms in Plants

Ethan J. Andersen ¹, Shaukat Ali ², Emmanuel Byamukama ², Yang Yen ¹ and Madhav P. Nepal ^{1,*}



INRAE

Breeding for resistance/tolerance in fruit crops: New trends
2023/02/28 Bénédicte QUILOT-TURION

➤ Durable management of resistance

Many Effector-Triggered Resistance genes with large effects have not been durable because of their specificity to pathogen genotypes

Specific resistances	Absolute, total, qualitative resistances	Monogenic Major gene	Prevents pathogen establishment	Triggers a strong plant response to neutralize the pathogen; not very robust Selection pressure on the pathogen selects more virulent strains: circumvention
	Partial, incomplete, quantitative resistances	Polygenic Several minor genes	Limits colonization and the impact of the presence of the pathogen	Causes disease reduction Longevity, durability of resistance
Non-specific resistances				to multiple diseases allowing plants to defend themselves against a broad spectrum of pathogens

➤ INRA-ResDur for durable resistance in grape vine

Resistance breakdown of

- Rpv3 : resistance to Plasmopara viticola (downy mildew)
- Run1 : resistance to Uncinula necator (powdery mildew)

Marker-assisted selection to stack resistance factors derived from multiple sources.

-> development of candidate cultivars two or three genes to control each disease

- Rpv1, Rpv3 and Rpv10 for downy mildew
- Run1, Ren3 and Ren3.2 for powdery mildew

INRA-ResDur: the French grapevine breeding programme for durable resistance to downy and powdery mildew

C. Schneider^a, C. Onimus, E. Prado, V. Dumas, S. Wiedemann-Merdinoglu, M.A. Dorne, M.C. Lacombe, M.C. Piron, A. Umar-Faruk, E. Duchêne, P. Mestre and D. Merdinoglu

Breeding for durable resistance to downy and powdery mildew
in grapevine

D. Merdinoglu*, C. Schneider, E. Prado, S. Wiedemann-Merdinoglu and P. Mestre

OENOne
VINE AND WINE
OPEN ACCESS JOURNAL
Volume 52 > Number 3 > 2018



INRAE

Breeding for resistance/tolerance in fruit crops: New trends
2023/02/28 Bénédicte QUILOT-TURION

➤ Summary

1. Mobilize the arsenal of modern tools
 - Sequencing, genotyping, genetic engineering
 - Digital phenotyping, NIRS
2. Ensure the durability of the resistance genes
3. Enlarge genetic diversity <- use **wild relatives**
4. Target new traits : eg **resilience**



➤ Enlarge genetic diversity : use wild relatives

2017

frontiers
in Plant Science

REVIEW
published: 04 April 2017
doi: 10.3389/fpls.2017.00460

Check for updates

Exploiting Wild Relatives for Genomics-assisted Breeding of Perennial Crops

Zoë Migicovsky* and Sean Myles

2022

Scientia Horticulturae 295 (2022) 110801

Contents lists available at ScienceDirect

ELSEVIER

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Exotic genes for solving emerging peach production challenges

Thomas M. Gradziel

Check for updates

2022

CellPress
OPEN ACCESS

Trends in
Biotechnology

Feature Review

Reap the crop wild relatives for breeding future crops

Abhishek Bohra ¹, Benjamin Kilian ², Shoba Sivasankar,³ Mario Caccamo ⁴, Chikelu Mba,⁵ Susan R. McCouch ^{6,*} and Rajeev K. Varshney ^{7,8,*}

2023

plants

MDPI

Review

Crop Wild Relatives: A Valuable Source of Tolerance to Various Abiotic Stresses

Aliki Kapazoglou ¹, Maria Gerakari ², Efstathia Lazaridi ², Konstantina Kleftogianni ², Efi Sarri ², Eleni Tani ^{2,*} 
and Penelope J. Bebeli ^{2,*}



INRAE

Breeding for resistance/tolerance in fruit crops: New trends
2023/02/28 Bénédicte QUILOT-TURION

➤ INRAE peach resistance program : green aphid and powdery mildew

2003

Theor Appl Genet (2003) 107:227–238
DOI 10.1007/s00122-003-1238-8

M. Foulongne · T. Pascal · P. Arús · J. Kervella

The potential of *Prunus davidiana* for introgression into peach [*Prunus persica* (L.) Batsch] assessed by comparative mapping

Trait/Resistance	Organism	Pathogen	Inoculation	Resistance/tolerance	Inheritance	Gene name	LG	Reference
<i>P. davidiana</i>								
Powdery mildew	Fungi	<i>P. pannosa</i>	Natural	Tolerance	Quantitative	QTLs	1, 2, 4, 6, 8	Foulongne et al. (2003)
Sharka	Virus	<i>Plum pox virus</i>	Controlled	Tolerance	Quantitative	QTLs	1, 2, 4, 5, 6,7	Rubio et al. (2013)
Green peach aphid	Insect	<i>Myzus persicae</i>	Natural	Tolerance	Quantitative	QTLs	1, 2, 3, 4, 5,6, 8	Sauge et al. (2012)
Root-knot nematode	Nematode	Meloidogyne spp.	Natural	Tolerance	Major gene		Not-mapped	Reighard and Loreti (2008)
Brown rot	Fungi	Monilinia spp.	Controlled	Tolerance	Quantitative		Not-mapped	Pascal et al. (1998)

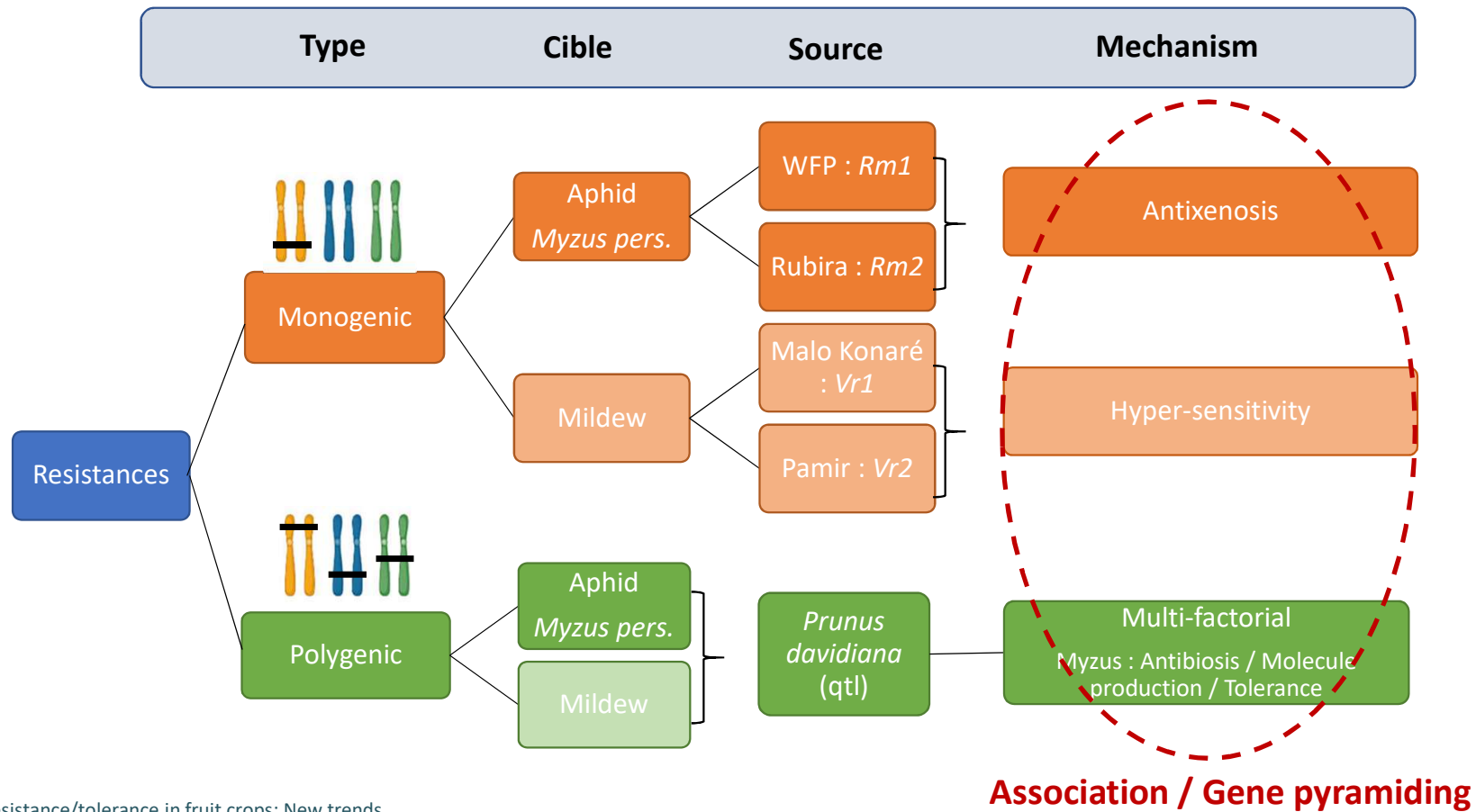


INRAE

Breeding for resistance/tolerance in fruit crops: New trends
2023/02/28 Bénédicte QUILOT-TURION

➤ INRAE peach resistance program : green aphid and powdery mildew

Major genes associated to minor genes for resistance durability



INRAE

Breeding for resistance/tolerance in fruit crops: New trends
2023/02/28 Bénédicte QUILOT-TURION

➤ Incorporation of resistance genes from wild species : a promising option

Aranzana et al. *Horticulture Research* (2019)6:58
<https://doi.org/10.1038/s41438-019-0140-8>

2019

Horticulture Research
www.nature.com/hortres

REVIEW ARTICLE

Open Access

Prunus genetics and applications after de novo genome sequencing: achievements and prospects

Maria José Aranzana¹, Véronique Decroocq², Elisabeth Dirlewanger², Iban Eduardo¹, Zhong Shan Gao³, Ksenija Gasic⁴, Amy Iezzoni⁵, Sook Jung⁶, Cameron Peace⁶, Humberto Prieto⁷, Ryutaro Tao⁸, Ignazio Verde⁹, Albert G. Abbott¹⁰ and Pere Arús¹

identify the valuable germplasm

very little information about resistance sources in non-crop *Prunus* relatives

-> systematic survey of *Prunus* wild relatives around the world

-> characterization of their **phenotype** for various resistance traits

-> introgression of the genetic factors linked to resistance into elite *Prunus* cultivars through interspecific crosses

The construction of a *Prunus* pan-genome would promote and accelerate the development of **appropriate genomic tools** for all *Prunus* species, including wild relatives



INRAE

Breeding for resistance/tolerance in fruit crops: New trends
2023/02/28 Bénédicte QUILOT-TURION

➤ Development of interspecific lines and molecular tool

2016

Tree Genetics & Genomes (2016) 12: 96
DOI 10.1007/s11295-016-1056-1



ORIGINAL ARTICLE

Marker-assisted introgression (MAI) of almond genes into the peach background: a fast method to mine and integrate novel variation from exotic sources in long intergeneration species

Octávio Serra¹ · José Manuel Donoso^{1,2} · Roger Picañol^{1,3} · Ignasi Batlle⁴ · Werner Howad¹ · Iban Eduardo¹ · Pere Arús¹

Horticulture
Research

2022

Article

Construction of a collection of introgression lines of “Texas” almond DNA fragments in the “Earlygold” peach genetic background

Naveen Kalluri¹, Octávio Serra², José Manuel Donoso³, Roger Picañol⁴, Werner Howad^{1,5}, Iban Eduardo^{1,5*} and Pere Arús^{1,5*}

HORIZON-CL6-2023-BIODIV-01-13 / Crop wild relatives for sustainable agriculture

WP1	CWR inventory and knowledge
WP2	CWR genotypic characterisation
WP3	Phenotypic characterisation
WP4	HTP genotyping of CWR core-collections
WP5	Genomics-based methods to integrate CWR in pre-breeding programs
WP6	Promotion and plans for sustainable conservation of CWR in situ populations



INRAE

Breeding for resistance/tolerance in fruit crops: New trends

2023/02/28 Bénédicte QUILOT-TURION

➤ An endless work, long and laborious

	# pathogens	# studied	included in breeding programs
Peach	70	9	Powdery mildew, brown rot, leaf curl, Bacterial spot, PPV, green peach aphid, root-knot nematodes
Cherry	61	2	Monilinia, bacterial canker
Apricot	59	5	Monilina, Bacterial canker, PPV, Apricot chlorotic leafroll phytoplasma

source WOS

Few resistance genes known
Old and emerging diseases
Too many traits to target one by one



INRAE

Breeding for resistance/tolerance in fruit crops: New trends
2023/02/28 Bénédicte QUILOT-TURION

➤ Summary

1. Mobilize the arsenal of modern tools
 - Sequencing, genotyping, genetic engineering
 - Digital phenotyping, NIRS
2. Ensure the durability of the resistance genes
3. Enlarge genetic diversity <- use wild relatives
4. Target an integrative new trait : eg **resilience**



➤ Why is resilience so relevant ?

Convergent selection targets in animals and plants

Farming challenges

- Resistances et tolerances to a large number of diseases and parasites

- Reduction of antibiotic/parasitic molecules

Examples of biomarkers of resilience:

- Milk production (ex. Elgersma et al. 2018)
- Blood tests (ex. Bai et al. 2020)
- Diet (ex. Putz et al. 2019)



Crop challenges

- Resistances et tolerances to a large number of diseases and parasites

- Reduction of pesticides

Biomarkers of resilience not defined yet:

- Aerial or root biomass?
- Photosynthetic activity?
- Evapo-transpiration?



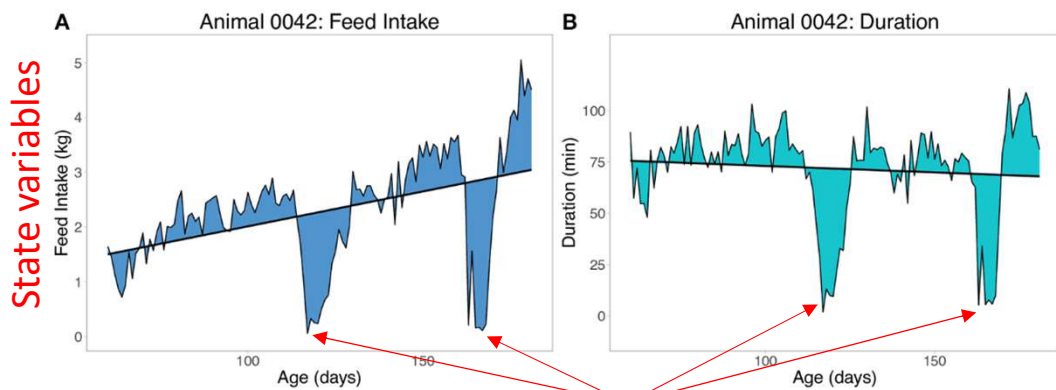
➤ Disease-resilience in animals : one step ahead?

« Polymicrobial natural disease model » versus « disease by disease »



Novel Resilience Phenotypes Using Feed Intake Data From a Natural Disease Challenge Model in Wean-to-Finish Pigs

Austin M. Putz¹, John C. S. Harding², Michael K. Dyck³, F. Fortin⁴, Graham S. Plastow³, Jack C. M. Dekkers^{1*} and PigGen Canada¹

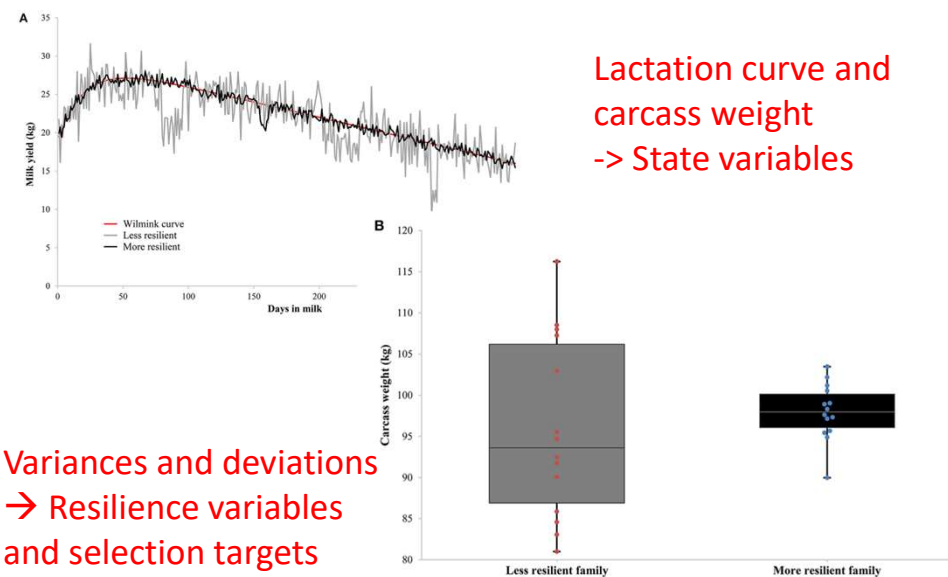


Sick animal



Opportunities to Improve Resilience in Animal Breeding Programs

Tom V. L. Berghof*, Marieke Poppe and Han A. Mulder



Lactation curve and carcass weight
→ State variables

Variances and deviations
→ Resilience variables
and selection targets



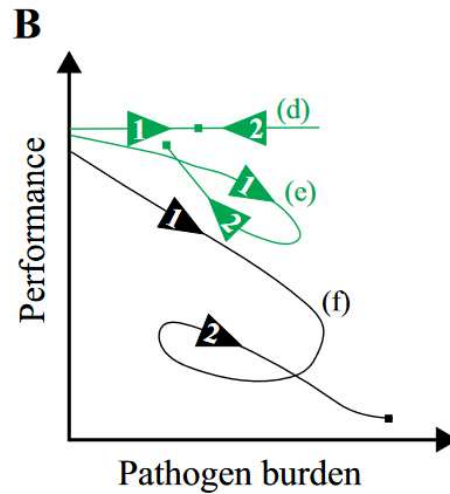
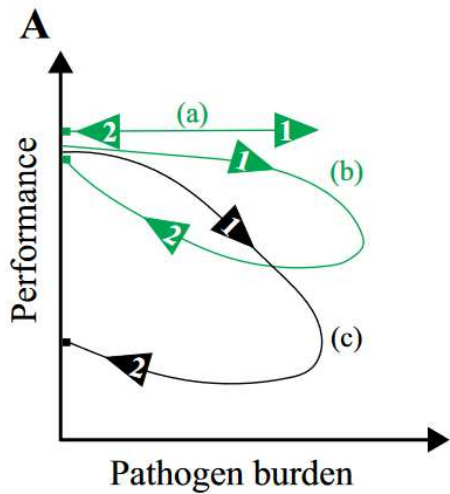
INRAE

Breeding for resistance/tolerance in fruit crops: New trends
2023/02/28 Bénédicte QUILOT-TURION

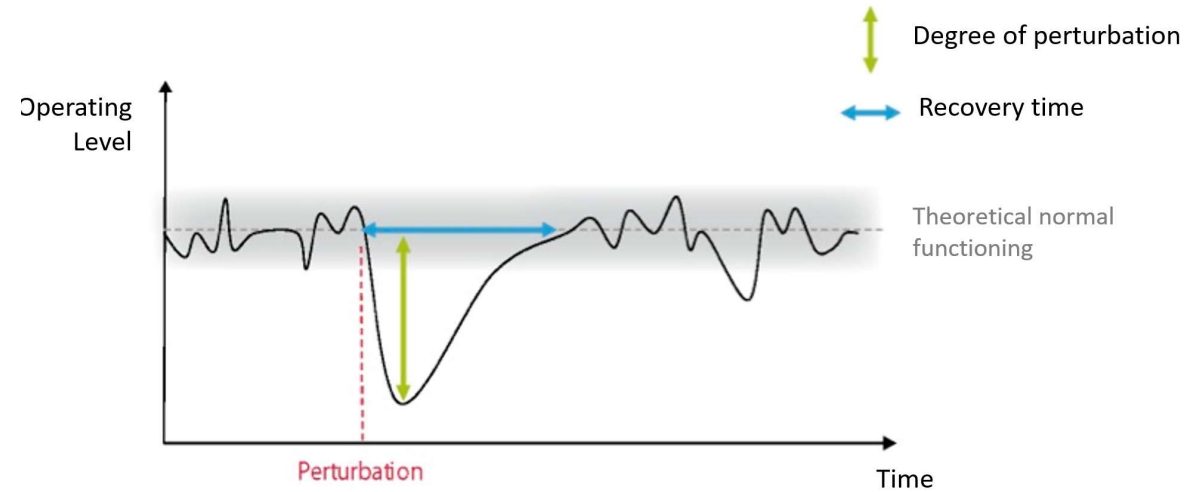
A source of inspiration

➤ Breeding for resilience : a durable way to manage the biotic pressures

— Resilient organism
— Non-resilient organism



(Bai and Plastow, 2022)



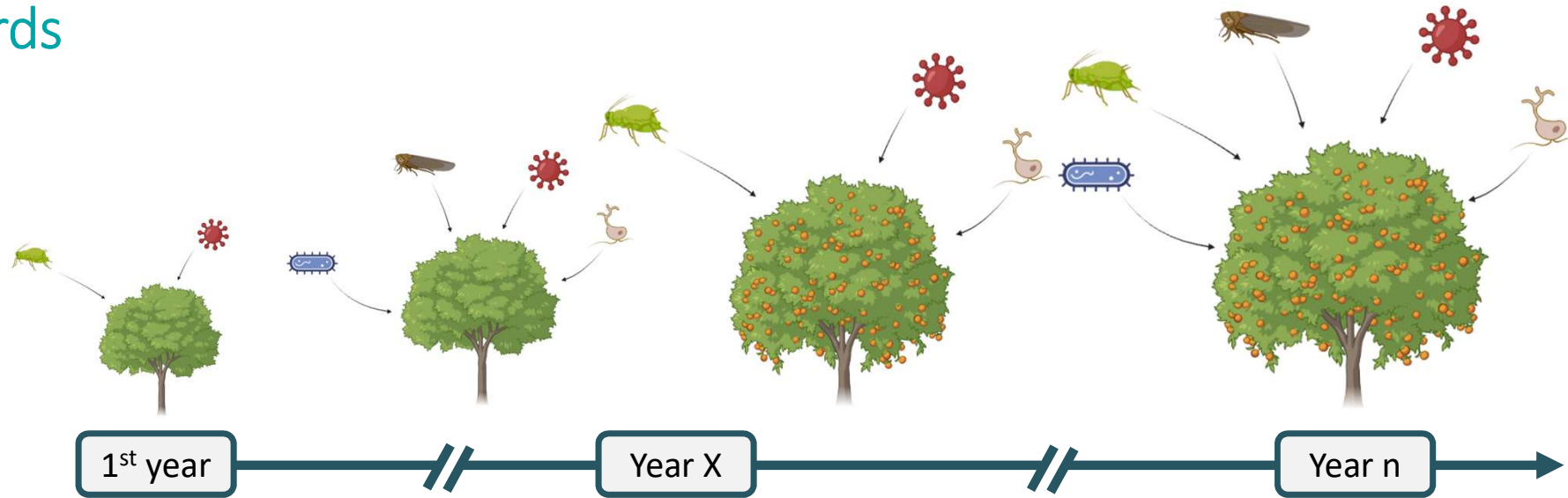
(Toubin et al. 2021)

RESILIENCE : the capacity of an organism to be minimally affected by disturbances or to rapidly return to the state pertained before exposure to a disturbance

➤ How to quantify resilience: multi-year and integrative monitoring of orchards

Multi-year monitoring of orchards

Pests/diseases damages

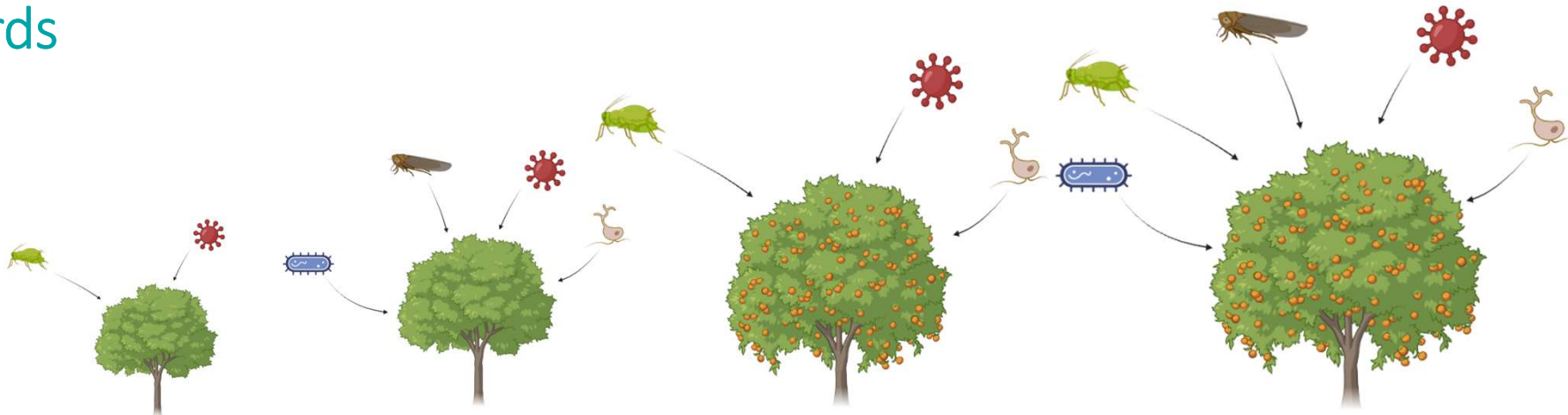


What are the impacts of these biotic stresses on tree health?
How to take these data into account in an integrative way?

➤ How to quantify resilience: multi-year and integrative monitoring of orchards

Multi-year monitoring of orchards

Pests/diseases damages

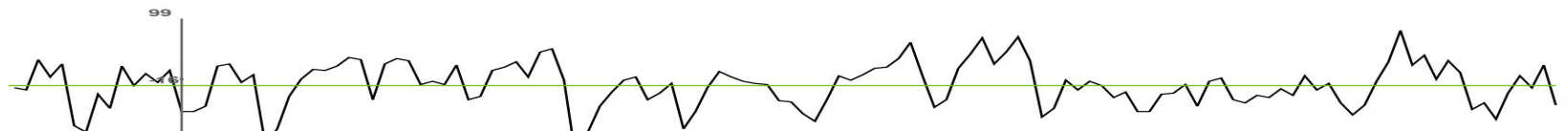


1st year

Year X

Year n

Vegetative growth



Production



Photosynthetic activity



Indicators of tree health: **Biomarkers of resilience**




INRAE

Breeding for resistance/tolerance in fruit crops: New trends
2023/02/28 Bénédicte QUILOT-TURION

> Approach


Uncover the fundamental principles of resilience in stone fruit trees

- Multi-trait observations among diversity panels
- Interactions between pest/diseases
- Quantify the impact of biotic stresses on tree health/vigor over the years
- Quantify risk factors with epidemiological covariables
- Summarizing multiple observations of symptoms into resilience metrics



Find genetic markers associated to disease resilience and/or resilience components (resistance/tolerance)

- Identify inheritance modes of resilience sub-trait and integrative traits
- Impact of the genetic, environment, G x E interactions effects
- Identify best-suited GWAS for multi-trait and multi-environment data



Explore the applications of the methods developed in the project for future disease-resilience breeding

- Defining “resilient ideotypes” as a new target for pre-breeding activities
- Discuss the feasibility of MAS for resilience traits
- Compare resilience characteristics between peach and apricot



➤ Plant material: multi-species core collections with a large genetic diversity

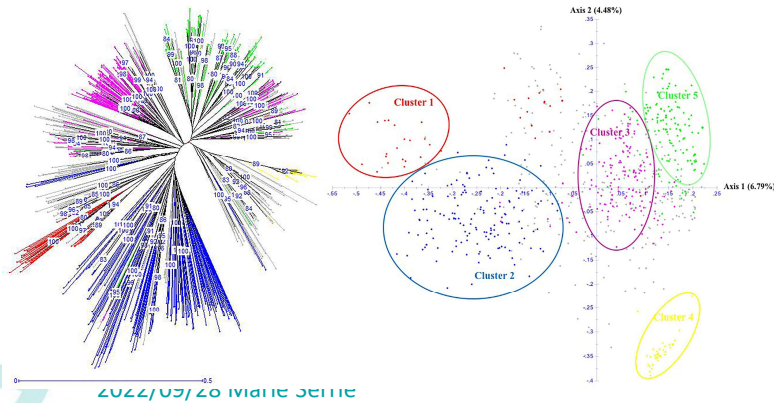


Apricot cultivated (*P. armeniaca*)

150 accessions
Resequenced with Illumina HiSeq 2000 NGS

Selecting accessions

- ✓ Genetic variability of 25 Microsatellites (*Bourguiba et al., 2012*)
- ✓ Within the five known genetic groups (*Bourguiba et al., 2020*)
- ✓ Incl. of modern accessions and hybrids from recombinant lines between phylogroups



2022/03/20 14:16:33

Breeding for resistance/tolerance in fruit crops: New trends

2023/02/28 Bénédicte QUILOT-TURIGNON
From Bourguiba et al. (2020)

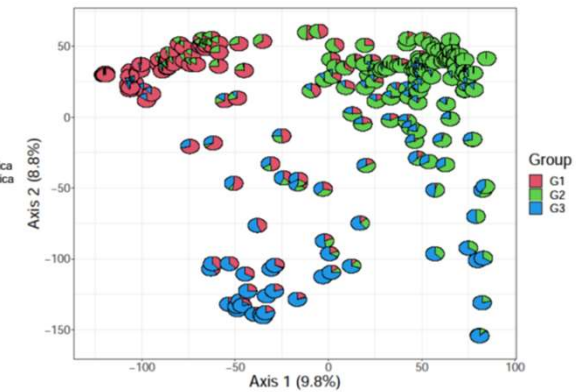
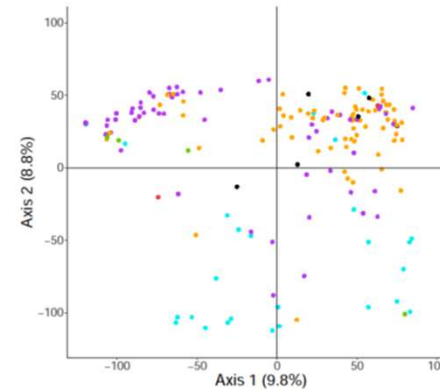


Peach cultivated and wild (*P. persica* + relatives)

206 accessions
Genotyped with 16k SNP chip

Selecting accessions

- ✓ SNP polymorphism with 9K chip (*Micheletti et al 2015*)
- ✓ Incl. of related species and interspecific
- ✓ 3 genetics groups
- ✓ Several geographical origin

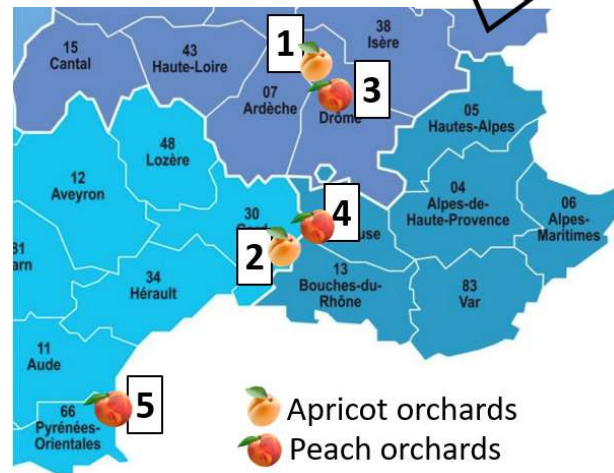


➤ Experimental design: multi-site with low pesticide conditions



Apricot cultivated (*P. armeniaca*)

5 single-shaft randomized blocks
4 to 5 years old orchards

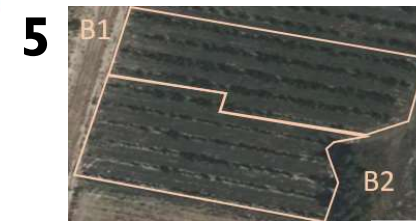


Apricot orchards
 Peach orchards



Peach cultivated and wild (*P. persica* + relatives)

1 or 2 single-shaft randomized blocks
3 years old orchards



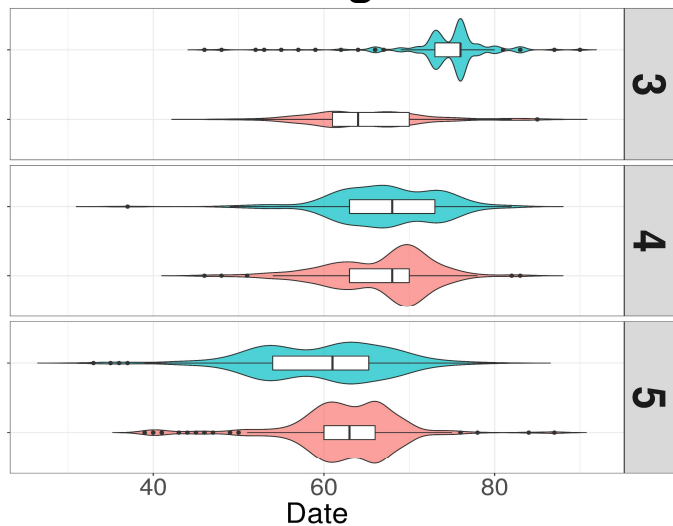
Management method:

Low and targeted phytosanitary protection



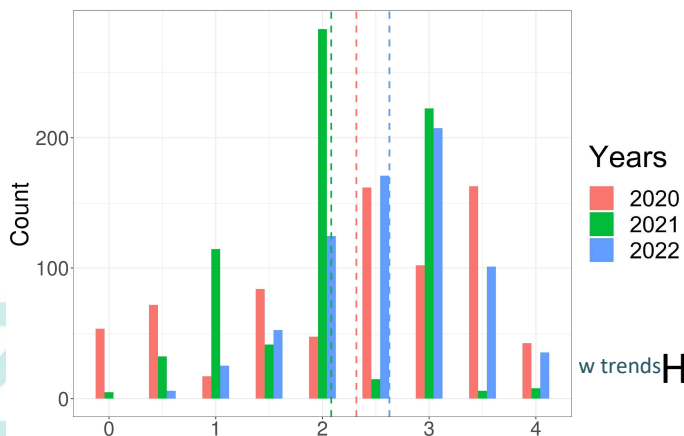
➤ A broad phenological diversity in the core collections

Blooming date



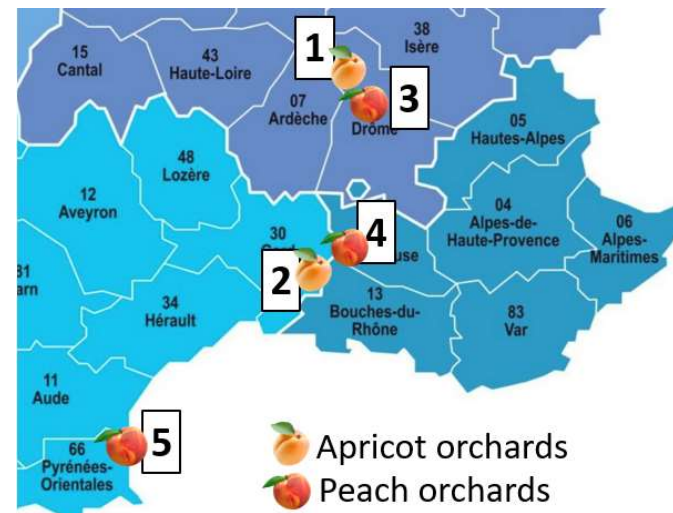
$H^2 : 0,74$



Flower density



w trends $H^2 : 0,63$

	2021	2022	
	0.672*	0.433*	2020
		0.475*	2021

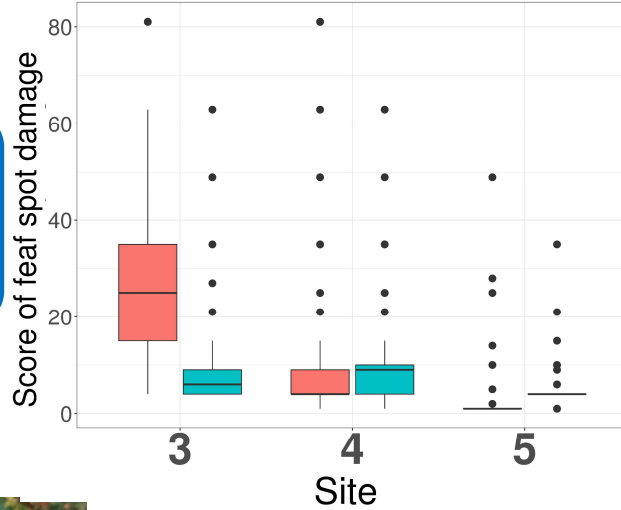


 Apricot orchards
 Peach orchards

➤ Strong effect of the environment: Necessity of multi-site and years trials



Shot hole



Iranian clones & Early Double Red: always the most sensitive



	3	5	
	0.577*	0.338*	4
2022			
	0.660*	0.557*	3
2021			

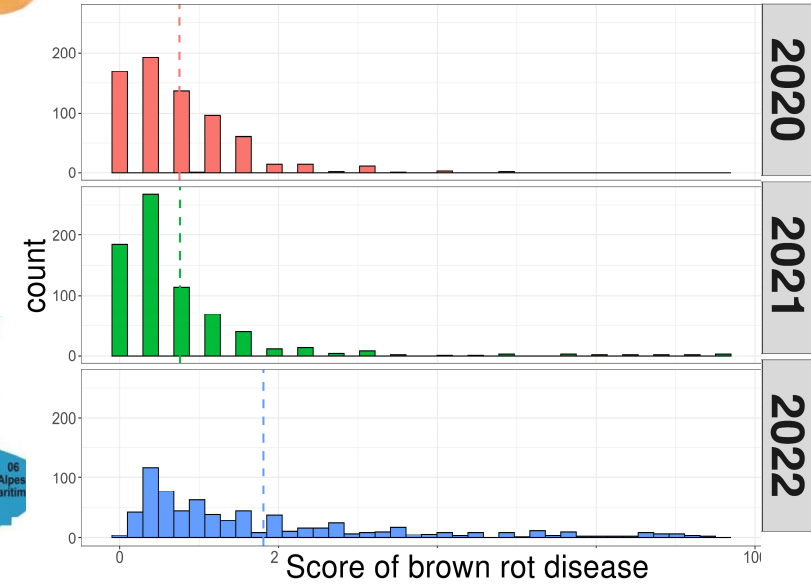
$H^2 : 0,31$

INR

Breeding for resistance/tolerance in fruit crops: New trends
2023/02/28 Bénédicte QUILOT-TURION

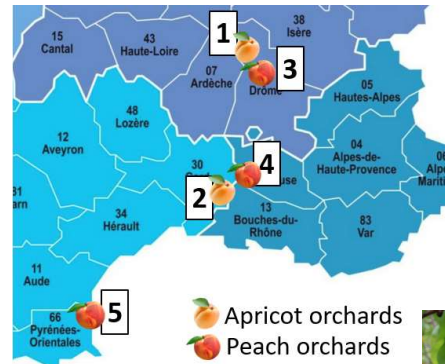


Monilia on flower and twigs

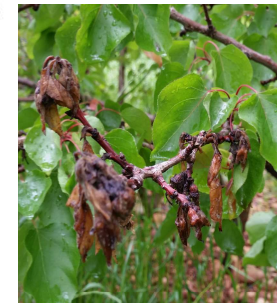


Years

2021
2022



Apricot orchards
Peach orchards



$H^2 : 0,43$

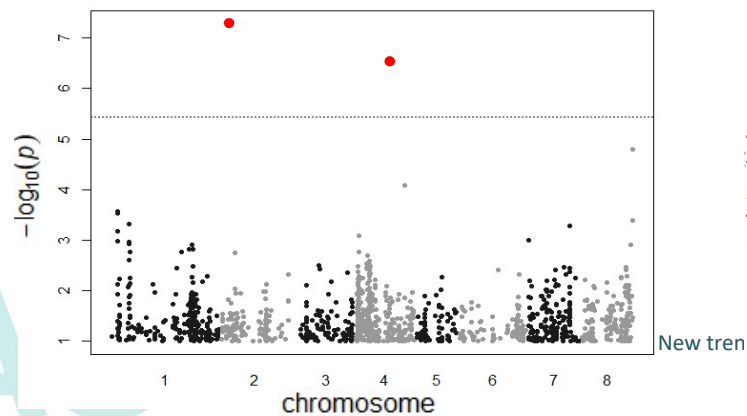
	2021	2022	
	0.421*	0.258*	2020
		0.004	2021

➤ First genome-wide association results on peach

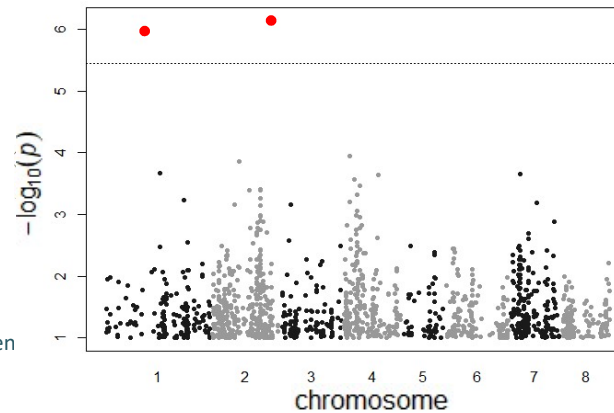
SNP detected	Trait	Chromosome	P-value	Effect size (β)
Peach_AO_0175780	Date of vegetative bud break	2	5.24e-08	-3.99
Peach_AO_0459115	Date of vegetative bud break	4	3.11e-07	-3.22
Peach_AO_0060498	Blooming date	1	1.1e-06	3.67
SNP_IGA_283581	Blooming date	2	7.3e-07	4.22
SNP_IGA_116812	Shot hole	1	2.79e-06	-0.27

- ✓ **MLMM method**
(Segura et al. 2012)
- ✓ **Bonferroni correction**
- ✓ **13,975 markers after filtering**

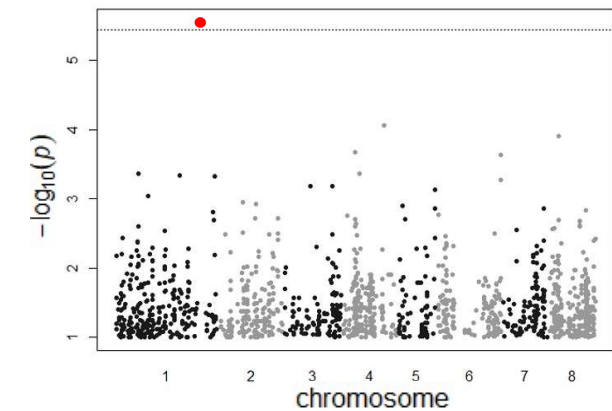
Date of vegetative bud break



Blooming date



Shot hole



➤ A new breeding approach : a better integration of the information

CLASSICAL SELECTION OF FRUIT TREES	OBJECTIVES FOR TOMORROW'S SELECTION
"Trait-by-trait" phenotypic selection of varieties with high yield and quality potential	Selection of resilient ideotypes assisted by high-throughput phenotyping and genetic information
Elite varieties with a wide geographical spectrum that are greedy in pesticides	Varieties able to cope with biotic stresses in a given environment
Exclusively visual phenotyping	Gain in precision and throughput and new descriptive variables by digital phenotyping
Sorting candidates for selection on adult plants	Early genetic diagnostics
Pyramiding of traits of interest, cross after cross within a restricted gene pool	Multi-trait modeling and optimization of the use of diversity
Resistance to rare diseases and only based on major genes	Consideration of so-called qualitative resistances and tolerances by quantitative genetics



➤ Acknowledgments

Team working



INRAE

Breeding for resistance/tolerance in fruit crops: New trends
2023/02/28 Bénédicte QUILOT-TURION

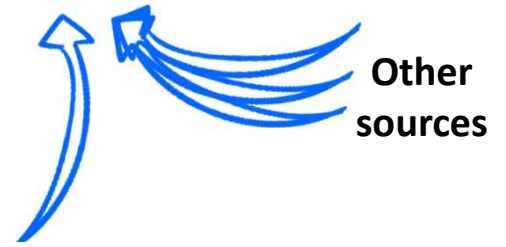
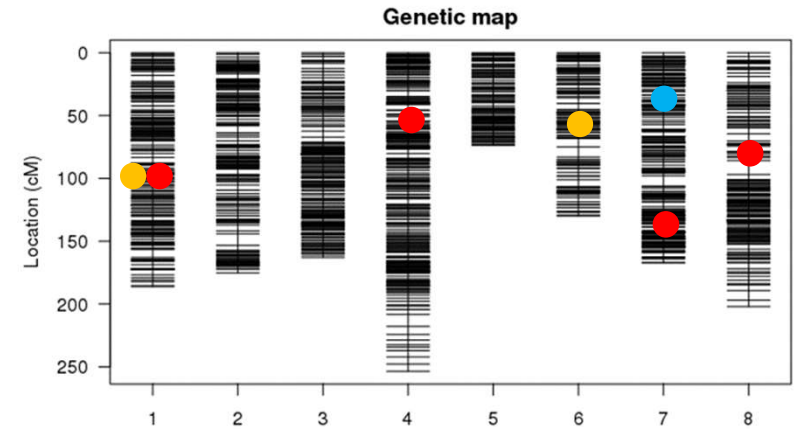
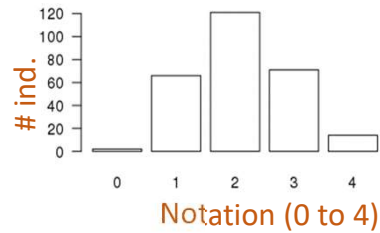
➤ How to detect partial resistance factors

EVALUATION of RESISTANCE LEVEL

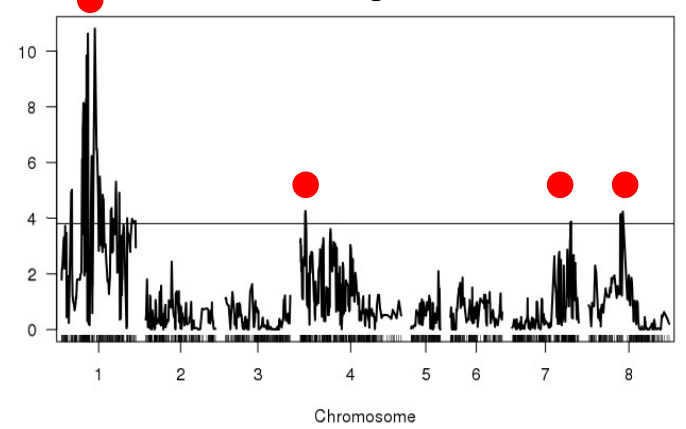
Controlled infections



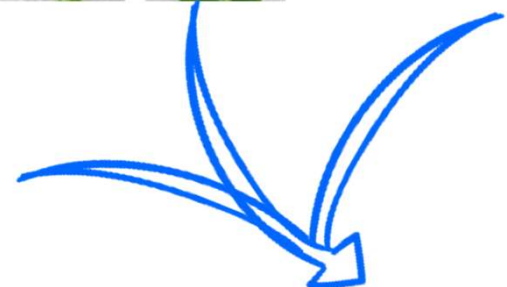
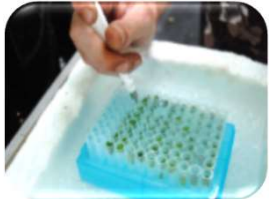
Observations in natural conditions



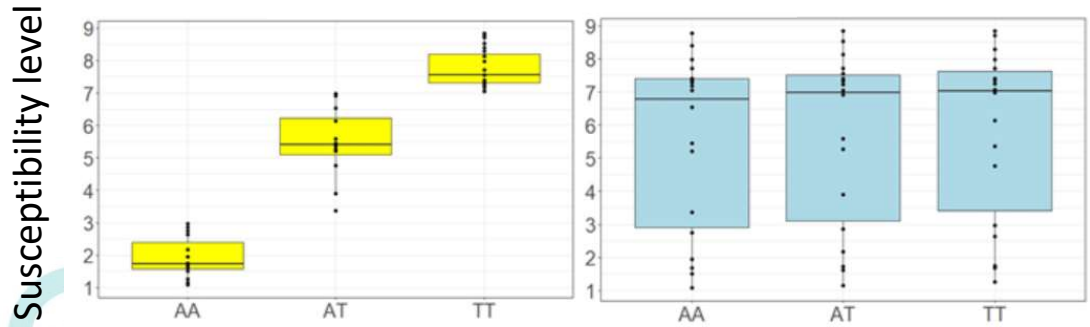
Location of areas of the genome controlling tolerance



GENOTYPING



PHENOTYPE-GENOTYPE LINK



Genotype groups at a marker on the genome

