BREEDING FOR RESISTANCE IN A CHANGING ENVIRONMENT –

DURABLE RESISTANCE: HOPES, PITFALLS, AND MANAGEMENT STRATEGIES

Moderator: Steven McKeand

Breeding poplars with durable resistance to *Melampsora larici-populina* leaf rust

A multidisciplinary approach to understand and delay pathogen adaptation

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Fourth International Workshop on the Genetics of Host-Parasite Interactions in Forestry
July 31 – August 5, 2011, Eugene, Oregon, USA

The protagonists

P. x interamericana — *P. x euramericana*

Populus

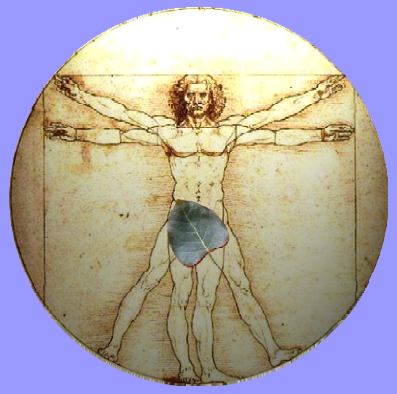
Melampsora larici-populina

P. trichocarpa X *P. deltoides* X *P. nigra*

The protagonists

Populus

Melampsora larici-populina



Larix

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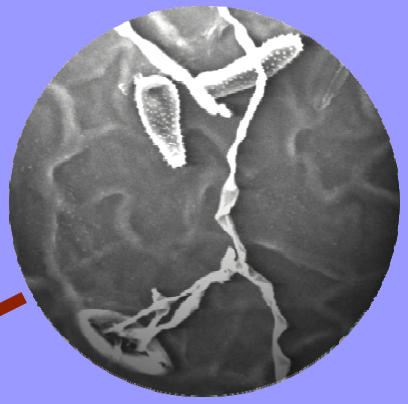

Melampsora larici-populina

Reproduction/ mating system										
Mixed "epidemic" genetic structure	High (3)	*Phytophthora sojae*	7 6 5	*Rhynchosporium secalis* *Mycosphaerella fijiensis, graminicola* *Venturia inaequalis* *Rhizoctonia solani* *Setosphaeria turcica* *Phaeosphaeria nodorum* *Leptosphaeria maculans* *Pseudocercosporella herpotrichoides*	8 7 6	*Blumeria graminis* *Bremia lactucae* *Phytophthora infestans*- new populations *Puccinia graminis* f. sp. *tritici* – pre 1930's *P. coronata* f. sp. *avenae* *Sclerospora graminicola* *Melampsora larici-populina, larici-epitea*	9 8 7	(3) (2) (1)	Effective population size
Outcrossing Sexual high genotype diversity Inbreeding	Medium (2)	*Pratylenchus* *Heterodera* *Armillaria mellea*	6 5 4	*Sporisorium reilianum*	7 6 5	*Ustilago hordei, maydis* *Tilletia* *Sclerotinia sclerotiorum*	8 7 6	(3) (2) (1)	
Asexual low genotype diversity	Low (1)	*Fusarium oxysporum* f. sp. *melonis, lycopersici, cubense* *Xanthomonas campestris* pv. *vesicatoria* Soil-borne viruses *Meloidogyne incognita*	5 4 3	*Colletotrichum graminicola* *Colletotrichum lindemuthianum* *Erwinia amylovora* Insect dispersed viruses	6 5 4	*Melampsora lini* *Magnaporthe grisea* *Cladosporium fulvum* *Puccinia coronata* f. sp. *avenae* – no alternative host *P. striiformis* *P. graminis* f. sp. *tritici, avenae* *P. triticina* – no alternative host *Xanthomonas oryzae* pv. *oryzae*	7 6 5	(3) (2) (1)	
Gene/genotype flow		Low (1)		Medium (2)		High (3)				
		Propagules soilborne, difficult to disperse ~ 5 meter total dispersal		Propagules waterborne, moderate dispersal ~100 m – within field		Propagules airborne, easily dispersed ~10 – 1000 km				
		Man-aided dispersal may modify risk								

Annu. Rev. Phytopathol. 2002. 40:349–79
doi: 10.1146/annurev.phyto.40.120501.101443
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Pathogen Population Genetics, Evolutionary Potential, and Durable Resistance

Bruce A. McDonald and Celeste Linde

Figure 4 (See figure on previous page) Scale of evolutionary risk organized according to reproduction/mating system, gene/genotype flow and effective population size. The organization of this diagram is modified from Figure 2 of Brent & Holloman (8). Effective population size (N_e) is on a 1–3 scale where 1 is small N_e, 2 is average Ne, and 3 is large N_e. Assignment of total risk value assumes that all effects are additive. This risk model assumes that mutation rates are constant and that selection is efficient for all pathogens. Placement of example pathogens is according to principles explained in the text or from Table 2.

Breeder's vocabulary and material

P. x interamericana — *P. x euramericana*

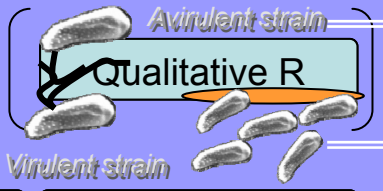
Populus

P. trichocarpa X *P. deltoides* X *P. nigra*

Breeding populations :

822	1760	2835
(ID, OR, WA, BC)	(IL>60%, IA, NE, OH, WI, MN, VT, MS, TN, MO, LA)	(France)

Resistance phenotypes :



Incompatible interaction

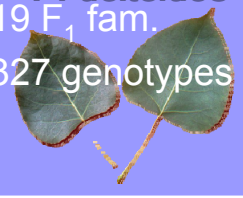
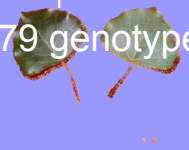
Compatible interaction

Quantitative R | Quantitative R | Quantitative R

Breeder's vocabulary and material

Populus

♂

	P. trichocarpa	*P. deltoides*	*P. nigra*
	16 F_1 fam. 602 genotypes	19 F_1 fam. 327 genotypes 	16 F_1 fam. 279 genotypes 
♀	38 F_1 fam. 2966 genotypes	55 F_1 fam. 2455 genotypes	40 F_1 fam. 1884 genotypes
	6 F_1 fam. 250 genotypes		18 F_1 fam. 936 genotypes

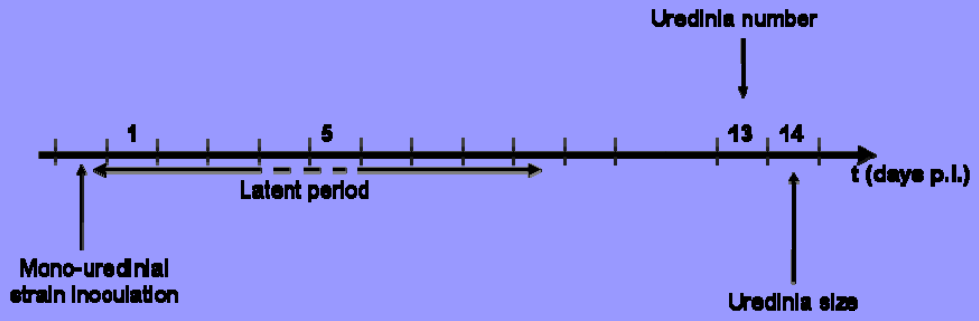
+ some backcrosses and F_2s

Breeder's vocabulary and material

Evaluating resistance

In the lab

In the field



Rust score on the most infected leaf

Pathogen adaptation

A reality

Why and how ?

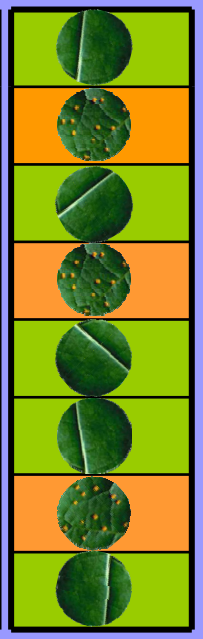
Breeding strategies

Pathogen adaptation
A reality

Several qualitative resistances inherited from P. deltoides did not prove to be durable

more than a few years after commercial deployment of interspecific hybrids :

1	cv. 'Ogy'	
	cv. 'Candicans'	
3	cv. 'Brabantica'	
4	cv. 'Unal'	
5	cv. 'Rap'	
6	87B12	
	cv. 'Beaupré'	
	cv. 'Hoogvorst'	

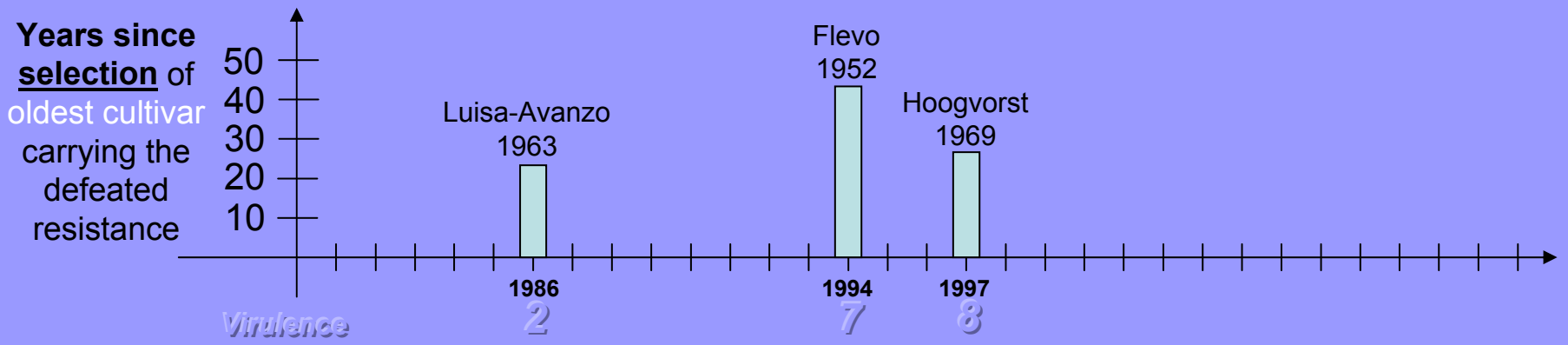


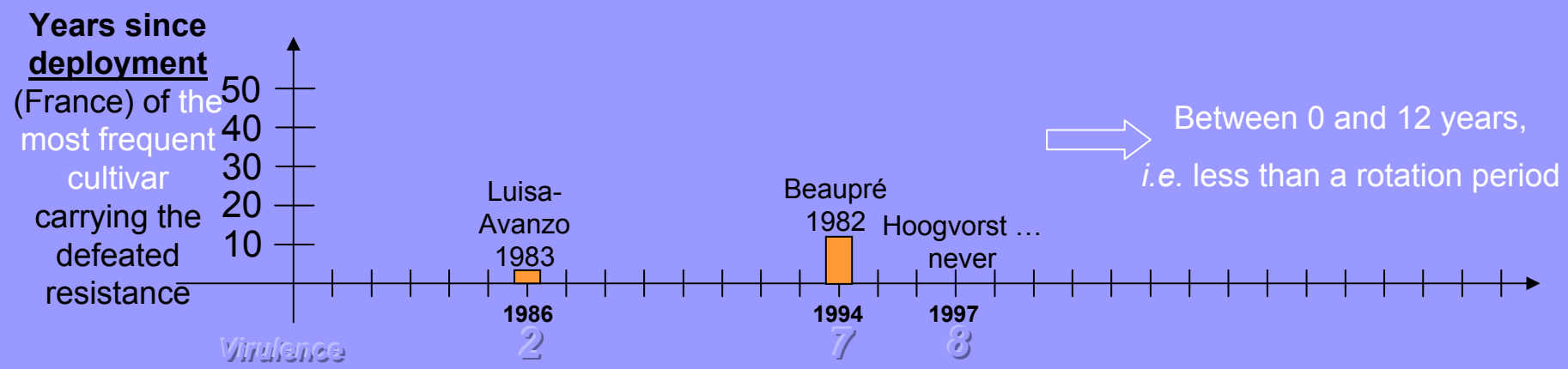
⇨ Pathotype 2-4-7

Pathogen adaptation
A reality

Several qualitative resistances inherited from P. deltoides did not prove to be durable

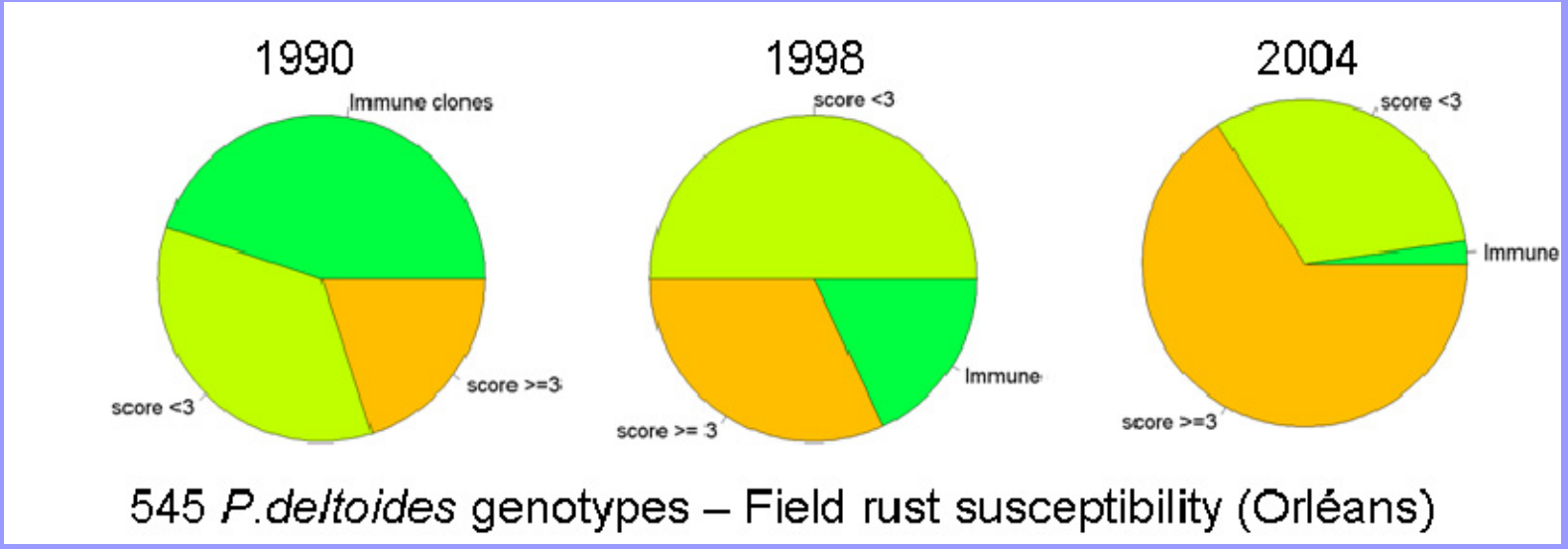
more than a few years after commercial deployment of interspecific hybrids :





Pathogen adaptation
A reality

Most qualitative resistances found in our P. deltoides breeding material are already defeated



Pathogen adaptation
A reality

Absence of qualitative resistance in the sympatric host P. nigra

adaptation as a fatality ?

Isolats

Plantes		Gard 1	2	3	4	5	6	7	8	9	10	11	Durance 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Drôme 1	2	3	4	5	6	7	8	9	10
Gard	G1	9	9	9	8	7	8	8	8	8	7	7	8	9	7	9	7	9	7	9	8	7	8	8	11	7	7	8	7	9	7	8	7	7	7	7	7
	G2	11	10	9	7	8	7	8	8	7	7	9	12	7	8	10	9	9	7	14	8	7	8	7	7	7	9	10	8	7	8	7	8	8	8	7	8
	G3	9	8	9	8	12	8	8	9	8	8	9	8	7	7	12	7	7	8	12	8	7	7	7	8	11	9	7	7	7	9	7	9	8	9	8	6
	G4	11	9	9	11	7	7	7	7	8	7	8	7	12	8	9	7	7	7	8	8	7	8	7	7	12	8	9	7	7	8	7	7	7	8	7	7
	G5	7	9	15	10	7	7	7	7	7	7	7	8	10	8	10	10	7	8	9	7	7	10	7	7	8	8	8	7	7	7	7	8	7	7	7	7
	G6	7	8	9	7	NT	7	10	8	8	7	6	6	NT	7	8	7	7	6	6	8	7	7	7	8	8	6	7	NT	8	8	7	8	8	8	8	6
	G7	9	8	9	8	8	8	9	10	11	8	9	8	10	9	7	9	7	7	8	9	7	11	8	8	8	8	7	8	7	7	7	8	8	7	7	7
	G8	12	9	9	9	8	7	9	9	7	7	10	7	7	9	8	10	9	10	8	8	7	7	7	7	7	10	9	8	7	8	7	7	8	7	8	7
	G9	7	7	9	7	7	7	7	7	7	7	6	8	10	7	9	10	7	7	9	7	7	8	7	7	7	8	9	7	7	7	7	7	7	7	7	7
	G10	9	7	9	11	8	7	9	8	7	7	7	7	10	9	9	7	9	7	9	8	7	8	7	7	8	8	10	8	7	8	7	9	7	12	7	7
	G11	9	8	9	7	7	8	8	8	8	7	12	8	9	9	11	7	9	10	9	7	7	7	7	7	7	7	10	7	8	7	8	8	8	8	7	7
	G12	9	7	10	7	7	7	8	7	7	8	7	8	7	7	7	10	9	11	9	7	7	11	7	7	8	9	10	7	7	8	7	7	9	7	7	7
	G13	7	7	9	7	7	7	8	7	7	9	6	13	8	7	9	7	7	12	9	7	7	8	7	7	9	9	7	7	7	8	7	7	8	7	7	7
	G14	7	8	11	9	7	7	9	7	7	7	10	9	10	10	10	9	7	10	9	7	7	9	7	7	7	9	10	7	8	7	8	7	9	7	7	7
	G15	7	8	8	7	8	7	7	7	7	7	6	6	7	7	9	7	7	7	7	7	7	8	7	6	9	7	7	7	7	7	7	7	7	7	7	7
	G16	9	8	12	8	7	7	8	7	7	7	12	14	9	8	9	13	9	9	9	7	7	9	7	6	7	12	7	7	7	7	7	7	8	7	7	7
	G17	9	7	9	9	7	7	7	7	7	7	12	14	11	7	11	9	9	10	10	7	7	9	7	6	7	9	9	7	7	7	7	7	7	7	7	7
	G18	7	7	9	7	8	8	9	8	8	7	9	14	9	8	9	12	9	7	8	7	7	9	7	7	11	9	9	8	7	7	7	7	7	7	7	7
	G19	9	7	9	9	7	7	8	7	7	7	7	7	9	8	9	10	9	9	9	7	7	8	7	7	7	8	7	7	7	7	7	7	7	7	7	7
	G20	7	9	9	8	7	7	10	8	8	7	6	6	10	8	8	9	7	7	7	7	7	7	7	7	7	9	7	7	7	8	7	7	8	7	7	7
	G21	9	7	13	10	7	7	7	7	7	7	6	13	8	7	7	10	9	9	14	7	7	14	7	6	7	13	7	7	7	7	7	7	7	7	7	7
	G22	7	12	13	9	7	7	9	7	8	7	7	10	8	7	9	7	7	9	12	7	7	13	7	7	8	9	7	7	7	7	7	7	7	8	7	7
	G23	7	9	8	9	7	7	7	7	7	7	12	14	9	8	8	7	7	9	10	8	7	7	7	7	6	7	10	7	7	7	7	7	7	7	7	7
	G24	NT	NT	NT	NT	NT	NT	8	8	8	7	NT	NT	NT	7	8	NT	NT	NT	NT	8	7	NT	7	8	8	NT	NT	NT	8	8	8	NT	8	9	8	NT
Durance	Du1	7	7	8	7	7	7	7	7	7	8	10	6	8	8	7	8	7	7	8	7	7	8	7	6	9	7	8	7	7	7	7	8	7	7	7
	Du2	7	10	7	9	9	8	7	7	7	9	7	10	10	9	9	10	7	9	9	8	9	9	7	8	9	9	7	8	8	8	8	6	9	7	7
	Du3	9	7	9	8	7	7	7	7	7	7	12	8	8	8	9	8	7	9	8	7	7	9	7	7	7	7	8	7	7	10	7	7	8	7	7	7
	Du4	8	9	9	7	7	7	9	8	8	8	10	7	10	9	10	10	7	7	10	8	7	7	7	8	8	9	7	7	7	8	7	8	8	8	7	7
	Du5	7	NT	7	NT	NT	NT	NT	NT	NT	8	6	7	NT	NT	12	7	NT	NT	NT	NT	7	NT	8	NT	NT	NT	NT	NT	7	NT	7	NT	8	NT	NT	NT
	Du6	9	7	7	7	7	7	8	7	7	7	7	10	9	8	10	9	7	7	9	6	7	9	7	6	8	9	7	7	8	7	8	7	7	7	7	7
	Du7	9	7	9	9	7	7	13	8	8	7	7	11	7	12	10	8	10	8	10	11	7	11	7	7	9	8	8	7	8	8	8	8	9	7	7	7
	Du8	9	7	9	10	7	11	NT	NT	NT	7	9	8	10	NT	7	10	9	11	8	NT	8	8	8	NT	NT	8	9	7	NT	NT	8	9	9	NT	NT	10
	Du9	10	7	7	10	NT	NT	NT	NT	NT	8	12	9	10	NT	10	10	10	10	10	NT	NT	9	NT	8	NT	NT	NT	10	NT	NT	NT	8	NT	8	NT	NT	NT
	Du10	9	10	13	10	13	13	NT	NT	NT	10	7	10	7	NT	7	7	10	7	13	NT	9	12	8	NT	NT	10	10	7	NT	NT	11	13	9	NT	NT	10
	Du11	10	8	9	9	9	10	8	10	11	8	9	8	8	7	9	9	9	10	NT	9	7	NT	8	7	12	NT	11	12	NT	8	7	10	8	8	9	8
	Du12	10	10	9	10	7	8	9	8	8	8	13	7	7	10	12	7	9	7	7	7	7	7	9	8	7	9	9	7	7	8	8	7	8	8	8	7
	Du13	9	7	7	9	7	7	8	9	9	9	7	7	10	8	10	10	9	9	8	7	8	10	8	7	7	8	10	7	8	7	7	8	8	8	7	7
	Du14	9	7	10	8	7	7	8	7	7	7	7	14	9	9	9	9	9	7	9	7	7	7	7	7	7	9	9	7	7	7	7	7	7	7	7	7
Drôme	Dr1	9	9	12	8	7	7	8	7	7	8	7	13	9	8	9	10	7	7	9	7	7	9	8	7	11	7	8	7	7	7	8	7	7	7	7	7
	Dr2	11	9	12	10	8	9	9	10	8	9	12	13	11	7	8	10	9	9	8	7	8	7	9	7	8	8	9	7	8	9	9	10	9	8	13	8
	Dr3	11	10	9	9	7	7	8	8	8	8	14	9	13	10	7	8	10	9	10	9	8	8	9	8	7	14	8	10	7	9	7	9	9	9	12	7
	Dr4	13	10	12	12	7	7	9	8	7	8	12	12	14	8	8	12	10	13	8	8	8	13	8	7	7	6	12	7	7	8	10	7	8	8	7	7
	Dr5	13	9	12	9	7	7	NT	NT	NT	NT	12	12	9	NT	11	10	9	7	8	NT	NT	8	NT	NT	NT	8	10	7	NT	NT	9	NT	NT	NT	NT	7
	Dr6	10	10	9	12	7	9	NT	NT	NT	8	7	12	11	NT	10	12	10	10	8	NT	8	8	8	NT	NT	7	7	8	9	NT	7	8	8	NT	NT	10
	Dr7	9	9	9	10	7	7	NT	NT	NT	8	11	8	10	NT	8	12	7	10	8	NT	7	7	7	NT	NT	13	10	7	9	NT	8	8	8	NT	NT	7
	Dr8	9	8	12	10	7	7	10	8	14	9	12	10	10	8	10	11	9	8	9	7	7	9	8	7	8	9	9	7	7	7	7	8	8	9	7	7

46 *P.nigra* hosts x 36 isolates

3 pops from Southern France

100% of the 1529 tested host x isolate interactions were compatible

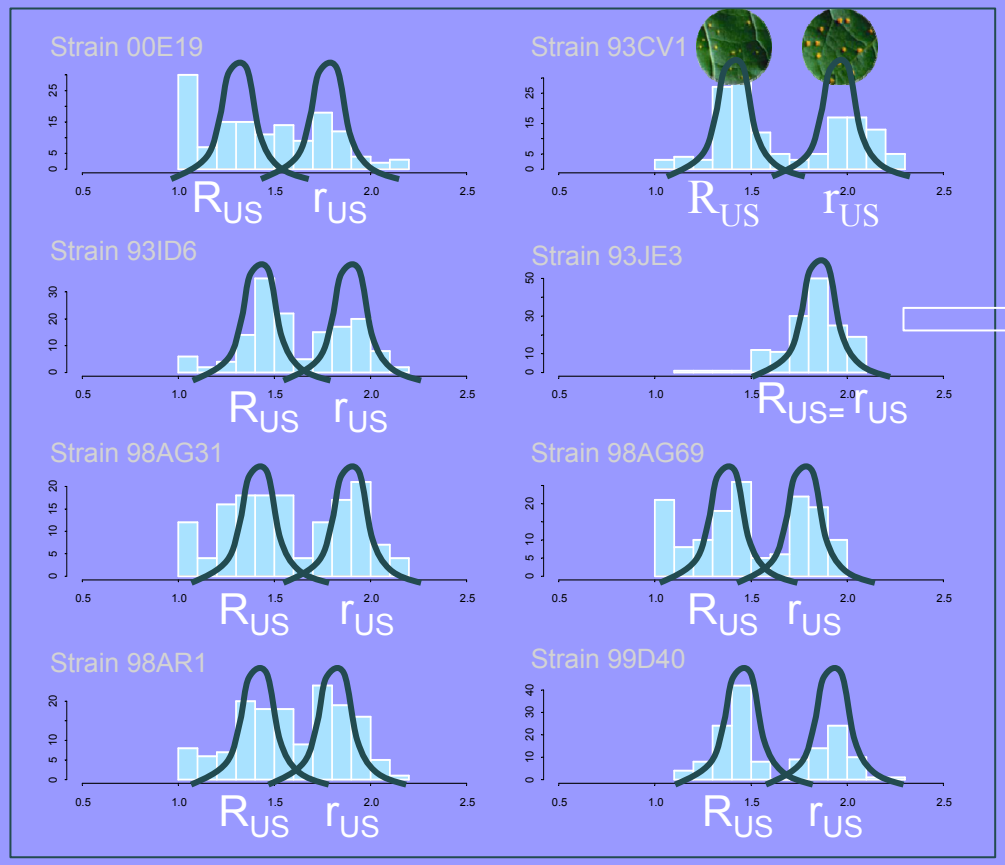
allopatry

sympatry

Pathogen adaptation
A reality

***Quantitative resistance* also can be defeated**

Exemple of a major quantitative resistance factor inherited from *P. trichocarpa



Distributions of clonal means for uredinia size in a *P. X interamericana* F_1 progeny

R_{US} defeating strain

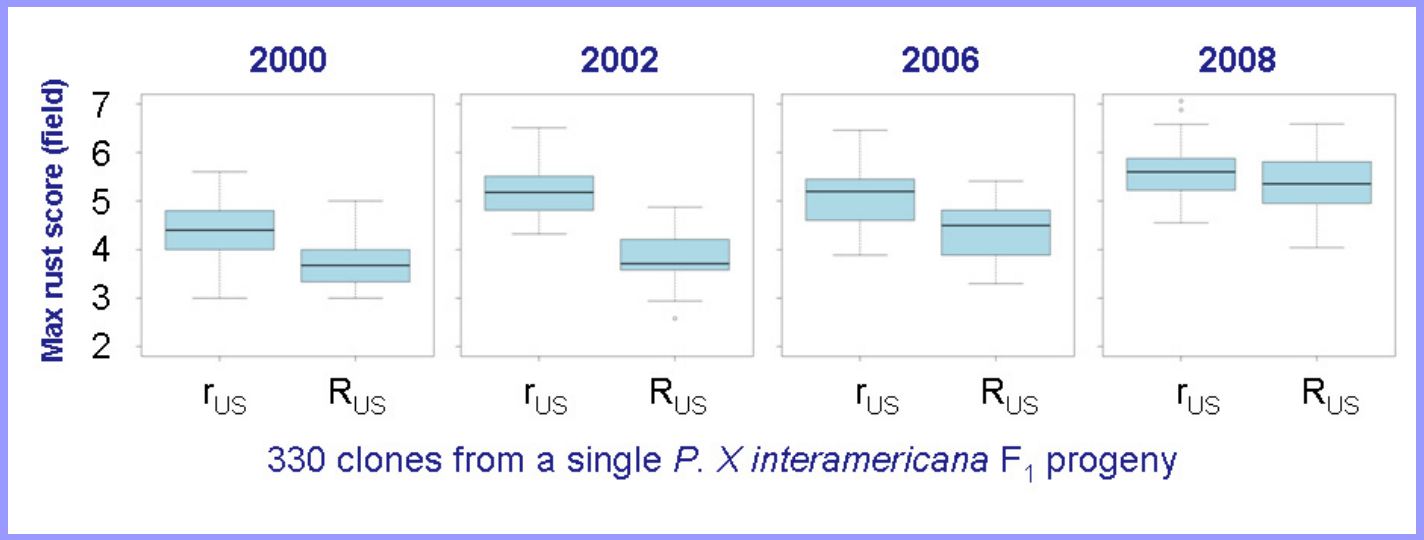
Collecting large uredinia on R_{US} host genotypes in our nursery revealed other R_{US} defeating strains

- Dowkiw *et al.*
Phytopathology 2003 Vol. 83 (4)
- Dowkiw and Bastien
Phytopathology 2004 Vol. 94 (12)
- Dowkiw et al.
Plant Pathology 2010 Vol. 59 (3)

Pathogen adaptation
A reality

Quantitative resistance also can be defeated

Exemple of a major quantitative resistance factor inherited from P. trichocarpa



$$\left(\begin{array}{l} r_{US} = r_{US} r_{US} \\ R_{US} = R_{US} r_{US} \end{array} \right)$$

Pathogen adaptation

A reality

Why and how ?

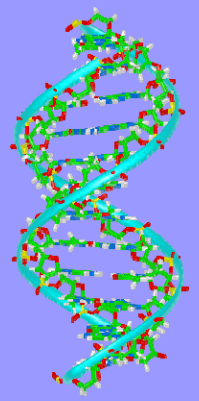
Breeding strategies

Pathogen adaptation
Why and how ?

From genes (host & pathogen) to the environment (incl. management)

Who are they ?
How many are they ?
How do they evolve ?
…

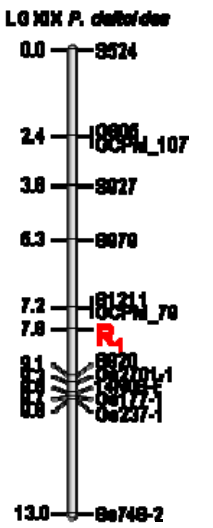
How does adaptation emerge ?
How does it maintain and spread ?
What role do wild/ornamental poplars play ?
What role does the alternate host play ?
…



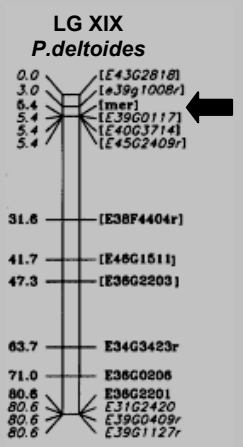
Pathogen adaptation
Why and how ?

From genes (host & pathogen) to the environment (incl. management)

Qualitative resistance inherited from *P. deltoides*



- Jorge *et al.*
New Phytol. Vol. 167 (1)

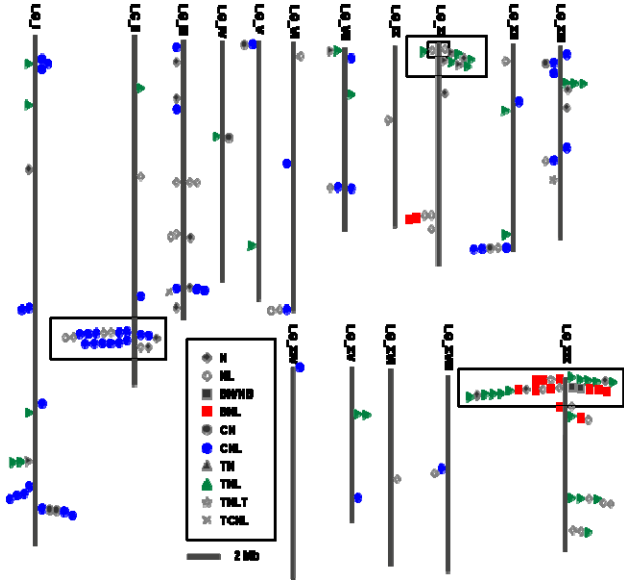


Cervera et al., Genetics, 2001

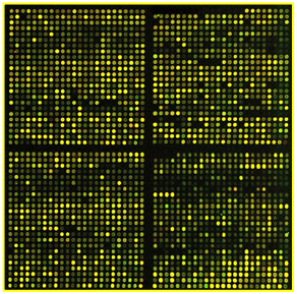
NBS-LRR RGA in

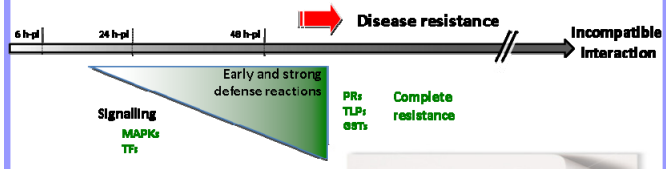
P. trichocarpa 'Nisqually-1'

-Kohler *et al.*
Plant Mol. Biol. 2008 Vol. 66 (6)



Transcriptomics





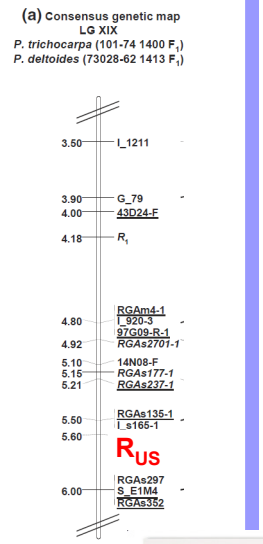
- Rinaldi *et al.*
Plant Physiol. 2007 Vol. 144

Pathogen adaptation
Why and how ?

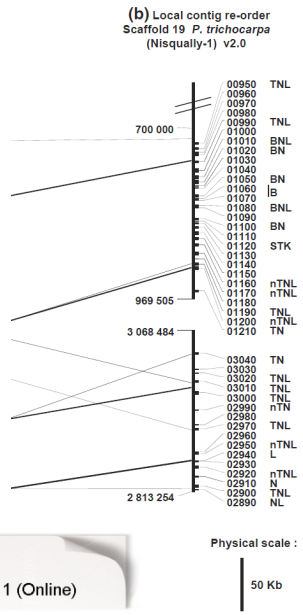
From genes (host & pathogen) to the environment (incl. management)

host

Quantitative resistance inherited from *P. trichocarpa*

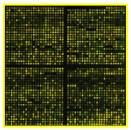


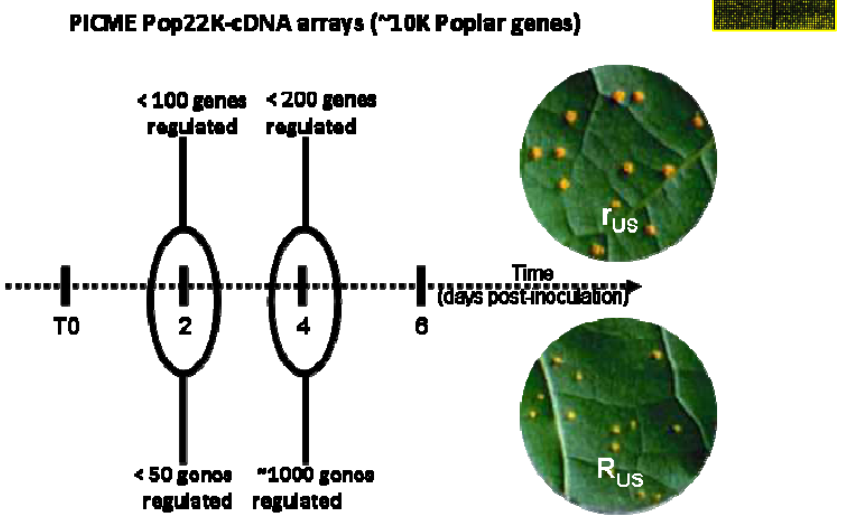
P. trichocarpa 'Nisqually-1' genome



-Bresson *et al.*
New Phytol. 2011 (Online)

Transcriptomics





Up-reg list contains all qualitative resistance toplist genes

Fourth International Workshop on the Genetics of Host-Parasite Interactions in Forestry

July 31 – August 5, 2011, Eugene, Oregon, USA

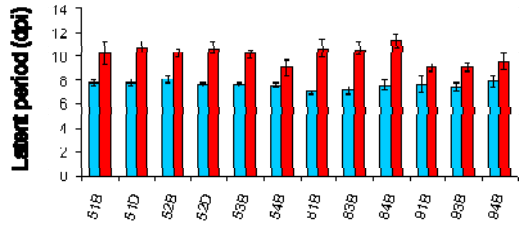
Pathogen adaptation
Why and how ?

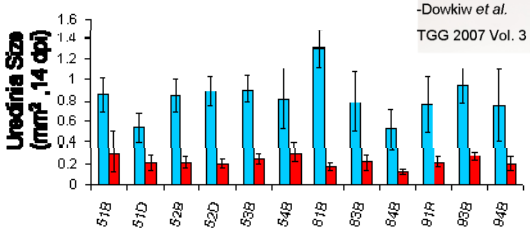
From genes (host & pathogen) to the environment (incl. management)

host

Other quantitative resistance factors

R-gene associated QR factors



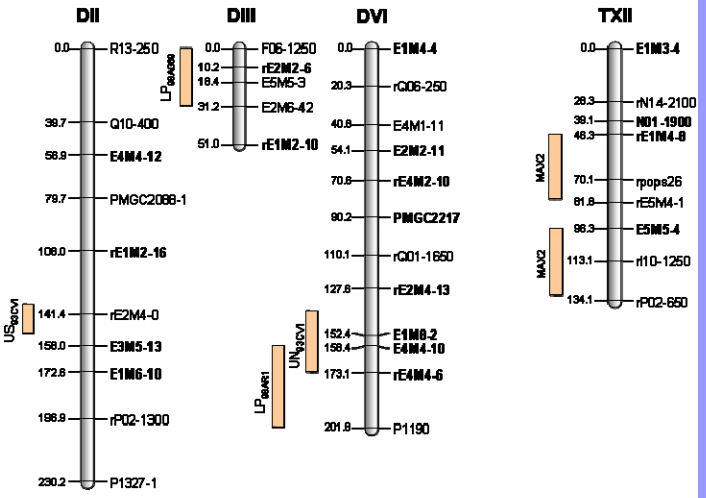


-Dowkiw *et al.*
TGG 2007 Vol. 3 (3)

***12 P. X interamericana* F_1 families (3 distinct mothers)**

Genotypes with/without a defeated qualitative resistance inherited from *P.deltoides*

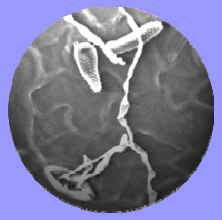
Small QTLs



- Jorge *et al.*
New Phytol. Vol. 167 (1)

Pathogen adaptation
Why and how ?

From genes (host & pathogen) to the environment (incl. management)



PNAS

Obligate biotrophy features unraveled by the genomic analysis of rust fungi

2011 Vol. 108 (22)

Sébastien Duplessis[a,1,2], Christina A. Cuome[b,1,2], Yao-Cheng Lin[c], Andrea Aerts[d], Emilie Tisserant[a], Claire Veneault-Fourrey[a], David L. Joly[e,3], Stéphane Hacquard[a,4], Joëlle Amselem[f], Brandi L. Cantarel[g], Readman Chiu[h], Pedro M. Coutinho[g], Nicolas Feau[e,5], Matthew Field[h], Pascal Frey[a], Eric Gelhaye[a], Jonathan Goldberg[b], Manfred G. Grabherr[b], Chinnappa D. Kodira[b,6], Arnegret Kohler[a], Ursula Kües[i], Erika A. Lindquist[d], Susan M. Lucas[d], Rohit Magni[j], Evan Mauceli[b], Emmanuelle Morin[a], Claude Murat[a], Jasmyn L. Pangilinan[d], Robert Park[k], Matthew Pearson[b], Hadi Quesneville[f], Nicolas Rouhier[a], Sharadha Sakthikumar[b], Asaf A. Salamov[d], Jeremy Schmutz[d], Benjamin Selles[a], Harris Shapiro[d], Philippe Tanguay[e], Gerald A. Tuskan[d,l], Bernard Henrissat[g], Yves Van de Peer[c], Pierre Rouzé[c], Jeffrey G. Ellis[j], Peter N. Dodds[j], Jacqueline E. Schein[h], Shaobin Zhong[m,7], Richard C. Hamelin[e],

MPMI Vol. 24, No. 7, 2011, pp. 808–818. doi:10.1094/MPMI-01-11-0006. © 2011 Th

***Melampsora larici-populina* Transcript Profiling During Germination and Timecourse Infection of Poplar Leaves Reveals Dynamic Expression Patterns Associated with Virulence and Biotrophy**

Sébastien Duplessis, Stéphane Hacquard, Christine Delaruelle, Emilie Tisserant, Pascal Frey, Francis Martin, and Arnegret Kohler

INRA, Institut National de la Recherche Agronomique, UMR 1136 INRA/Université Nancy, Interactions Arbres/Micro-organismes, Centre INRA de Nancy, 54280 Champenoux, France

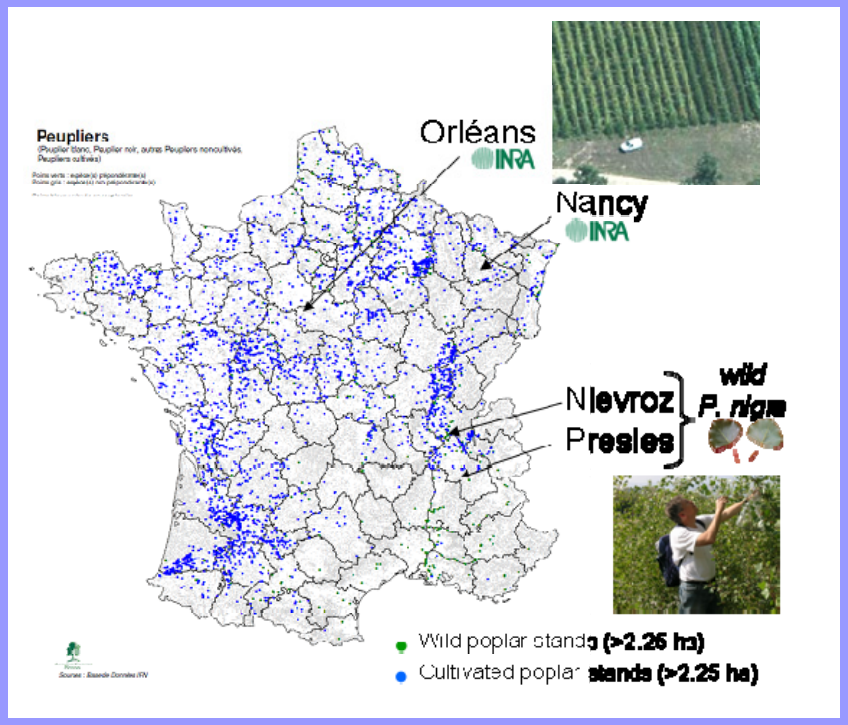
Only candidate effectors for the moment : >1000 small secreted proteins, 74% are species-specific

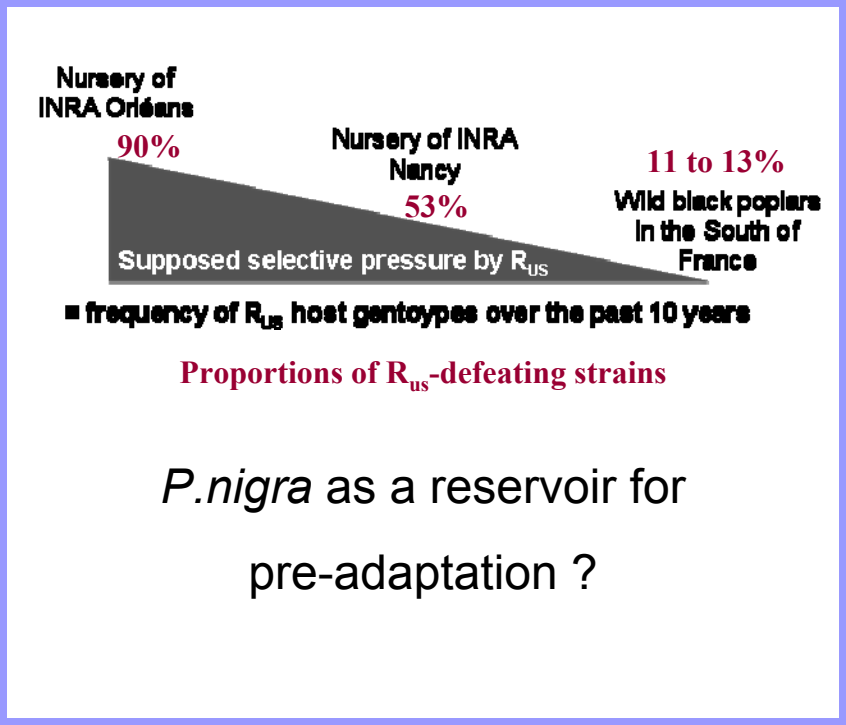
Several homologs of genes encoding haustorially expressed secreted proteins and avirulence factors identified in *M.lini*

Pathogen adaptation
Why and how ?

From genes (host & pathogen) to the environment (incl. management)

How does adaptation emerge ?
How does it maintain and spread ?
What role do wild/ornamental poplars play ?
What role does the alternate host play ?





P.nigra as a reservoir for pre-adaptation ?

Pathogen adaptation
Why and how ?

From genes (host & pathogen) to the environment (incl. management)

How does adaptation emerge ?
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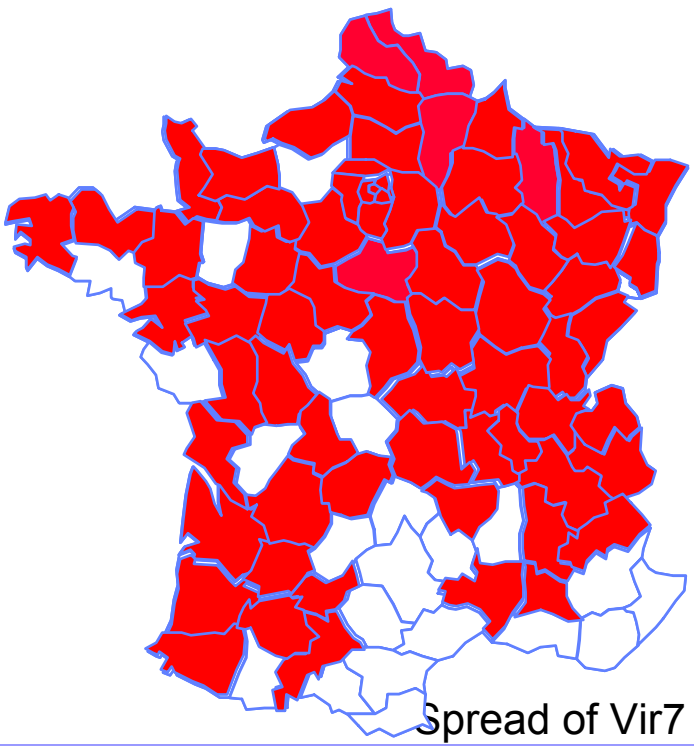
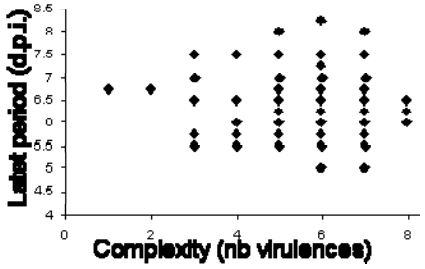


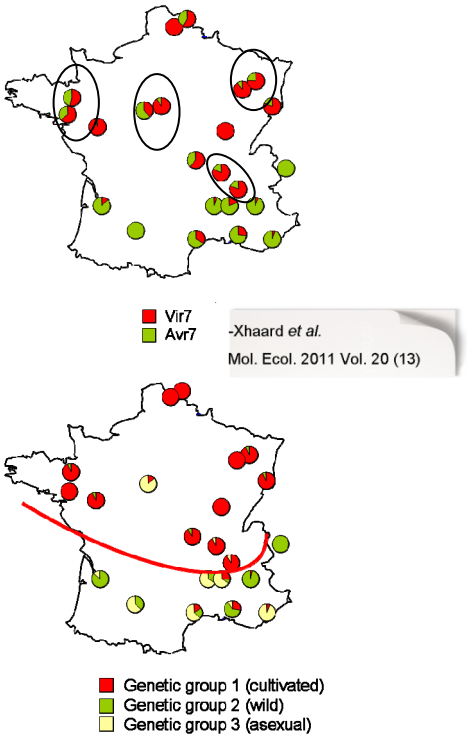
Spread of Vir7 strains

First pathotypes identified containing Vir7

1996	1999
3-4-7	4-7
3-4-5-7	1-2-3-4-5-7
1-3-4-5-7 (98%)	
1-3-4-5-6-7	



200 isolates isolated from larch



Pathogen adaptation

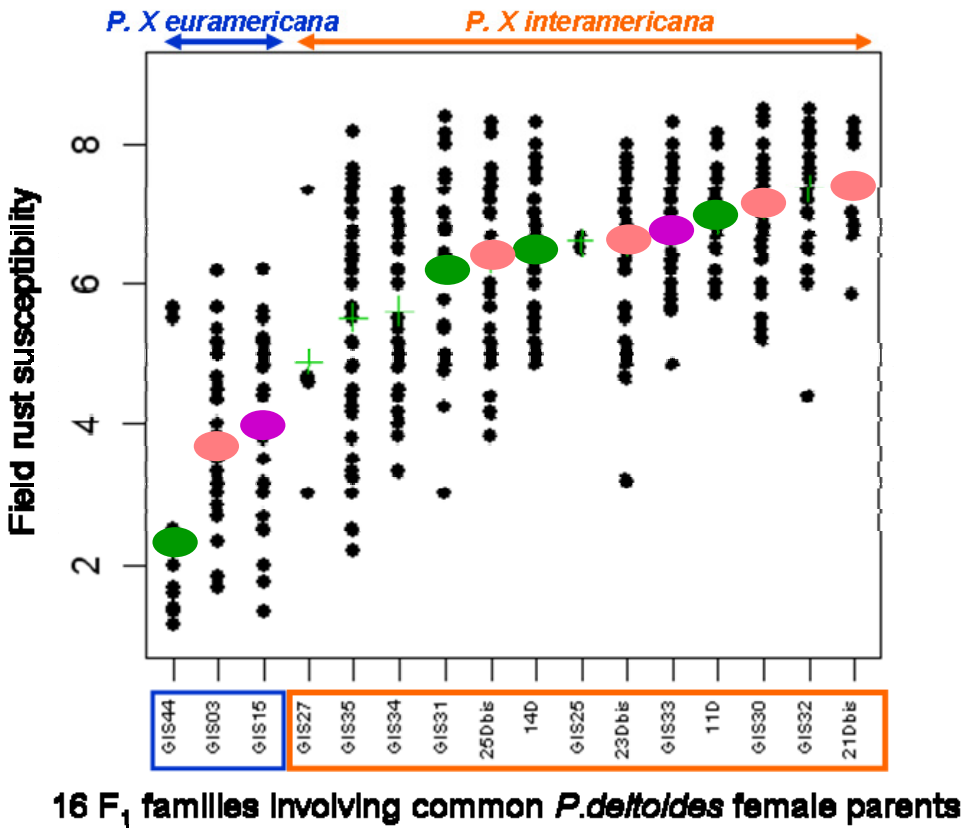
A reality

Why and how ?

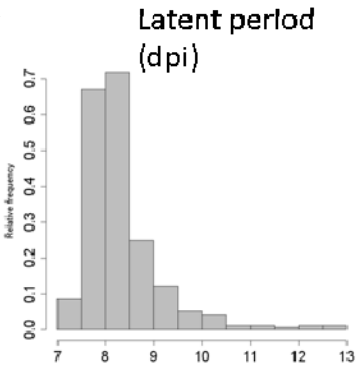
Breeding strategies

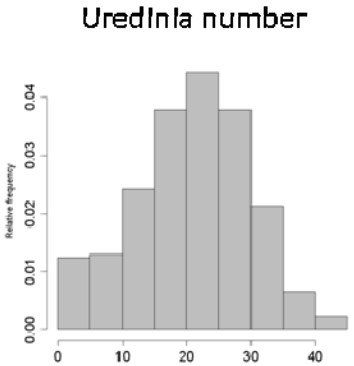
Pathogen adaptation
Breeding strategies

Using co-evolved quantitative resistance from *P. nigra*



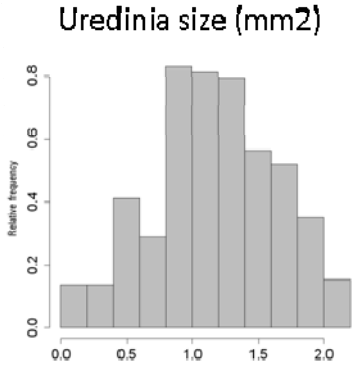
16 F_1 families involving common *P.deltoides* female parents





348 *P.nigra* genotypes

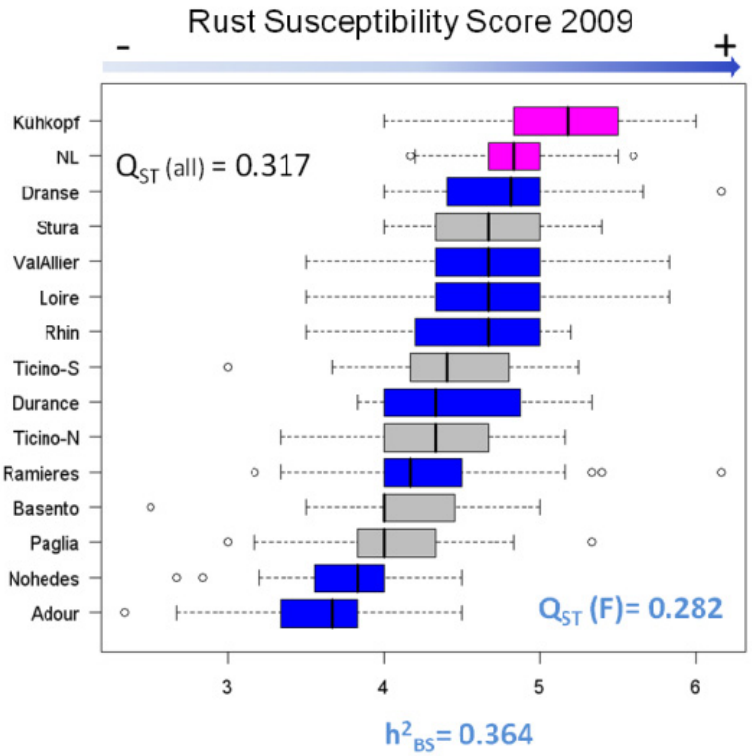
Lab test (1 strain)



Pathogen adaptation
Breeding strategies

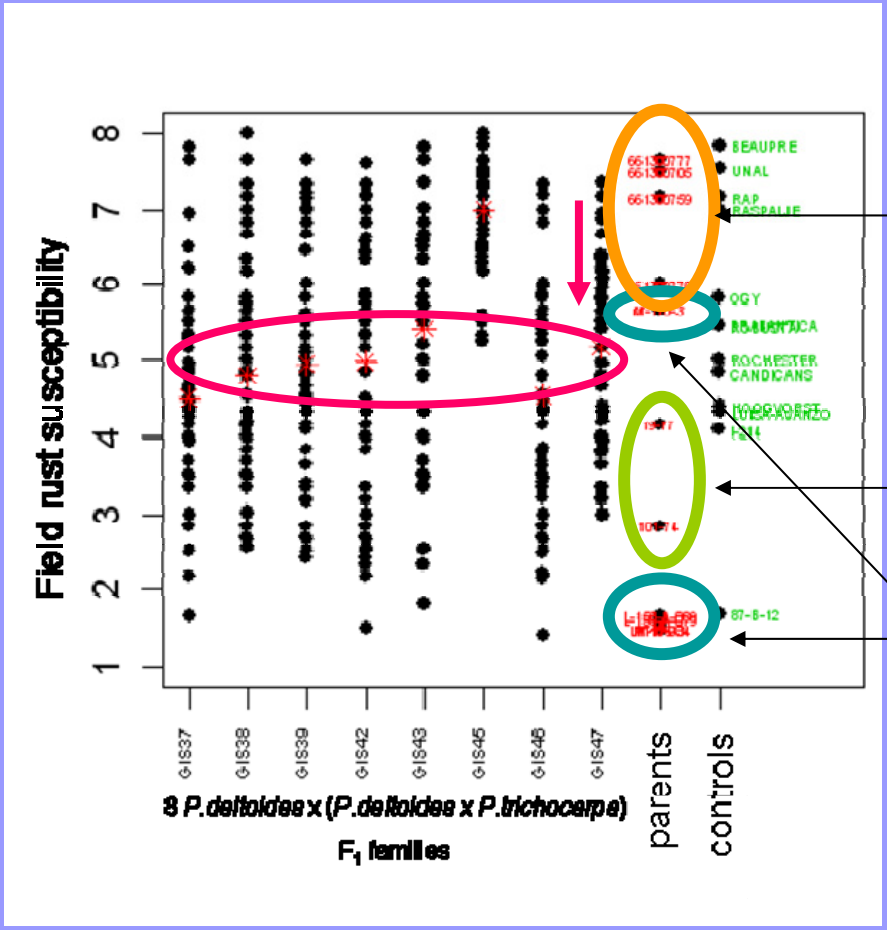
Using co-evolved quantitative resistance from *P. nigra*

Evoltree *P.nigra* Collection (1100)



Pathogen adaptation
Breeding strategies

Backcrossing to a parental species



P. deltoides X *P. trichocarpa*

P. trichocarpa

P. deltoides

Pathogen adaptation
Bredding strategies

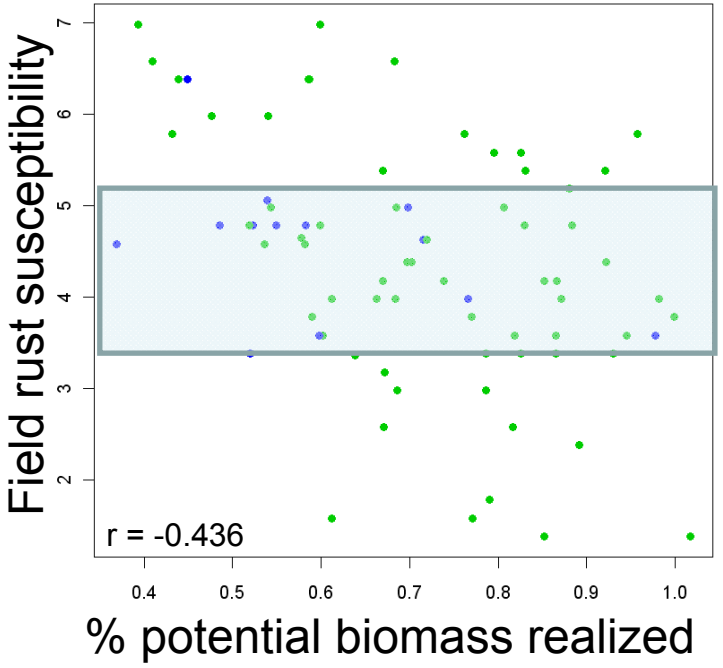
Living with the pathogen …

Using

'fungicide treated vs. non-treated'

field experiments

(cloned material)



Resistance ?
Escape ?
Tolerance ?

Pathogen adaptation

Tank you for your attention

+ Fabien, Bénédicte …

The research leading to these results received funding from (e.g.)

- the European Community's Seventh Framework Program (FP7/ 2007-2013) under the grant agreement n° 211868 (Noveltree Project)

- the French « Bureau des Ressources Génétiques » and the French Ministry for Agriculture and Fisheries (Programme 142, sous-action 27, CTPS)

- INRA