A new CpGV isolate overcoming Cydia pomonella *resistance to Granulovirus: improvement of the virus efficiency by pressure of selection on resistant hosts.*

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Cydia pomonella, a major pest on apple and pears

Photo Graciela Quintana, INTA

Worldwide pest under temperate climates (except Far east)

1-4 generations per year

Many *C.p.* populations have become resistant to chemicals

CpGV, Cydia pomonella Granulovirus

- First isolated in Mexico in 1964
  → Mexican isolate
- Kills the larvae by ingestion

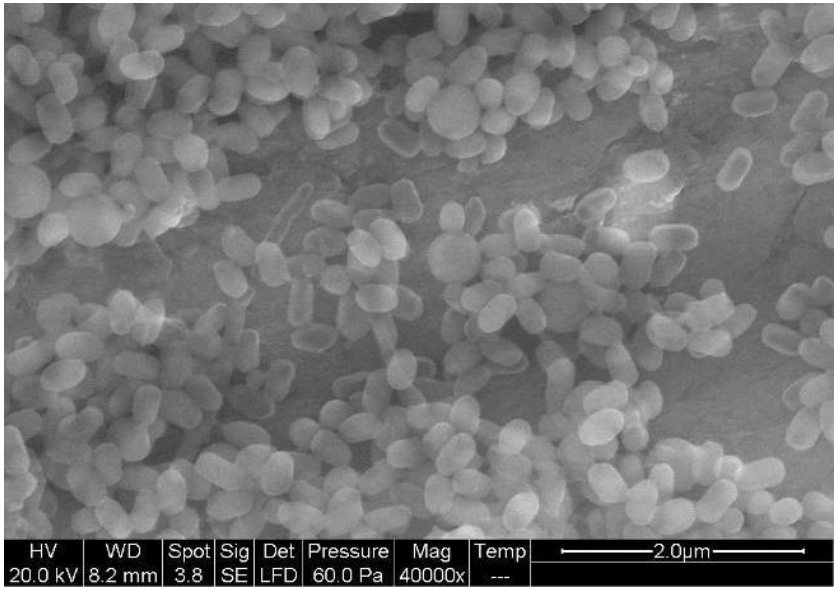


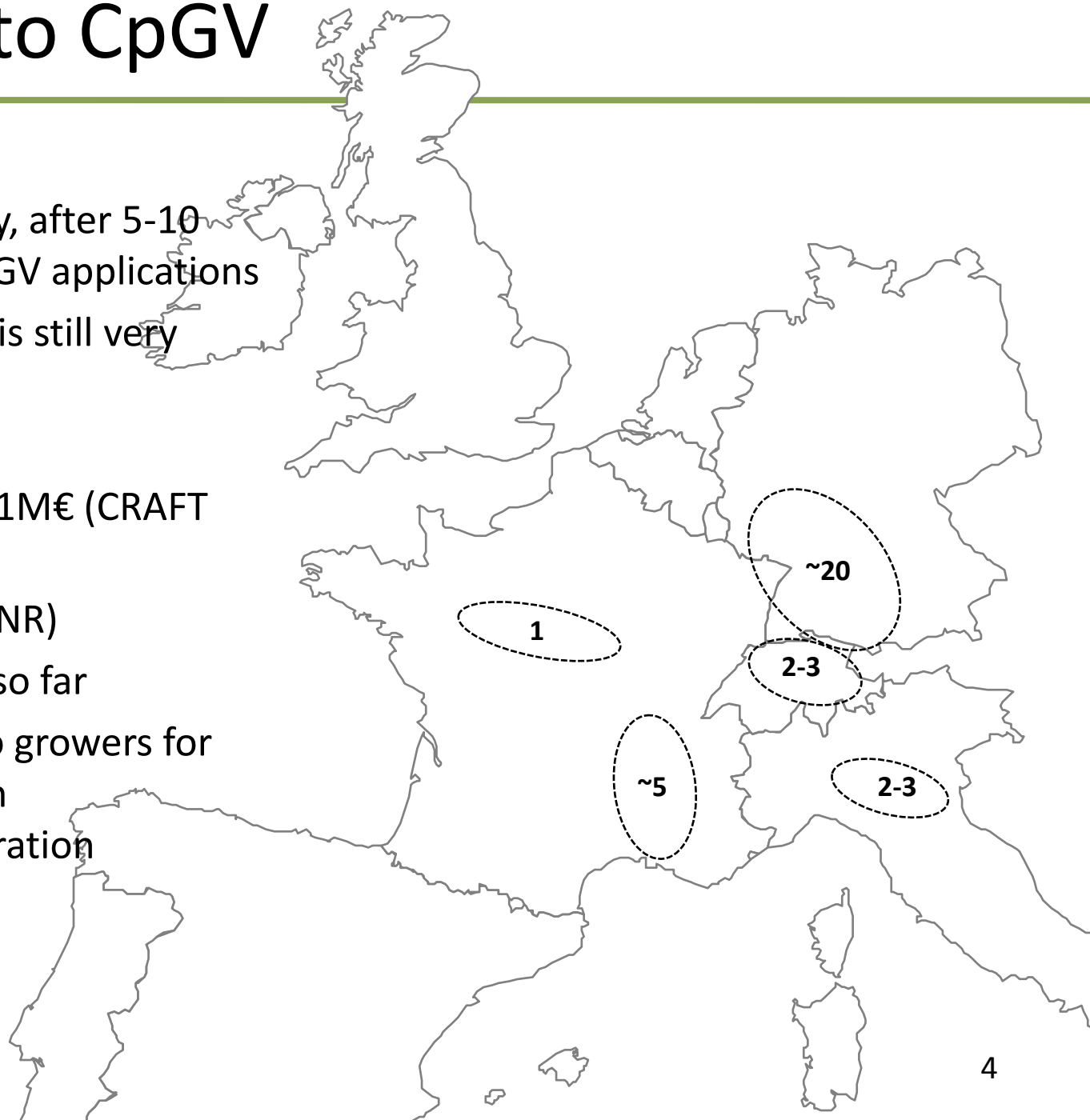
Photo Marie Berling, Ecole des Mines d'Alès

- > 100,000 super-developed hectares sprayed every year
- In organic orchard (20%) and in IPM (80%)

Resistance to CpGV

- First records in 2004
- Organic orchards only, after 5-10 years of exclusive CpGV applications
- Number of locations is still very limited

- EU Com has granted 1M€ (CRAFT Project)
- and France 0.4M€ (ANR)
- Arysta spent 0.2 M€ so far
- Recommendations to growers for resistance prevention
  → choose your generation



Methodology

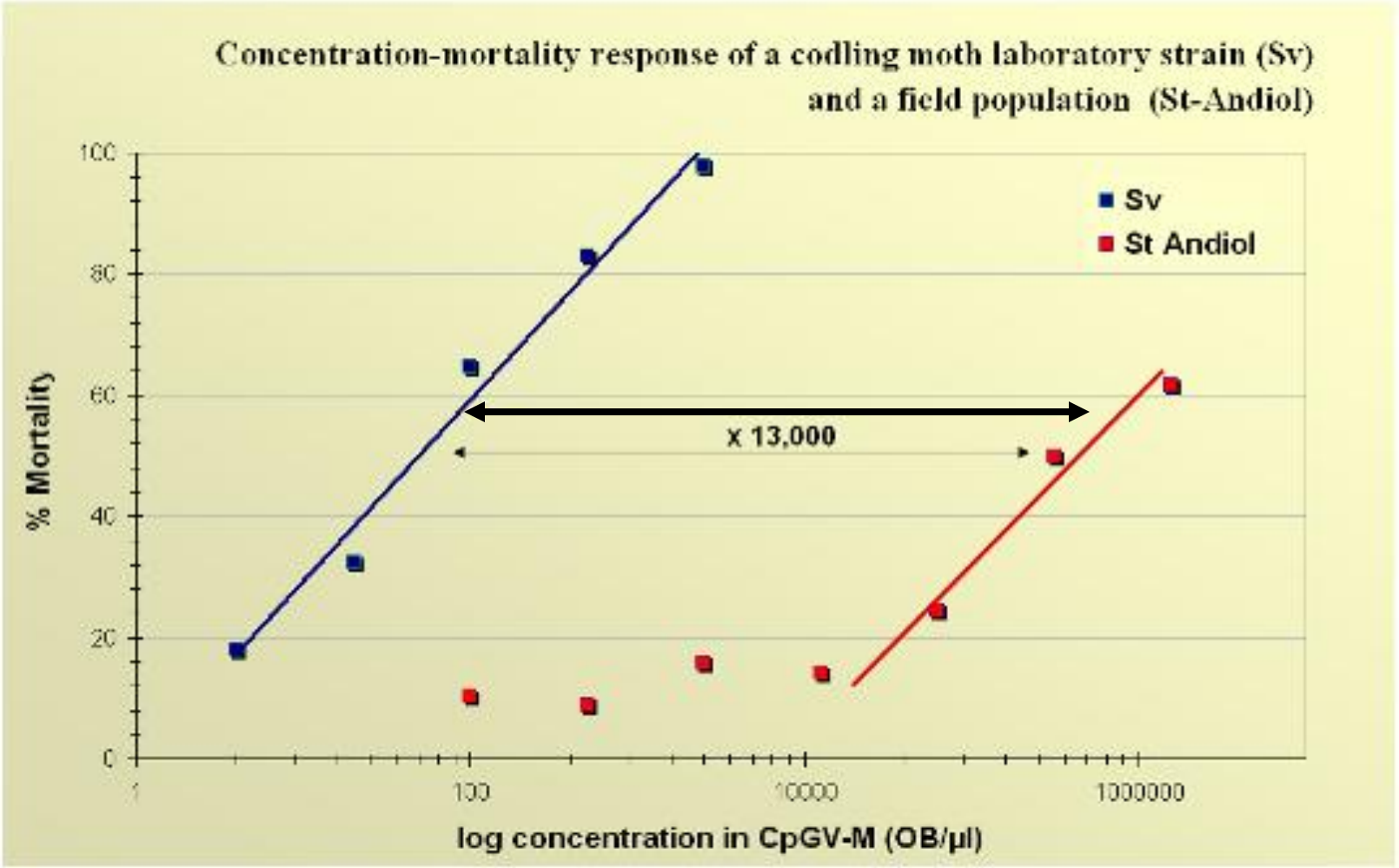
For resistance assessment

- Insect collection in fields where lowered efficacy was recorded and lab-tests for resistance

For research on new virus isolates

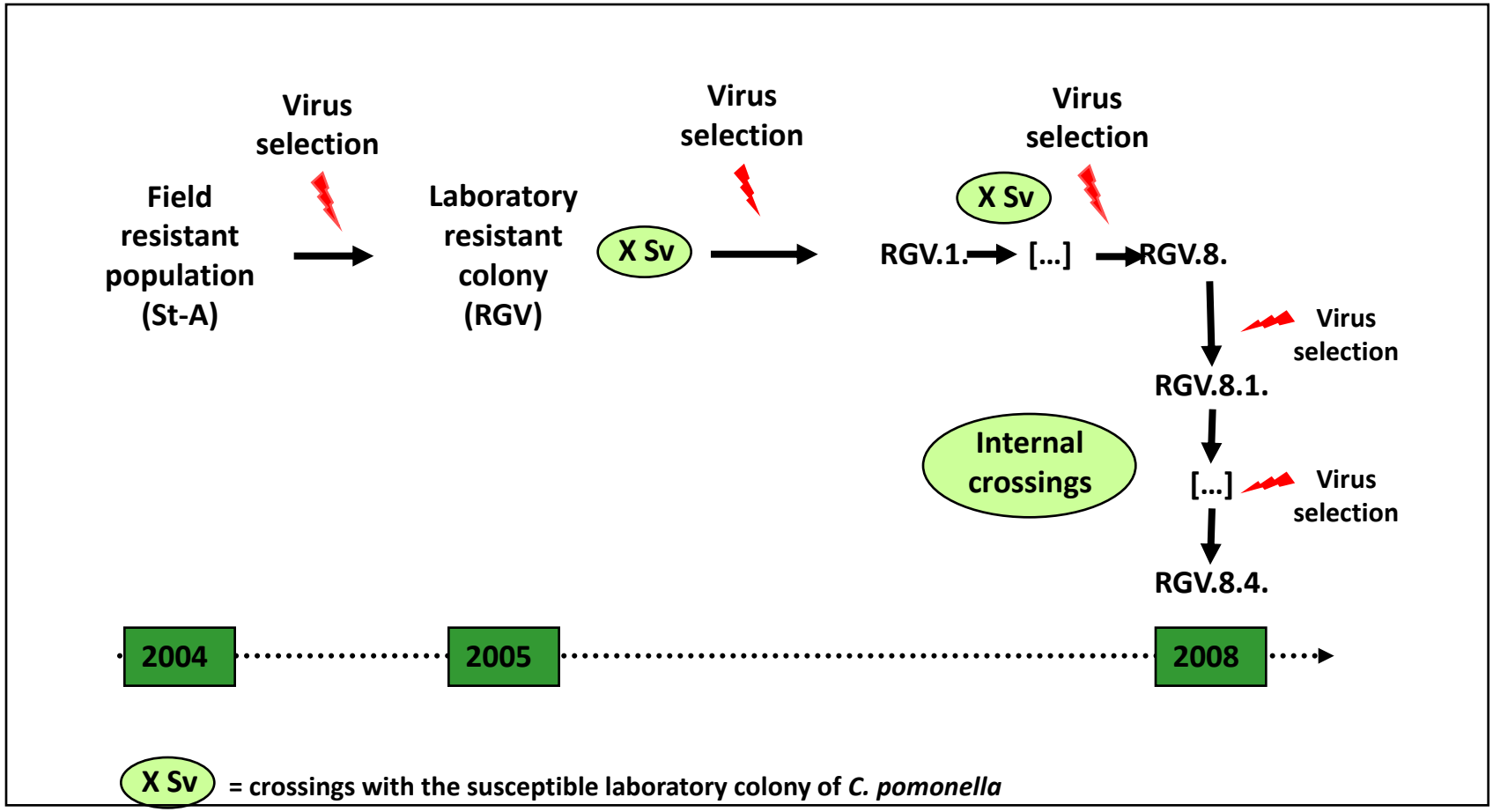
- Establishment of a resistant insect colony in the lab
- Screening of different virus isolates and selection of one or several promising isolates
- Purification and enhancement by propagating on the resistant colony
- Field trials

Starting point



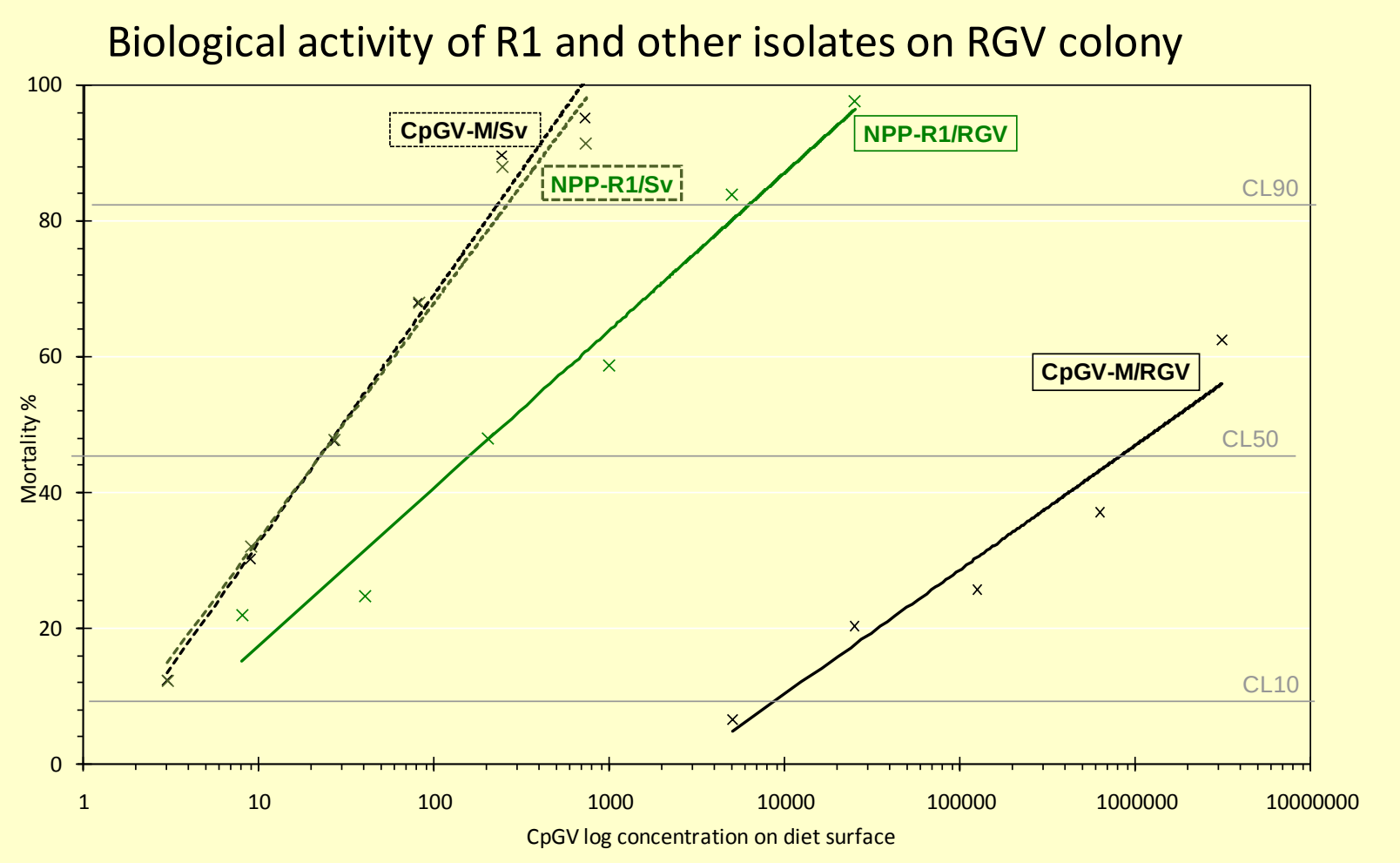
→ More than 13,000-fold a resistance factor

Introgression of resistance into a colony

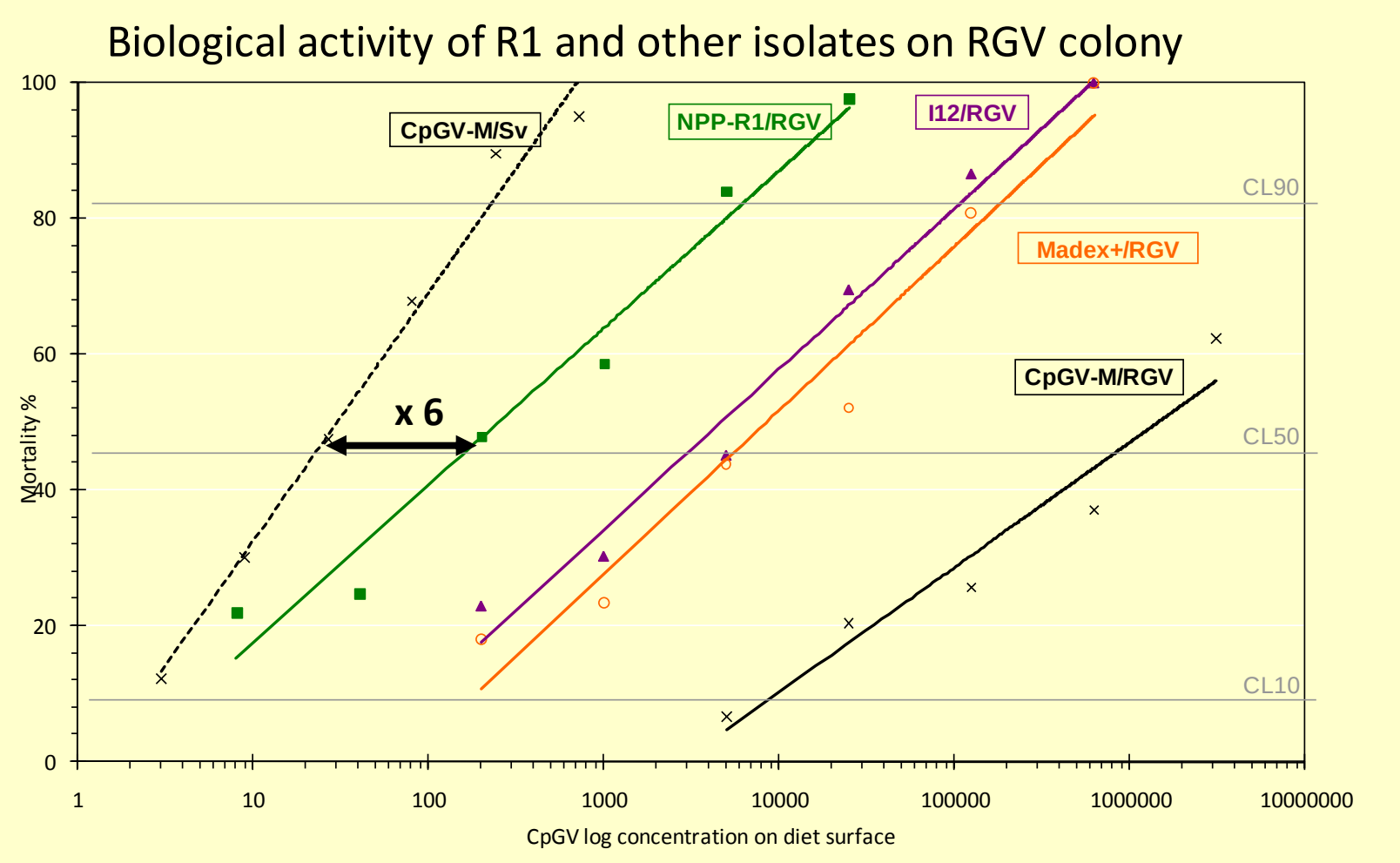


→ More than 50,000-fold a resistance factor after introgression

NPP-R1 in the lab



NPP-R1 in the lab



→ Same efficacy on susceptible colony as the Mexican isolate

→ Very promising efficacy on the 'RGV' resistant colony

NPP-R1 and I-12 in the field (2007)

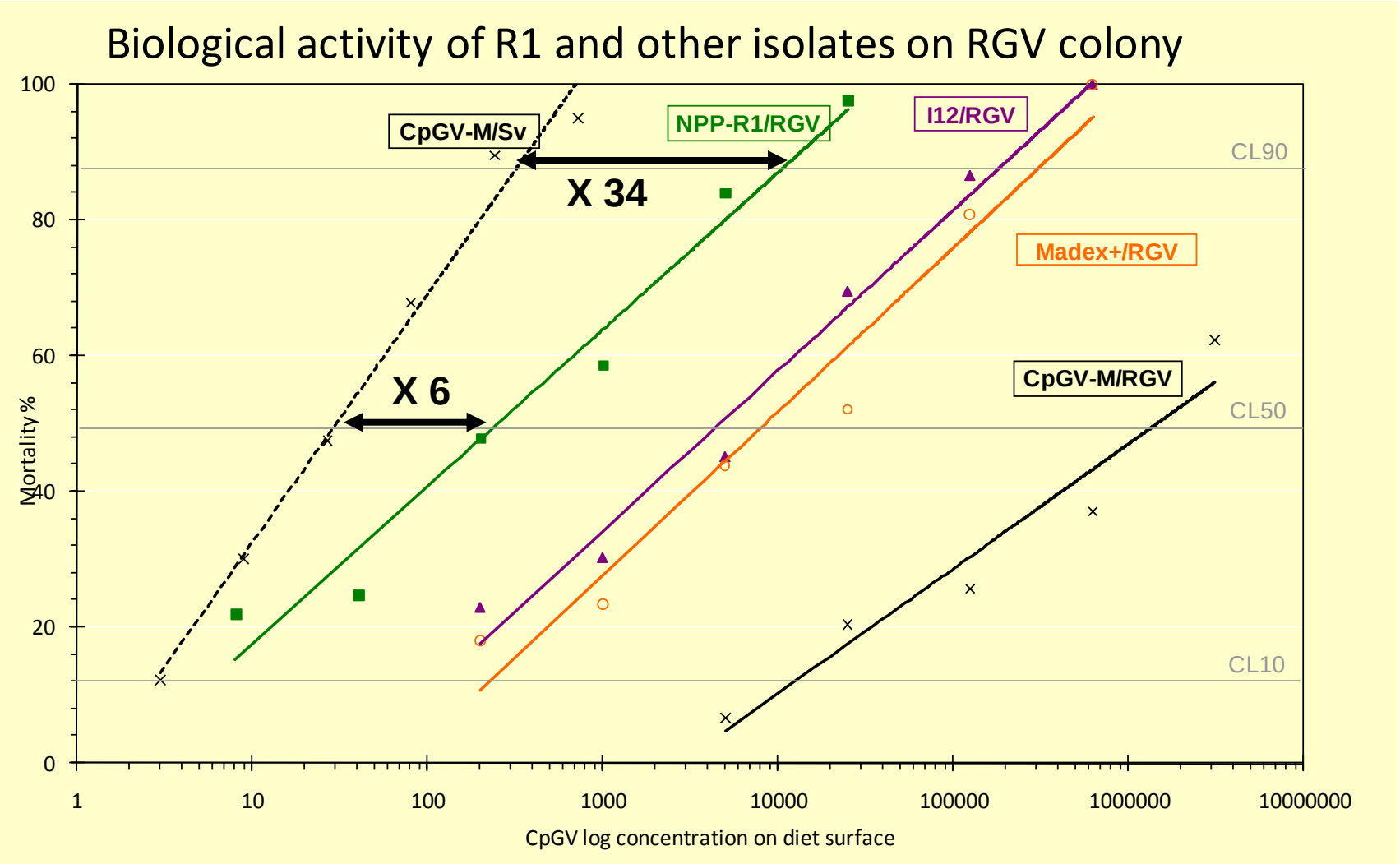
Trial in Germany (Neustadt)

- No resistance
- Efficacy of R1 and I-12 was almost zero

Trial in France

- Resistance
- Efficacy of R1 and I-12 was almost zero

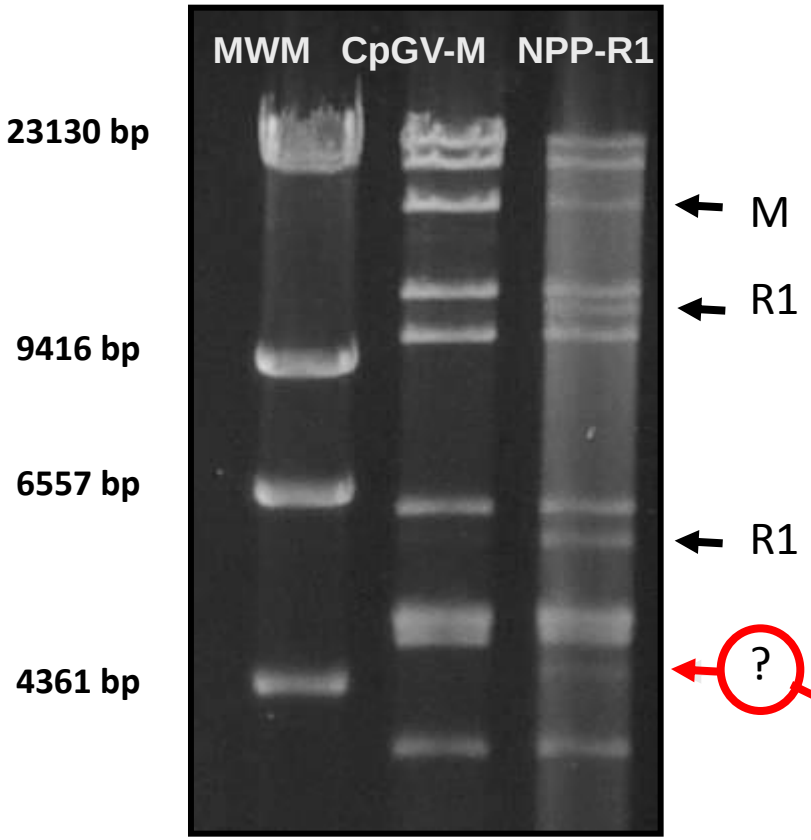
NPP-R1 and I-12 in the field (2007)



→ NPP-R1 needs improvement to be competitive in the field

Starting point: NPP-R1 restriction profile

Digestion with EcoRI

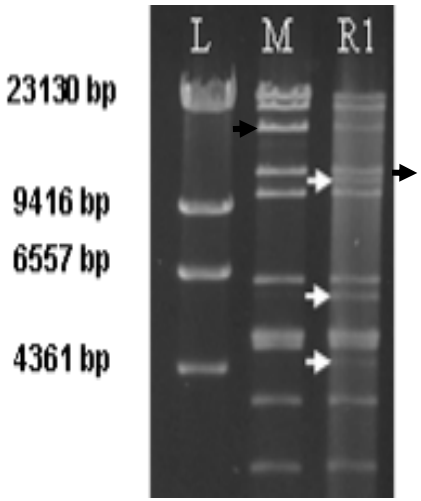


NPP-R1 is a mixture of at least 2 major genotypes:

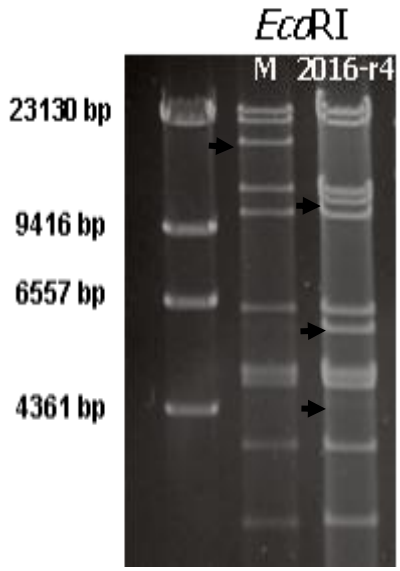
- M-Type = 30%
- "R1"-Type = 70%

Purification – enhancement of NPP-R1

Virus purification by successive passages on a selective host, the RGV colony



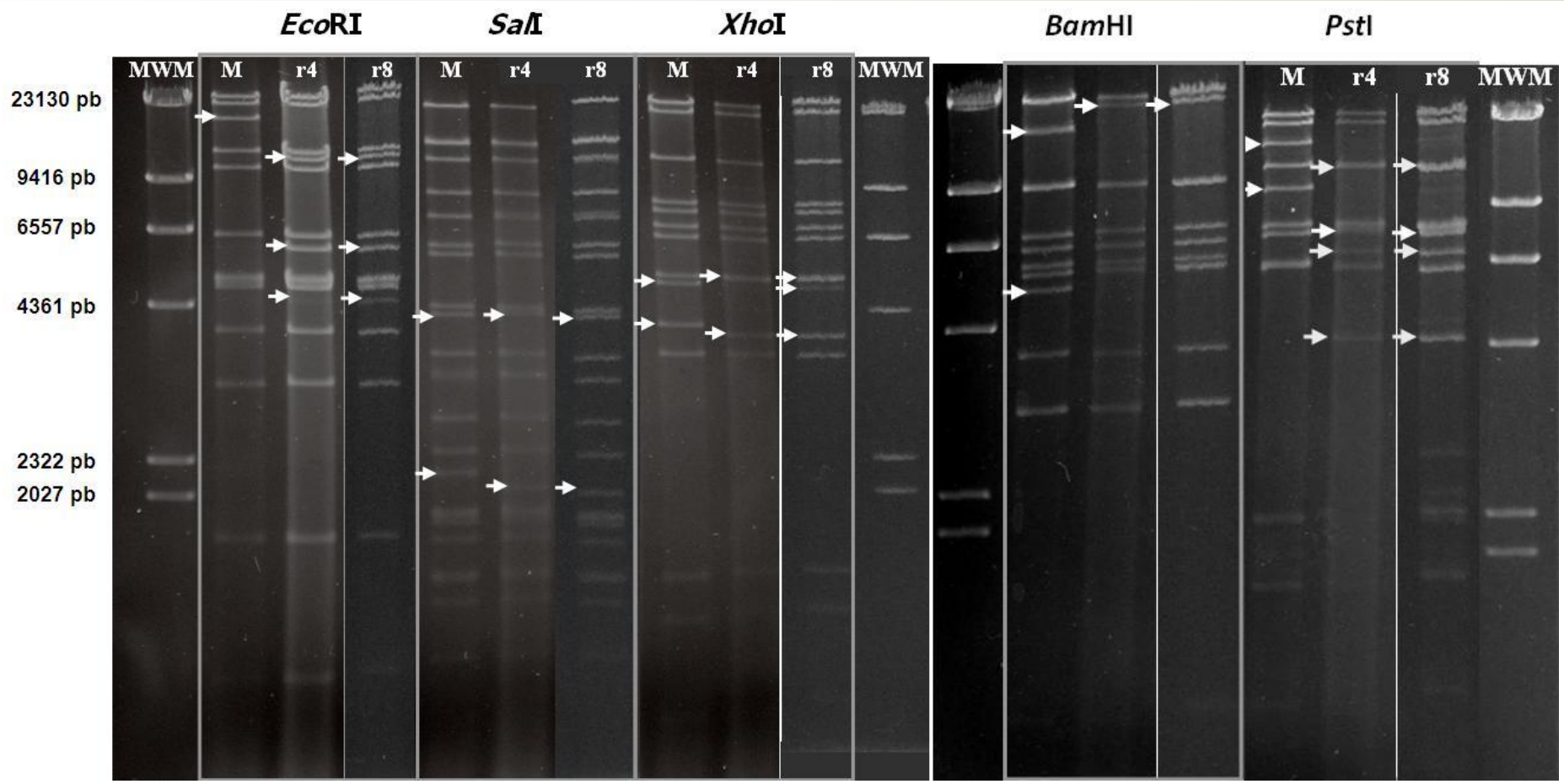
Mixture



R1 pure

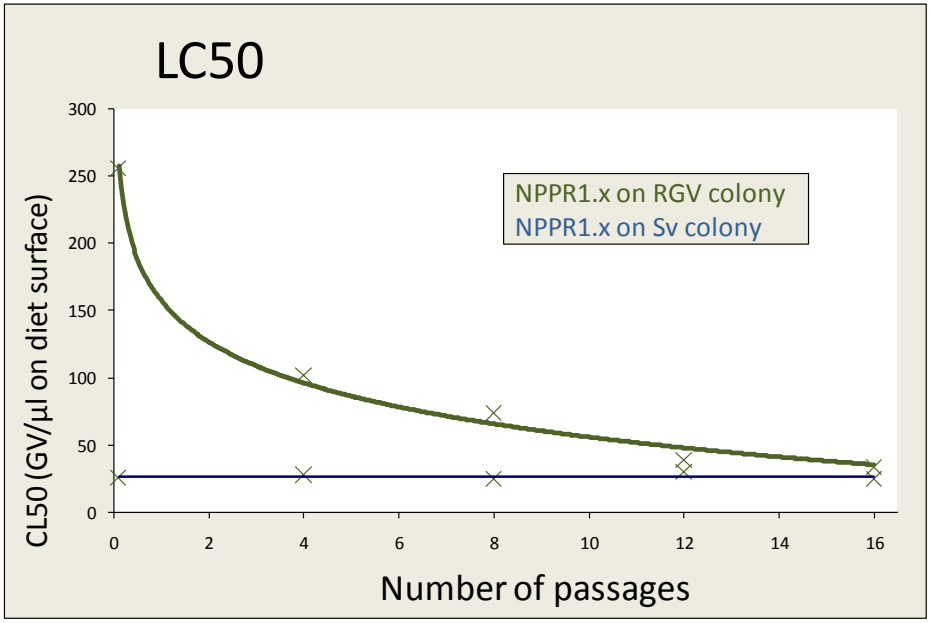
→ In 4 passages, reduction of the M-type to an indistinguishable level.

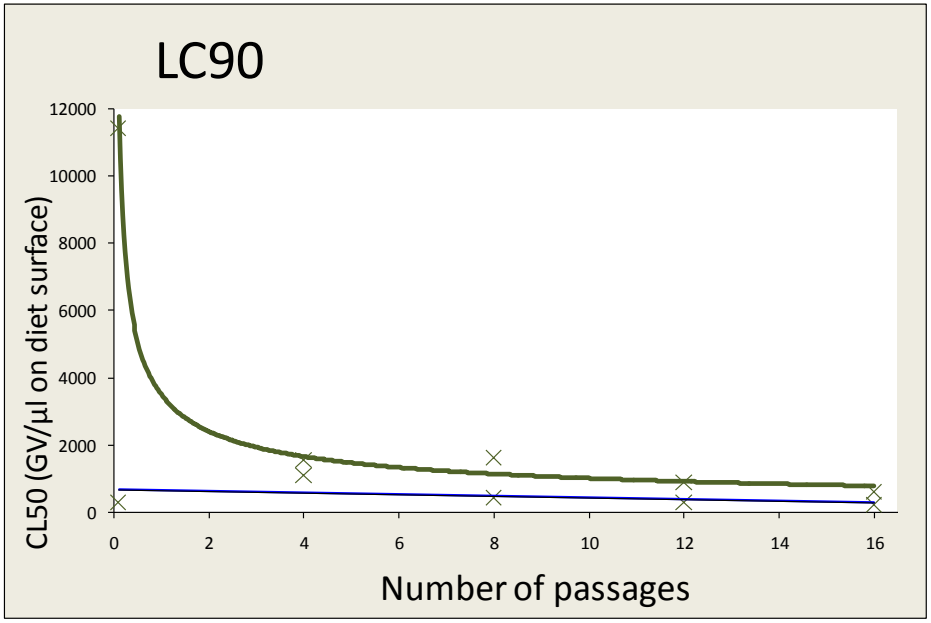
Complete restriction profile of NPP-R1



→ Same conclusion on 4 other enzymes (*Sal*I, *Xho*I, *Bam*HI, *Pst*1)

Biological enhancement of NPP-R1



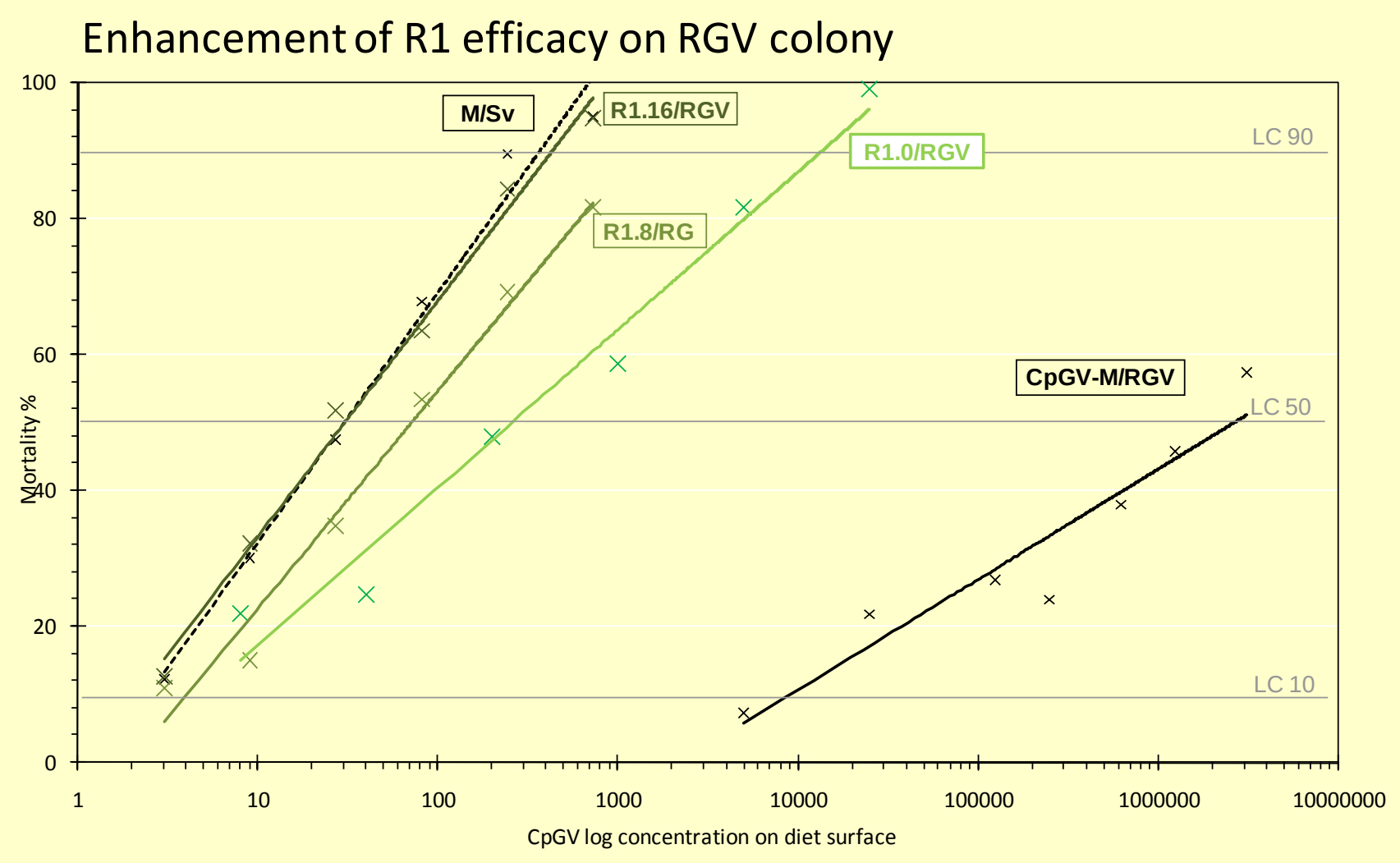


	LC50	min-max	ratio	LC90	min-max	ratio
CpGV-M / Sv	**29**	**(14-49)**		**377**	**(232-726)**	
NPP-R1.0	167	(91-278)	5.7	12800	(5950-38000)	34.0
NPP-R1.4	102	(63-147)	3.5	1570	(1010-2970)	4.2
NPP-R1.8	75	(43-114)	2.6	1630	(993-3300)	4.3
NPP-R1.12	39	(20-65)	1.3	921	(550-1850)	2.4
NPP-R1.16	34	(16-59)	1.2	636	(365-1400)	1.7

→ NPP-R1 should be competitive in the field

→ R1.8 was the last version available for the trials

Biological enhancement of NPP-R1



2008 field trials protocol

Untreated control

CpGV-M → to assess resistance of the population

I-12 at 10^{13} CpGV /ha

Madex Plus at 10^{13} CpGV /ha → normal dose of Carpovirusine / hectare

NPP-R1.8 at 10^{13} CpGV /ha

I-12 at $5x10^{13}$ CpGV /ha

Madex Plus x 5 → to check if 1L/ha is the maximum efficacy

NPP-R1.8 x 5

→ All CpGV solutions are Carpovirusine with the specific isolate (except in Germany were Madex 3 was used as positive control)

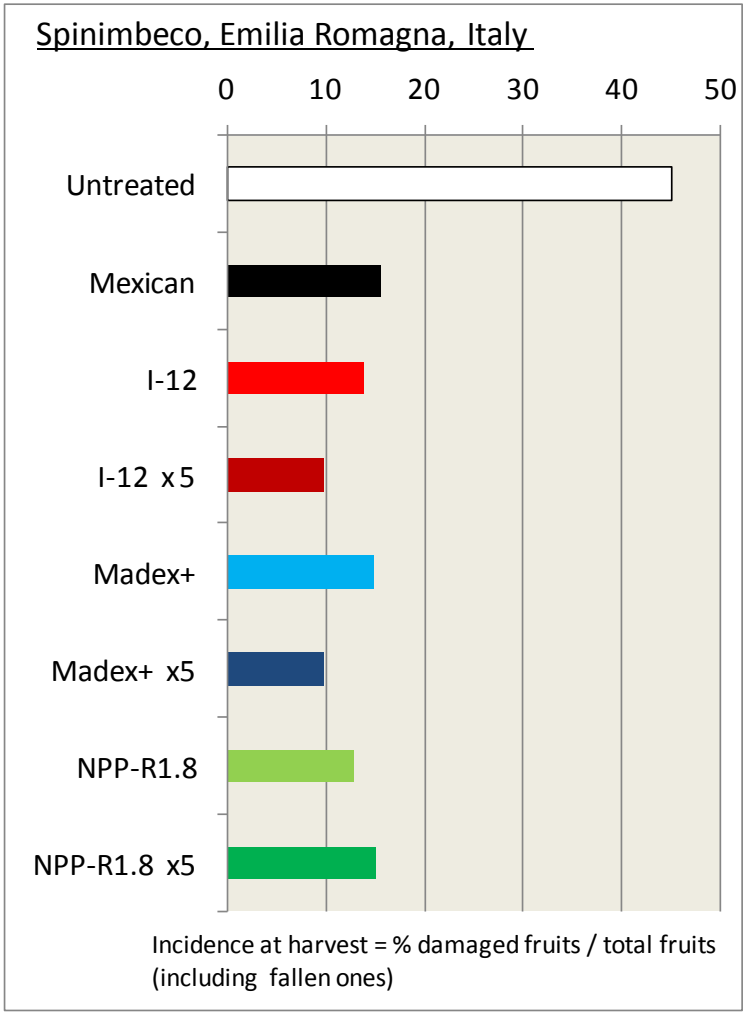
→ Corrugated cardboard bands were used for checking effect on populations

→ 7 trials on apple in Italy (2), Germany (2), France (3→1)

→ Locations were populations have been proven resistant (except 1 trial Germany)

Spinimbeco, Emilia Romagna, Italy

	Code	Damage 23/6		Efficacy G1	Incidence at harvest		Efficacy harvest	Traps healthy	Traps infected
1	Untreated	12.1	a		45.0	a		6.5	0.5
2	Mexican	3.3	b	67.3	15.6	b	65.6	1.8	1.8
3	I-12	2.4	b	78.9	13.8	b	69.7	0.8	0.3
4	I-12 x 5	1.3	b	89.6	9.7	b	78.7	0.3	0.5
5	Madex+	2.8	b	68.7	14.8	b	67.3	0.5	0.3
6	Madex+ x5	3.4	b	71.6	9.7	b	78.5	0.3	0.3
7	NPP-R1.8	1.3	b	88.2	12.8	b	71.7	0.3	0.0
8	NPP-R1.8 x5	0.7	b	93.7	15.1	b	66.8	0.0	0.0



Conclusions

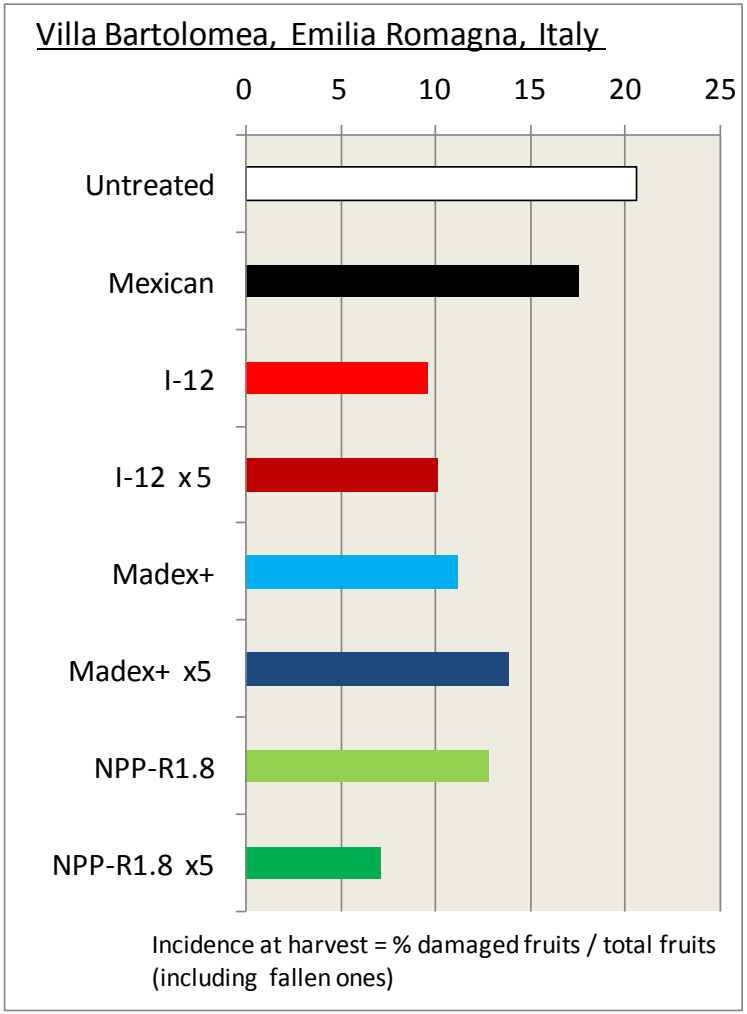
- No resistance.
- Extreme Codling moth pressure on harvest
- Same efficacy of the 3 new isolates for damage control
- No dose effect
- Good trend for population control

Villa Bartolomea, Emilia Romagna, Italy

	Code	Damage 27/6		Efficacy G1	Incidence at harvest		Efficacy harvest	Traps healthy	Traps infected
1	Untreated	7.0	ns		20.6	a		0.5	0.0
2	Mexican	5.3	ns	36.0	17.5	ab	15.0	1.0	0.8
3	I-12	2.5	ns	48.5	9.6	ab	53.4	0.8	0.5
4	I-12 x 5	1.6	ns	60.3	10.1	ab	51.0	1.5	0.0
5	Madex+	4.3	ns	37.5	11.2	ab	45.6	0.0	0.8
6	Madex+ x5	2.5	ns	58.3	13.8	ab	33.0	1.5	1.0
7	NPP-R1.8	1.2	ns	60.9	12.8	ab	37.9	0.0	0.3
8	NPP-R1.8 x5	0.3	ns	70.6	7.1	b	65.5	0.0	0.0

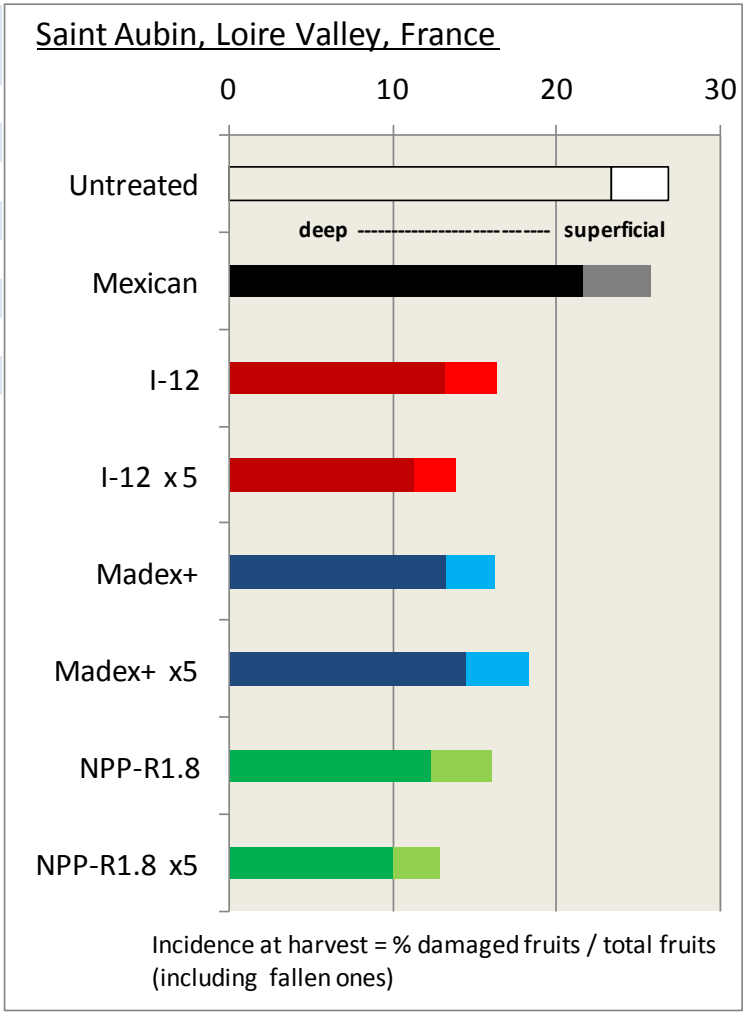
Conclusions

- Resistant population
- Very high Codling moth pressure on harvest
- Good efficacy trend for the 3 new isolates
- No clear dose effect
- Good trend for R1 at the end of G1 and for population control



St Aubin le dépeint, Loire Valley, France

	Code	Total damage		Deep damage		Superf. damage		Efficacy on total	Efficacy on deep	Traps healthy	Traps infected
1	Untreated	26.8	a	23.3	a	3.4	ns			3.3	0.0
2	Mexican	25.7	a	21.6	a	4.1	ns	3.8	7.4	2.3	0.3
3	I-12	16.3	b	13.1	b	3.2	ns	39.1	43.7	1.3	0.0
4	I-12 x 5	13.9	b	11.3	b	2.6	ns	48.2	51.7	0.3	0.0
5	Madex+	16.2	b	13.3	b	2.9	ns	39.6	43.2	1.3	0.3
6	Madex+ x5	18.2	b	14.4	b	3.7	ns	32.0	38.1	1.0	0.0
7	NPP-R1.8	16.0	b	12.3	b	3.6	ns	40.4	47.1	0.5	0.0
8	NPP-R1.8 x5	12.8	b	10.0	b	2.8	ns	52.3	57.2	0.8	0.3



Conclusions

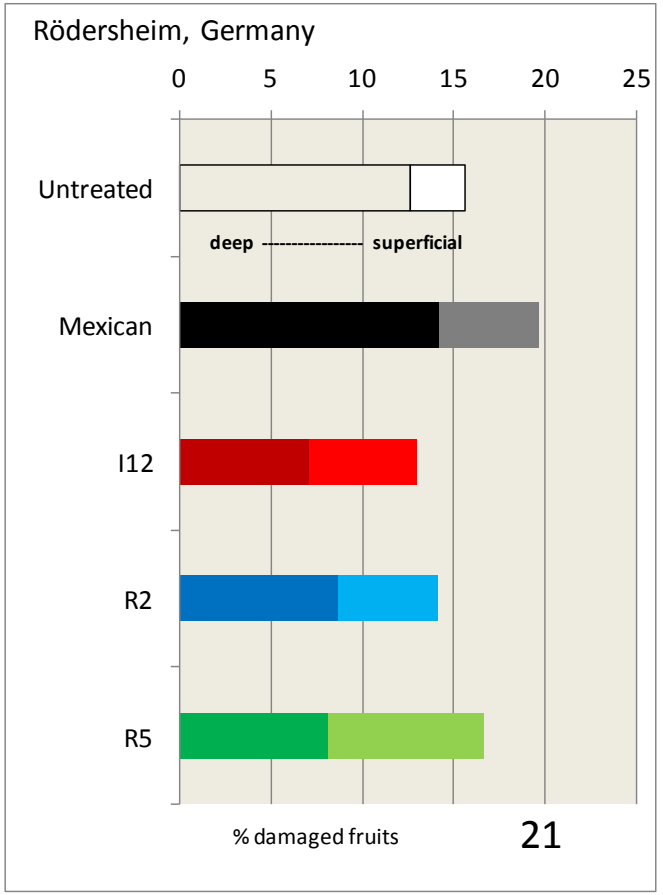
- Resistance population
- Very high Codling moth pressure on harvest
- The 3 new isolates provide good control of the damage (deep ones and total)
- No clear dose effect
- Good trend for R1

Rödersheim, Germany

	Code	Deep damage G1	Stopped damage G1	Total damage G1	Efficacy G1	Deep damage harvest	Stopped damage harvest	Total damage harvest	Efficacy harvest	Traps healthy	Traps infected
1	Untreated	8.1	2.7	10.8		12.6	3.0	15.6		0	0
2	Mexican	2.9	4.5	7.4	65%	14.2	5.4	19.6	-13%	6	0
3	I-12	0.7	2.1	2.8	91%	7.1	5.9	13.0	44%	2	0
4	Madex +	1.6	3.9	5.6	80%	8.6	5.5	14.1	31%	3	0
5	NPP-R1.8	1.1	5.4	6.5	86%	8.1	8.5	16.6	36%	1	0

Conclusions

- Population seems susceptible to M at G1 but not on harvest
- High pressure
- Excellent efficacy on 1st generation, very limited on 2nd
- I12 seems to work better
- Good trend for population control (traps)



General conclusion

- improvement of the virus efficiency by pressure of selection on resistant hosts was very successful in the lab
  - We are eager to test NPP-R1.16 in the field
  - New hosts (insect populations) will be necessary in the future

- Now alternate strains exist for overcoming resistance in the field,
  - The limited efficacy of the new isolates for controlling the damage is balanced by a good level of population control
  - Both parameters are showing that recovery from a situation of resistance is taking more than one season

- Field trials did not distinctly show the supremacy of NPP-R1 observed in the lab
  - Tremendous Codling moth pressure and high variability of the organic orchards do not provide enough discriminant data
  - At the moment we don’t know whether 1 isolate will be able to overcome resistance everywhere or if we will need several to adapt to local conditions
  - This is a crucial point for our future strategy

Thank you for your attention!