Genetic diversity of Belgian *Phytophthora ramorum* isolates

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ILVO - Plant (Crop Protection)
http://www.ilvo.vlaanderen.be/

Northern part of Belgium (Flanders) is one of the largest producers of *Rhododendron* in Europe

Results of the annual federal survey (FAVV)

- ± 50 major nurseries sites inspected
- 100-500 samples annually
- 20-25% of Belgian samples positive
- Main host plants:
  Rhododendron (>80%) and *Viburnum*

Global research genotyping *P. ramorum*

		Number of isolates	**Primer pairs**	**Different genotypes**	**Dominant genotype**
Ivors et al. 2004	AFLP	67 USA 18 EU	4	16 USA 15 EU	USA: 1
Ivors et al. 2006	SSLP	71 USA 80 EU	12	4 USA 7 EU	USA: 1 EU: 1
Prospero et al. 2007	SSLP	323 USA	10	33 USA	USA: 1 (OR)
Mascheretti et al. 2008	SSLP	292 USA	9	35 USA	USA: 3 (CA)

Objective

Determine the genetic diversity within the population of Belgian *P. ramorum* isolates

Materials and methods

1. **Amplified Fragment Length Polymorphism (AFLP)**

- 79 isolates (2002-2006)

- Protocol:
  - ❑ Adapted from Ivors et al. (2004)
  - ❑ Five pairs of selective primers
  - ❑ 2-3 replicates per *P. ramorum* isolate (reproducibility)

Materials and methods

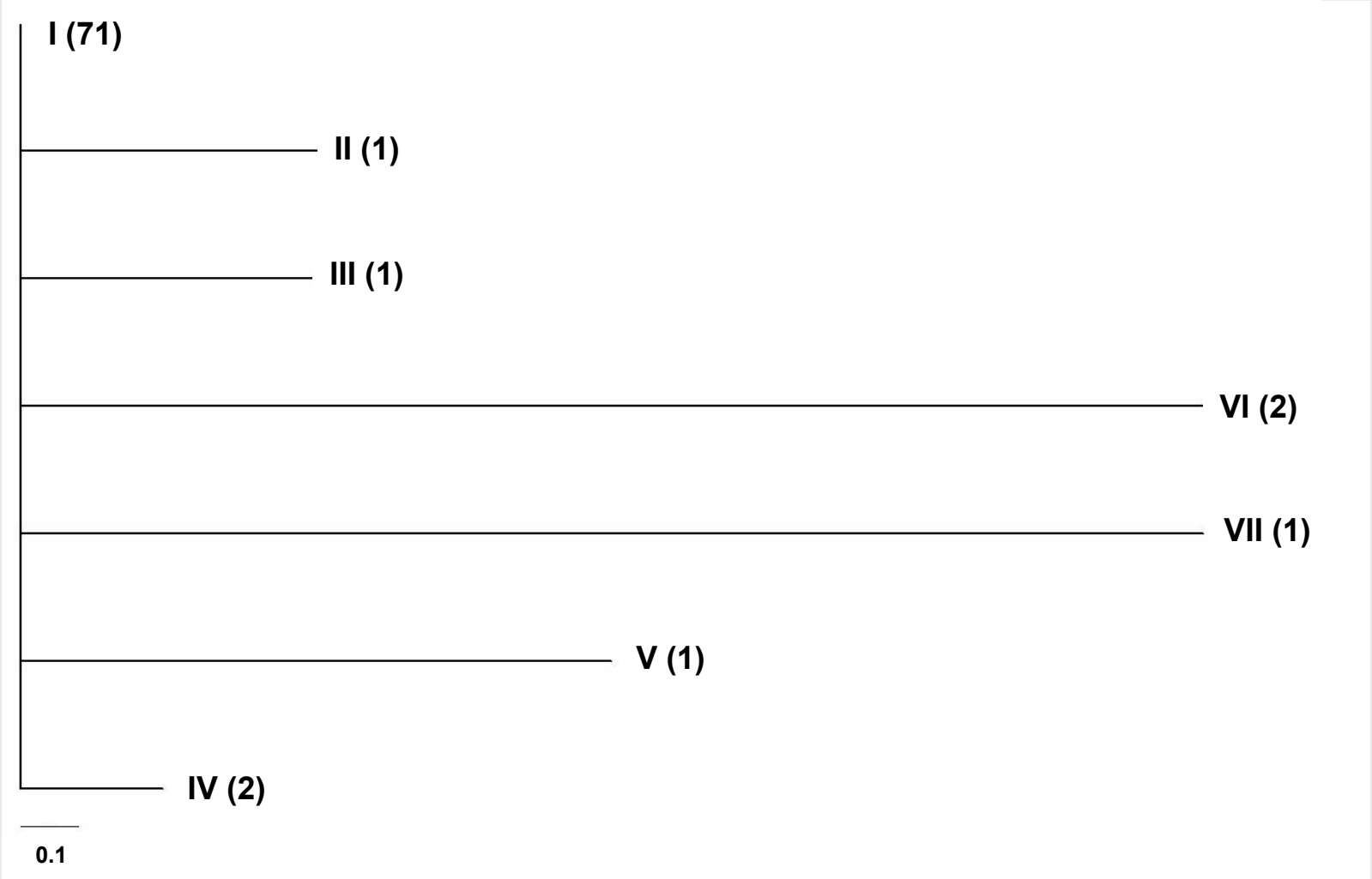
2. SSLP (microsatellite polymorphisms)

- 314 isolates (2002-2007)

- Protocol:
  - ❑ Screening of isolates with 6 selected SSR markers:
    - ✓ 3 from Ivors et al. (2006)
    - ✓ 3 from A. Vercauteren: screening of 132 candidate polymorphic SSR (screening based on polymorphisms within selected EU isolates)
  - ❑ Confirmation of polymorphic status of isolates

Results AFLP

- 5 primer pairs → 222 fragments →13 polymorphic
- 71 isolates (90%) belong to a single genotype
- 7 genotypes identified
- no correlation between genotype and place, year



Neighbour-joining cluster analysis

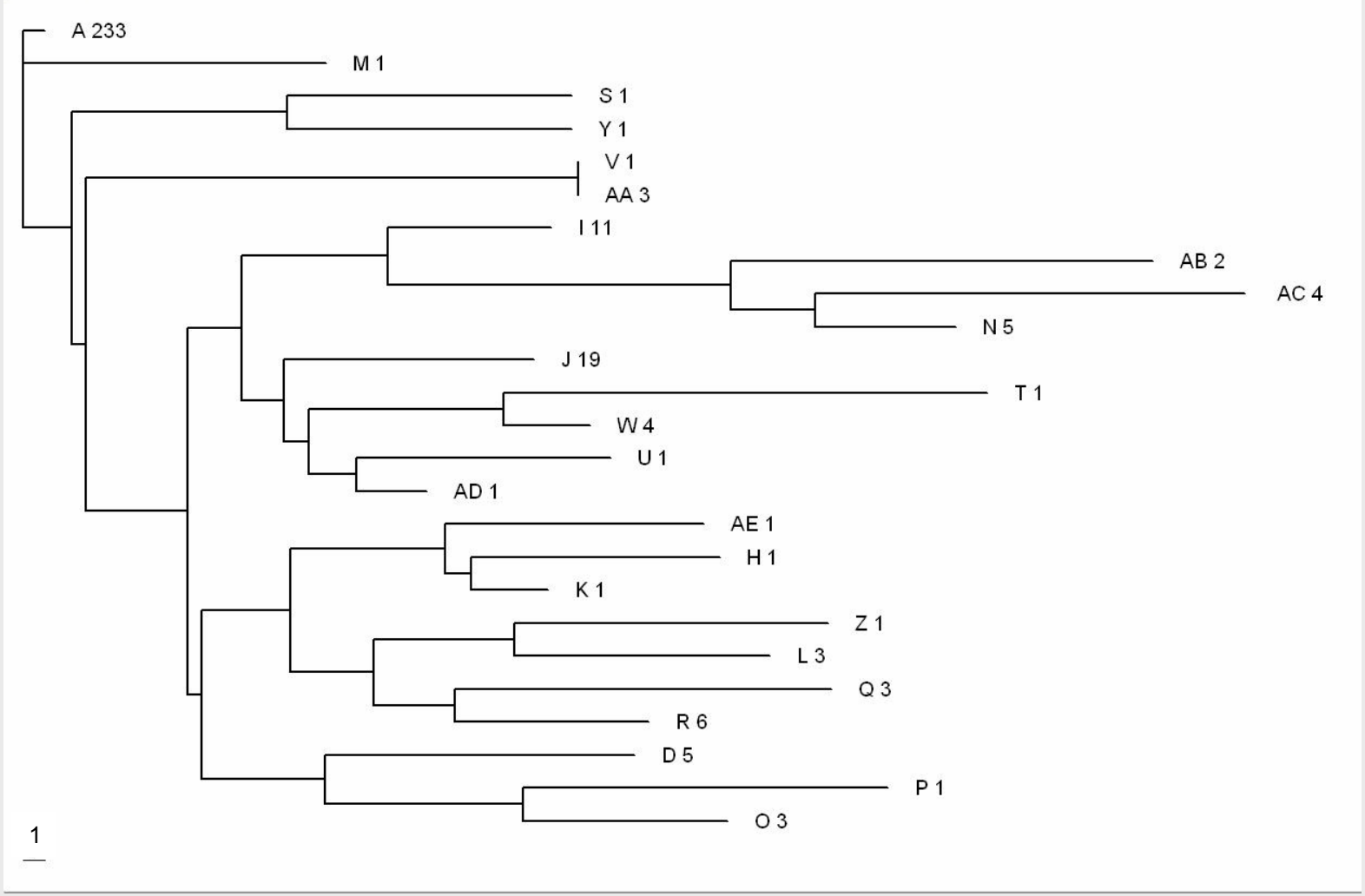
Results microsatellites

- 5 out of 6 EU-polymorphic primer pairs show polymorphisms within BE isolates
- 233 out of 314 isolates (74%) belong to one genotype
- 26 genotypes identified

Microsatellite primer pair code

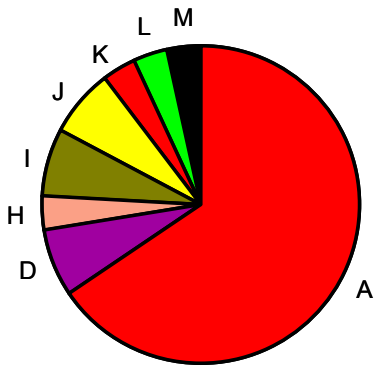
Genotypes

	Ivors et al.				Vercauteren				
	18	64	82		2199				512
A	262	398	137	242	186	202	217	265	156
D	262	**400**	137	242	186	202	217	265	156
H	262	398	137	**248**	186	202	217	265	156
I	262	398	137	242	186	202	217	**249**	156
J	262	398	137	242	186	202	217	**260**	156
K	262	398	137	**238**	186	202	217	265	156
L	262	398	137	**244**	186	202	217	**260**	156
M	262	398	137	242	-	-	217	265	156
N	262	398	137	242	186	202	**212**	**249**	156
O	262	**400**	137	242	186	202	217	**260**	156
P	262	**400**	137	**244**	186	202	217	**260**	156
Q	262	398	137	**244**	186	202	217	**270**	156
R	262	398	137	**244**	186	202	217	265	156
S	262	398	137	242	186	202	217	265	**167**
T	262	398	**133**	242	186	202	217	**270**	156
U	262	398	137	242	186	202	217	**254**	156
V	**260**	398	137	242	186	202	**222**	265	156
W	262	398	137	242	186	202	217	**270**	156
X	262	398	137	242	186	202	**212**	265	156
Y	262	398	137	242	186	202	217	265	**171**
Z	262	398	137	**228**	186	202	217	**260**	156
AA	262	398	137	242	186	202	**222**	265	156
AB	262	398	137	**246**	186	202	**212**	**249**	156
AC	262	**402**	137	242	186	202	**212**	**249**	156
AD	262	398	137	242	186	202	217	**243**	156
AE	262	398	137	**246**	186	202	217	265	156

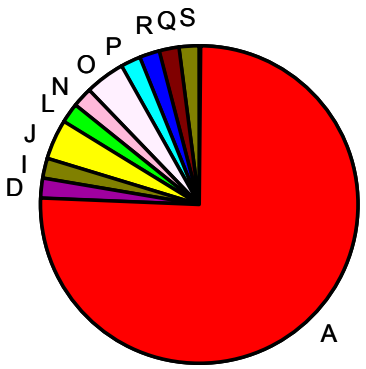


Neighbour-joining cluster analysis

Genotype composition of Belgian *P. ramorum* population



2002 (29)



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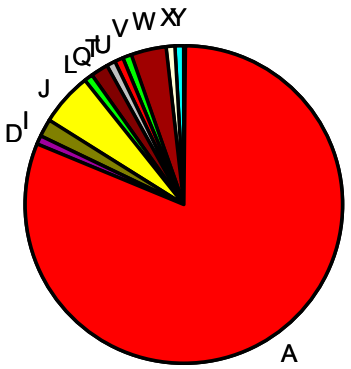

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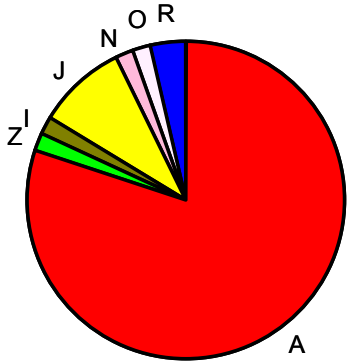

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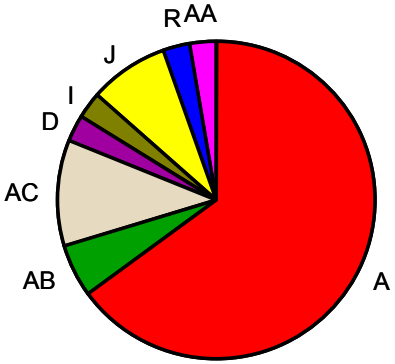

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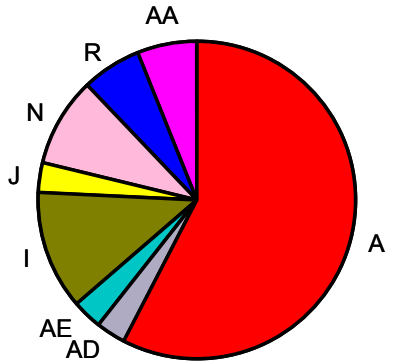
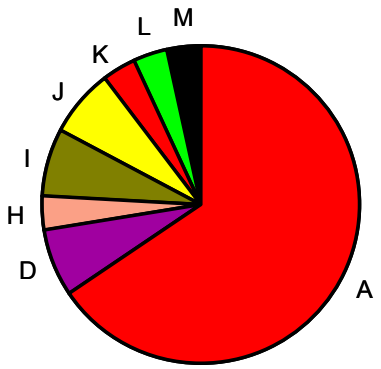


2007 (33)

Resistance to fungicide metalaxyl-M

Year	Percentage resistant isolates
2002	29
2003	17
2004	56
2005	95
2006	84
2007	59

Genotype composition of Belgian *P. ramorum* population



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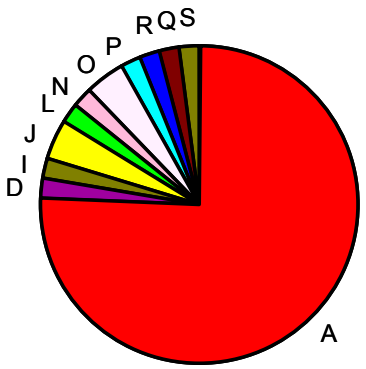

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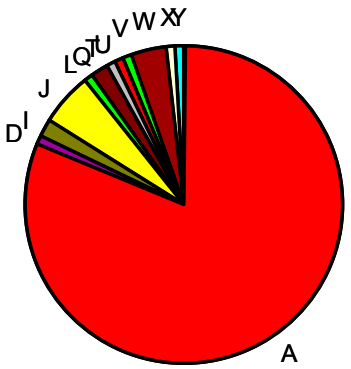

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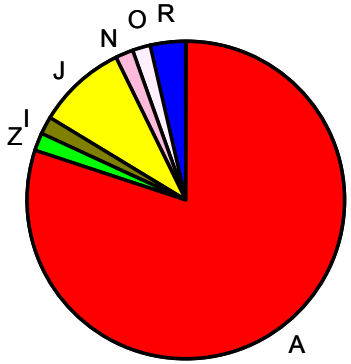

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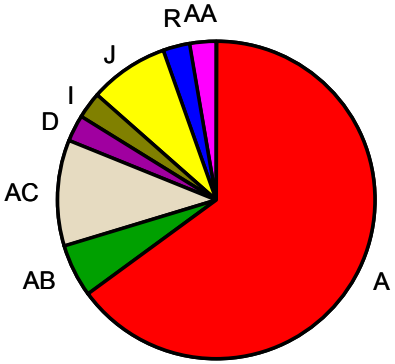
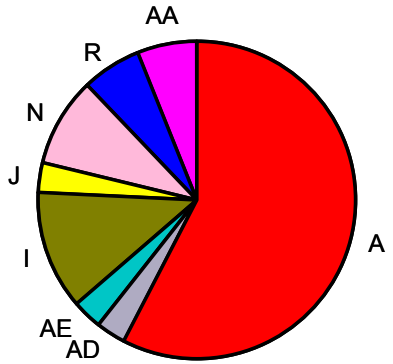
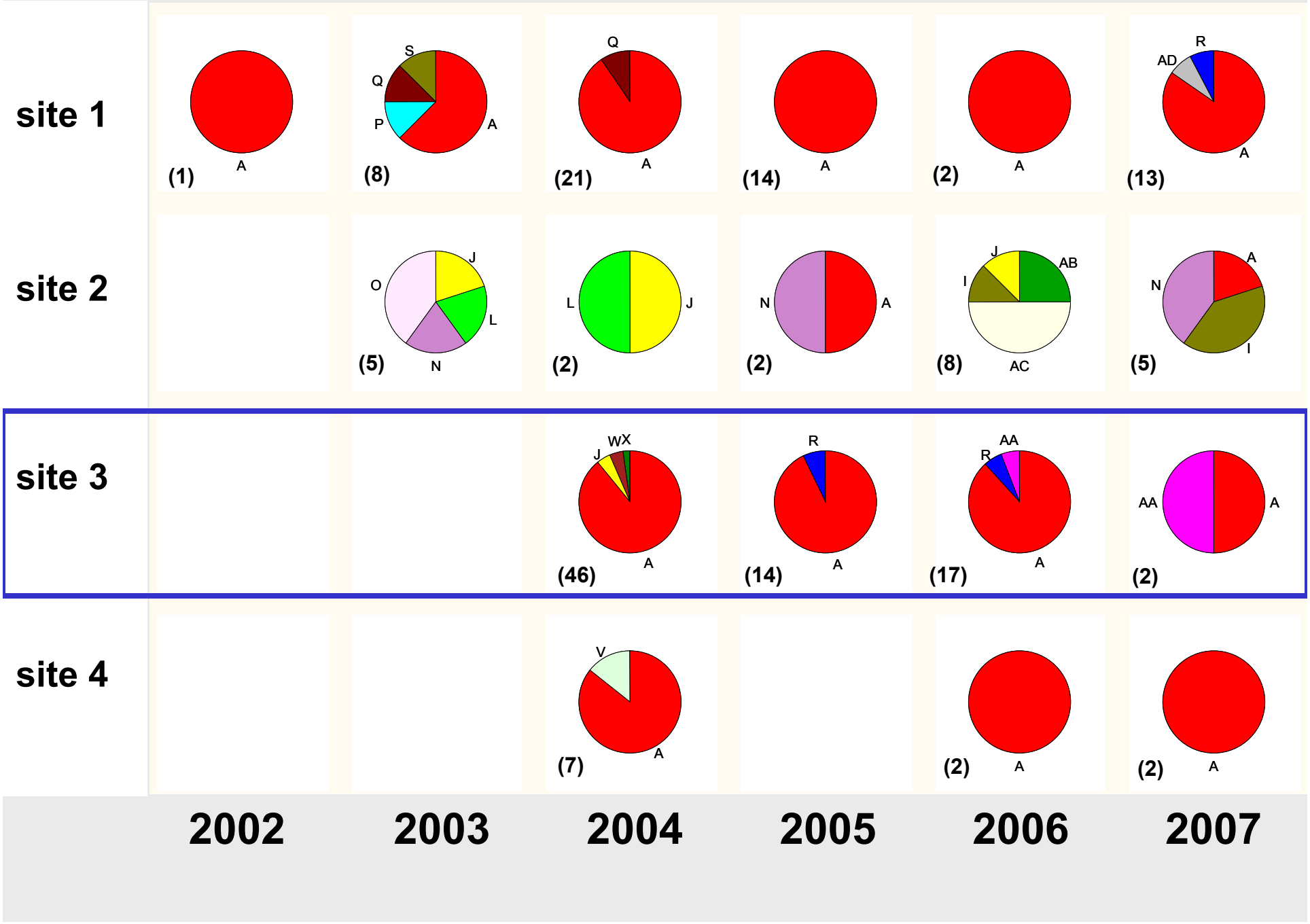


2006 (37)

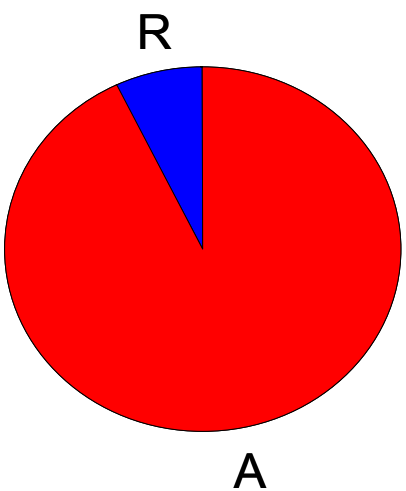
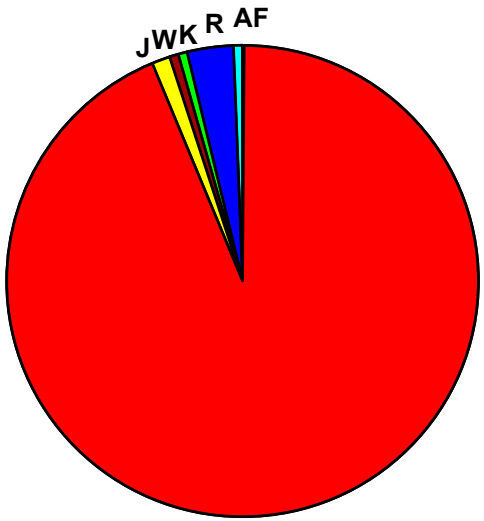


2007 (33)

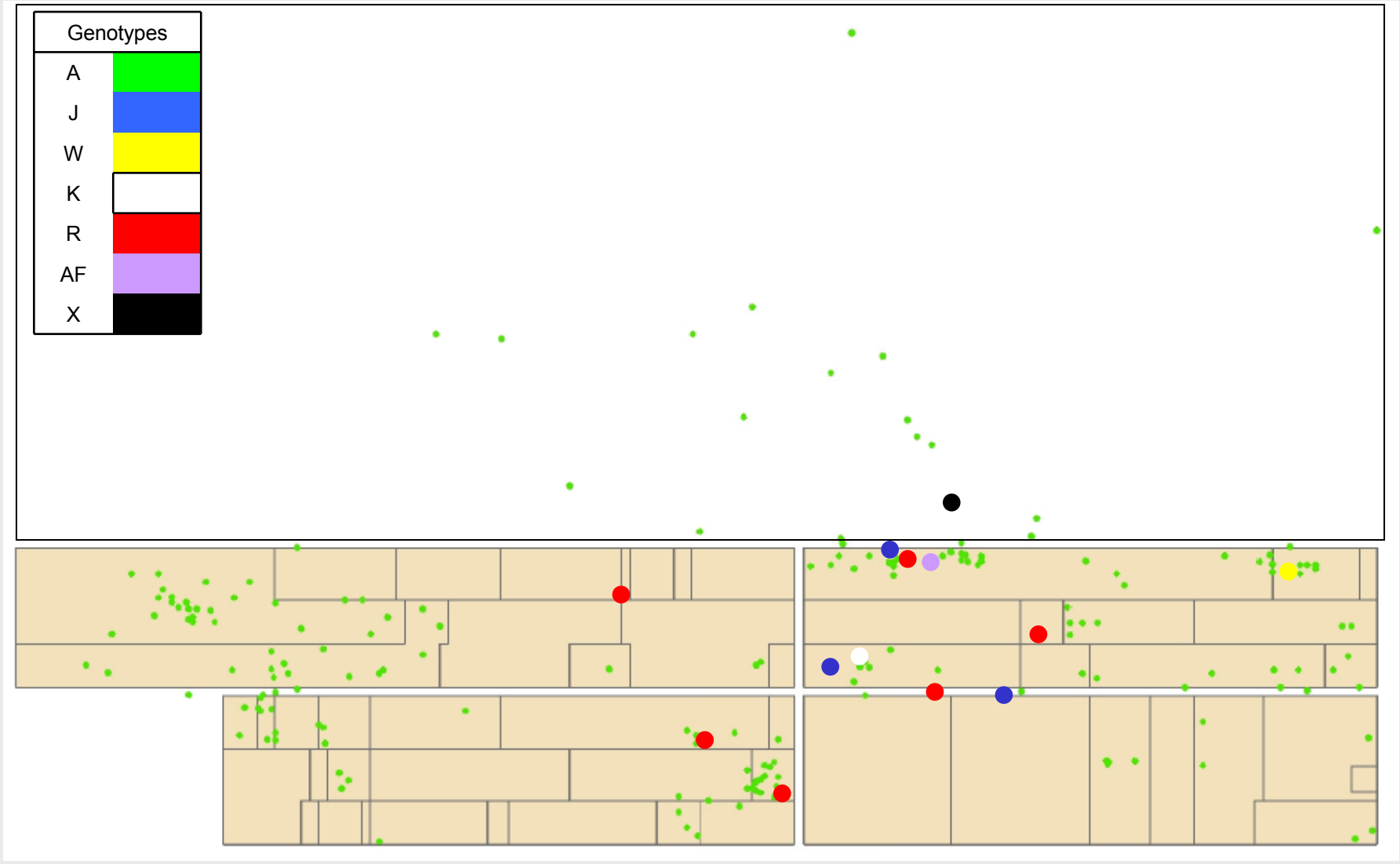
Local occurence of genotypes



2004-2005: 1399 samples were collected → 331 *P. ramorum* isolates found

	2004	2005
Federal survey	 (46)	 (14)
Project	 (157)	 (174)

Field surveyed in 2004



10m

Conclusions

- Multiple *P. ramorum* genotypes in Belgium (based on AFLP and SSLP)
- One genotype is dominant in Belgium (AFLP: 90% – SSLP: 74%)

  → founder genotype
- Other genotypes (6/79 isolates with AFLP – 25/314 isolates with SSLP)

  → mostly single isolates
- Evolution: some genotypes disappear, new genotypes arise
- Bottleneck in 2004-2005 : metalaxyl-M resistance
- Local specialization: 15/26 genotypes found at single nurseries
- Application to study of epidemiology → dispersal of genotypes in a field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