Resistance behaving badly!

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ROTHAMSTED RESEARCH

BBSRC bioscience for the future

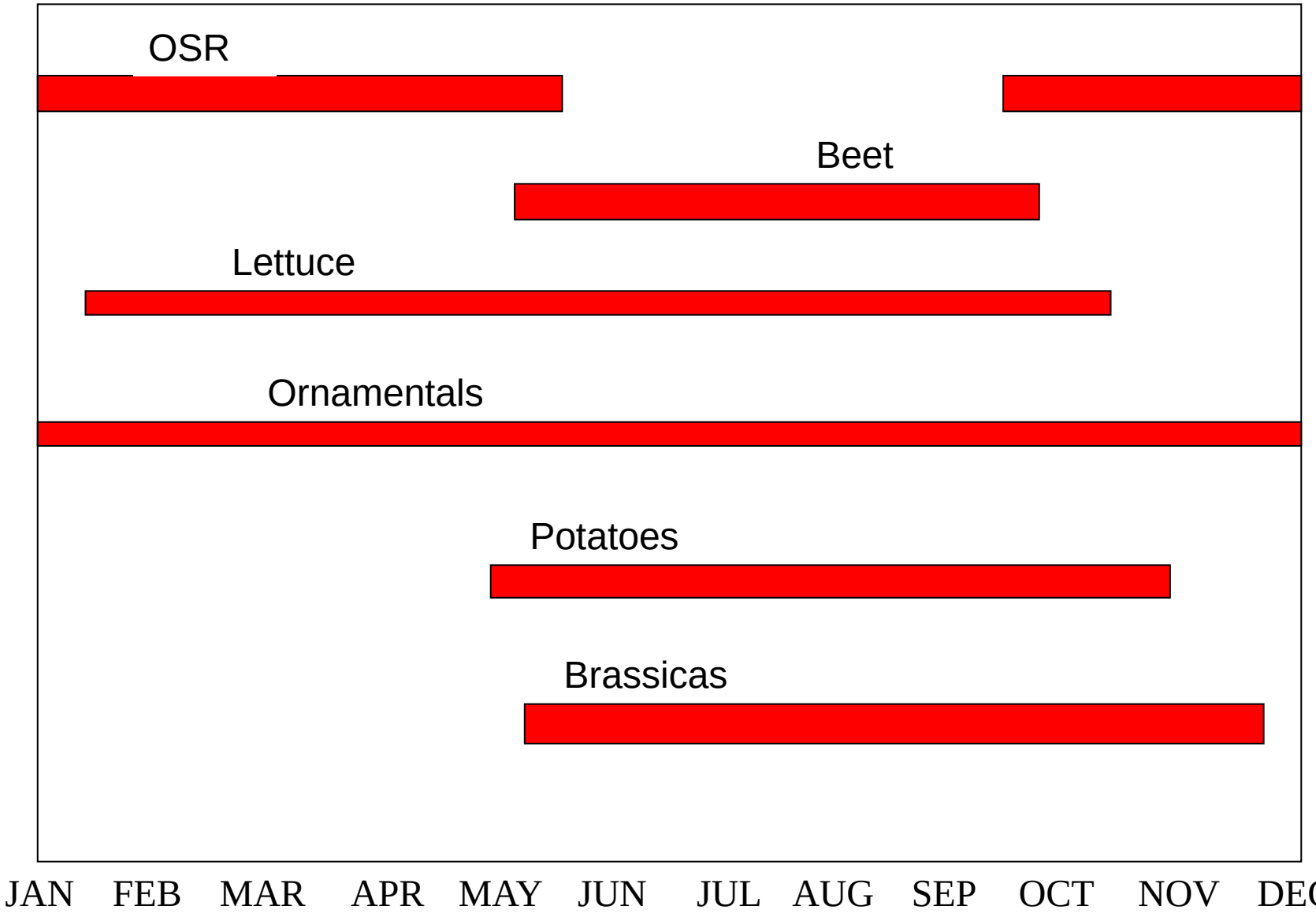
University of Hertfordshire

IRAC, Jealotts Hill, March 2013

Peach-potato aphid, *Myzus persicae*

- Highly polyphagous
- Parthenogenetic on field crops
- Sexual cycle on peach

Seasonal dynamics of *M. persicae* on host plants in the UK



Resistance mechanisms in *M. persicae*

Overproduced carboxylesterase (E4/FE4)

S, R1, R2 and R3 ‘phenotypes’

- organophosphates, monomethyl carbamates

Modified acetylcholinesterase

MACE (S431F)

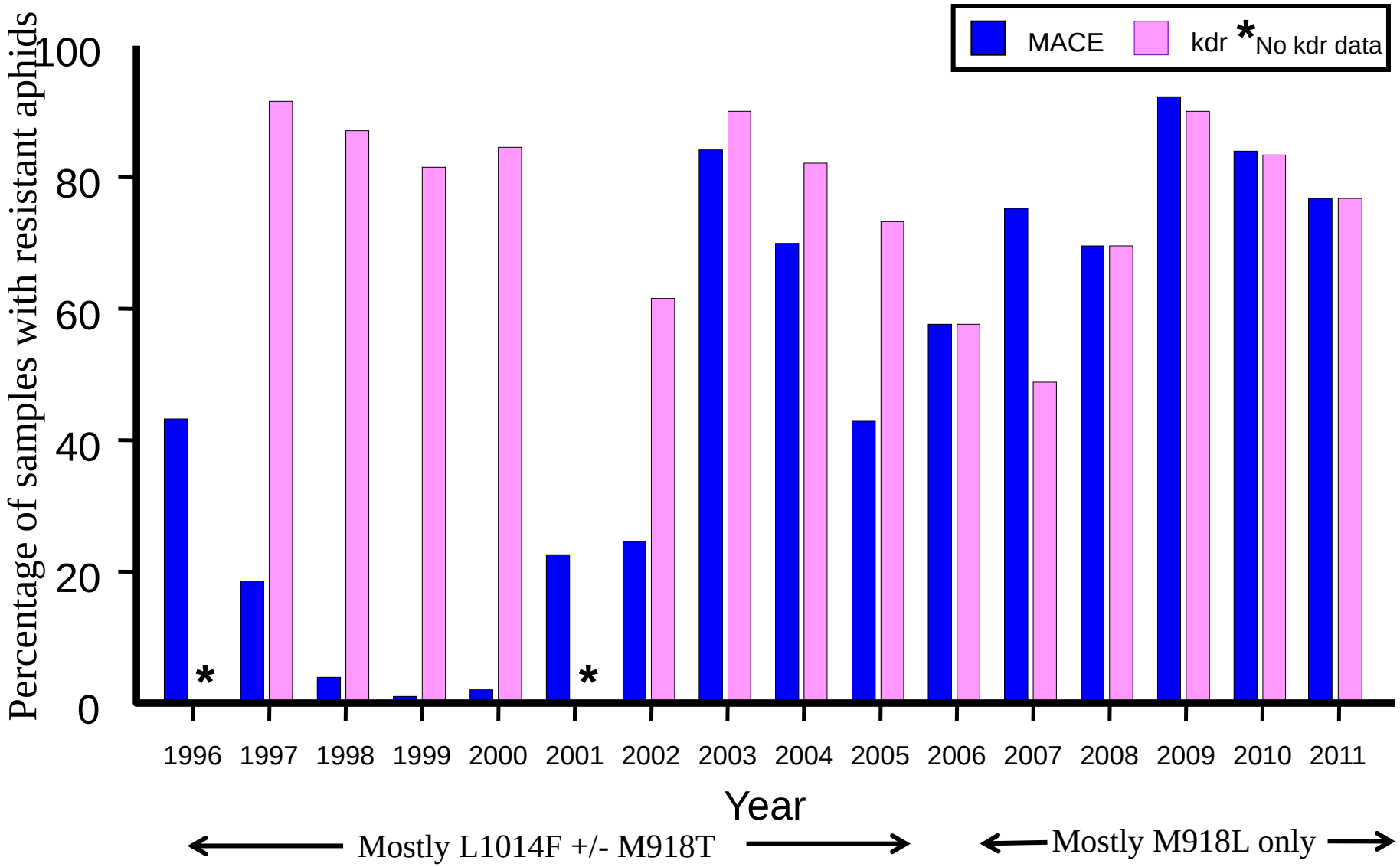
- dimethyl carbamates (pirimicarb)

Modified voltage-gated sodium channel

Kdr (L1014F), Super-kdr (M918T or M918L)

- pyrethroids

Dynamics of the MACE and Knockdown resistance mechanisms in field-caught *M. persicae* in the UK



Resistance mechanisms in *M. persicae*

Mechanism	Potential 'genotypes'
Overproduced carboxylesterase (E4/FE4) S, R1, R2 and R3 'phenotypes' - organophosphates, monomethyl carbamates	4
Modified acetylcholinesterase MACE (S431F) - dimethyl carbamates (pirimicarb)	3
Modified voltage-gated sodium channel Kdr (L1014F), Super-kdr (M918T or M918L) - pyrethroids	18(?)
Total possible multi-locus genotypes	216(?)

But very few of these possible combinations have been found!

What drives these dynamics?

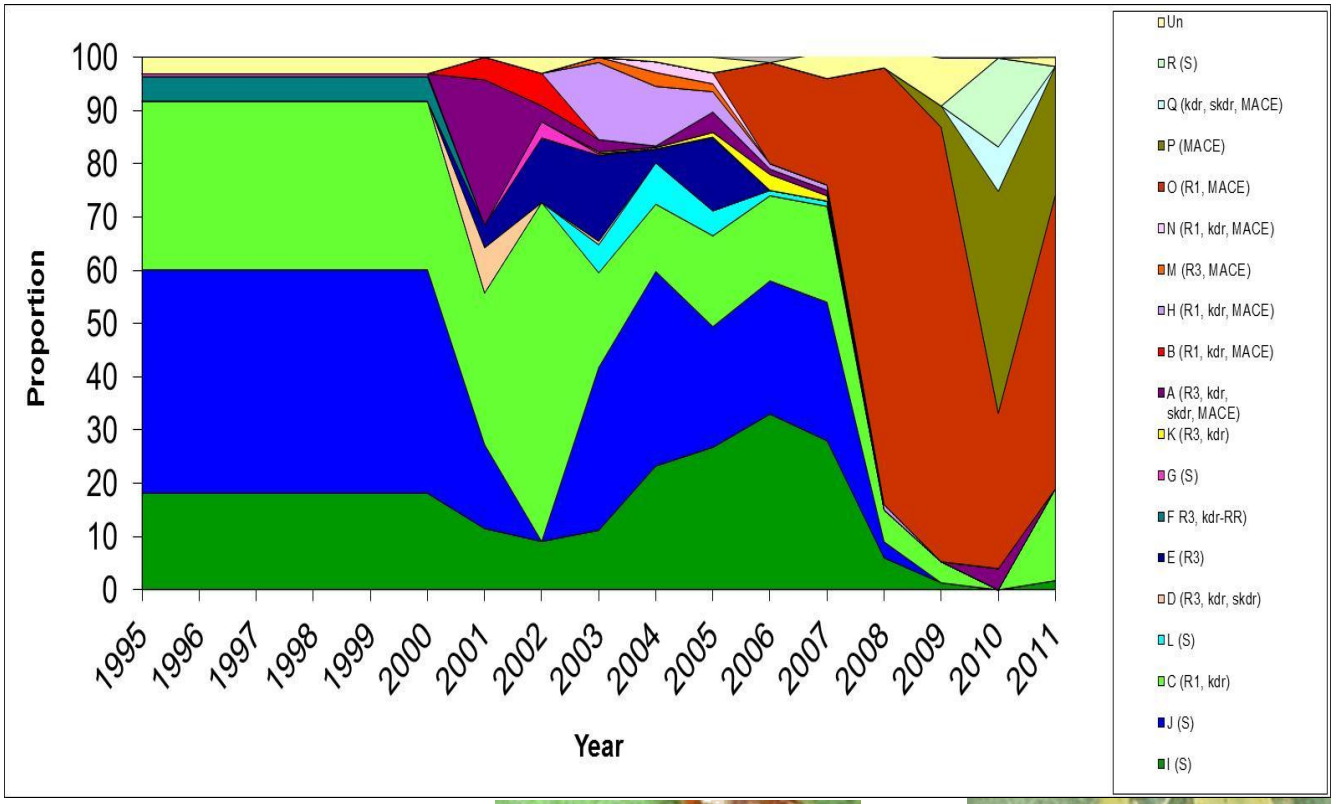
Insecticide use MUST play an important role

BUT

genotypic variation is extremely limited, and resistance dynamics can’t be readily related to patterns of insecticide use

Something else is going on!

Temporal changes in clonal composition



Clones ‘O’ and ‘P’ share the same multi-locus resistance genotype:

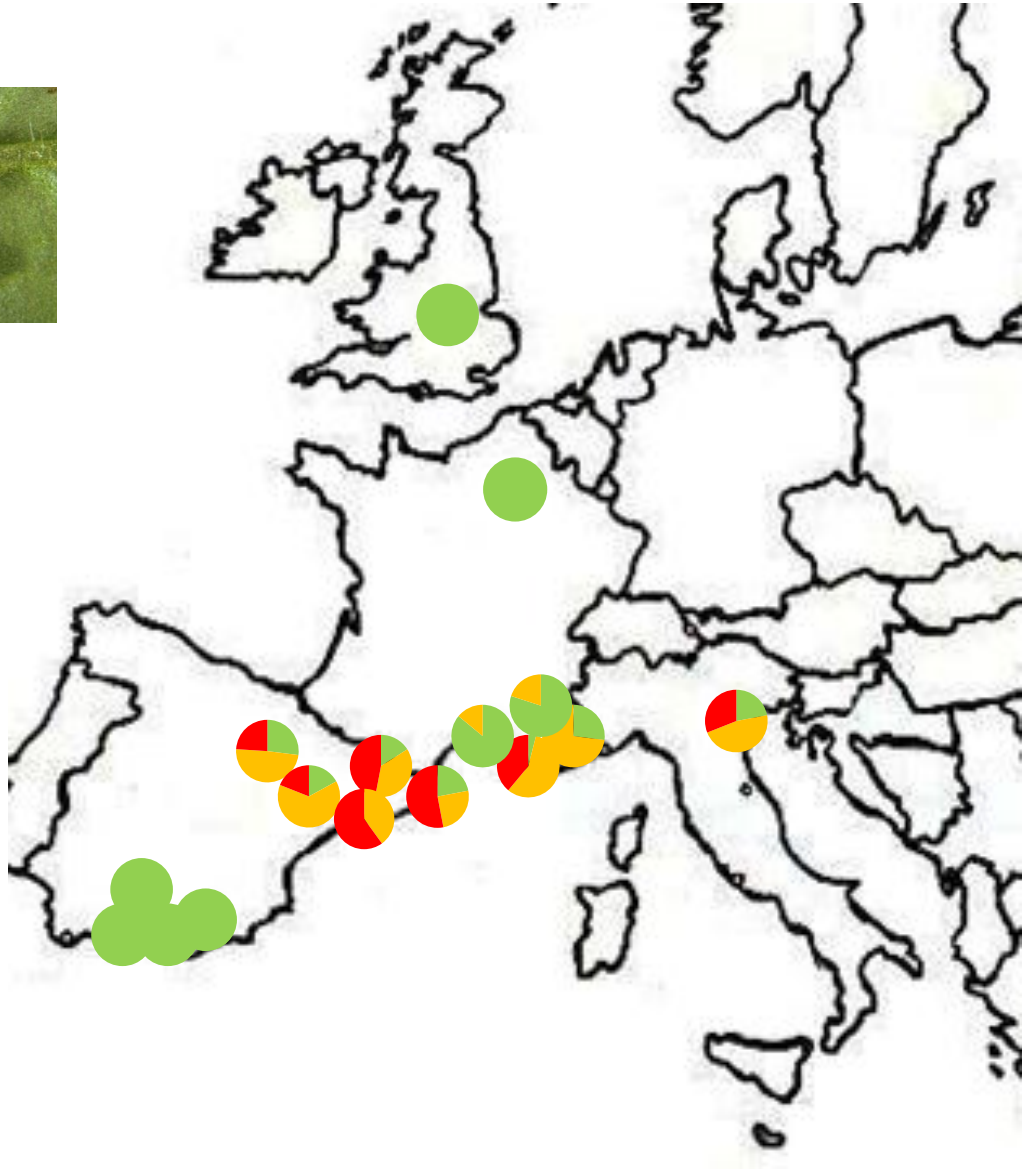
Overproduced carboxylesterase	R1
MACE	S431F/+
Knockdown resistance	M918L/+

This genotype accounts for 80-90% of all individuals in the UK

In a parthenogenetic species:

- Resistance genes appear in a particular clonal background and cannot transfer to a new one other than by recurrent mutation or sexual recombination in another part of the species' range.
- Dynamics of resistance depends on an interplay of insecticide use and overall competition between clones in the face of other environmental challenges (overwintering, host range etc).
- Resistance genes present in a highly competitive clonal background (e.g. 'O' and 'P') can persist at high frequencies irrespective of what insecticides are being used. This greatly limits opportunities for managing resistance.
- New resistance genes (e.g. ones conferring neonicotinoid resistance) can still invade, but competition with existing clones may limit their ability to establish and spread.

Neonicotinoid resistance in *Myzus persicae* - 2011



Green – SS

Orange – SR

Red - RR

Grain aphid, *Sitobion avenae*

- Major pest on wheat
- Adult size 1.3 - 3.3mm
- Majority of population spends entire year on cereals and grasses
- Colonies of wingless aphids develop on leaves and emerging ears
- Direct damage via feeding and indirect damage via transmission of plant viruses
- Autumn migrants infest winter cereals and grasses

Life cycle of *S. avenae*

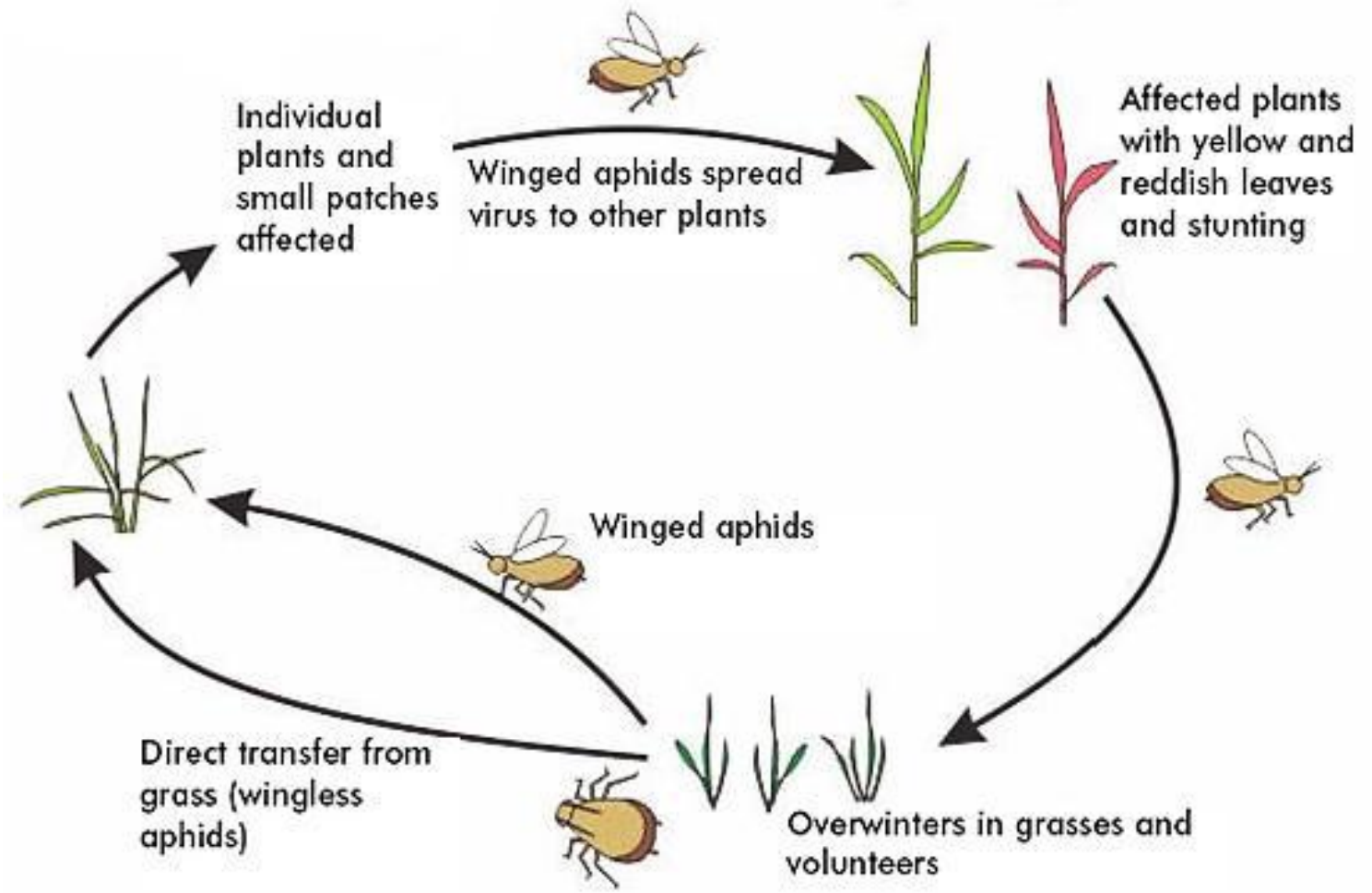


Image from HGCA website

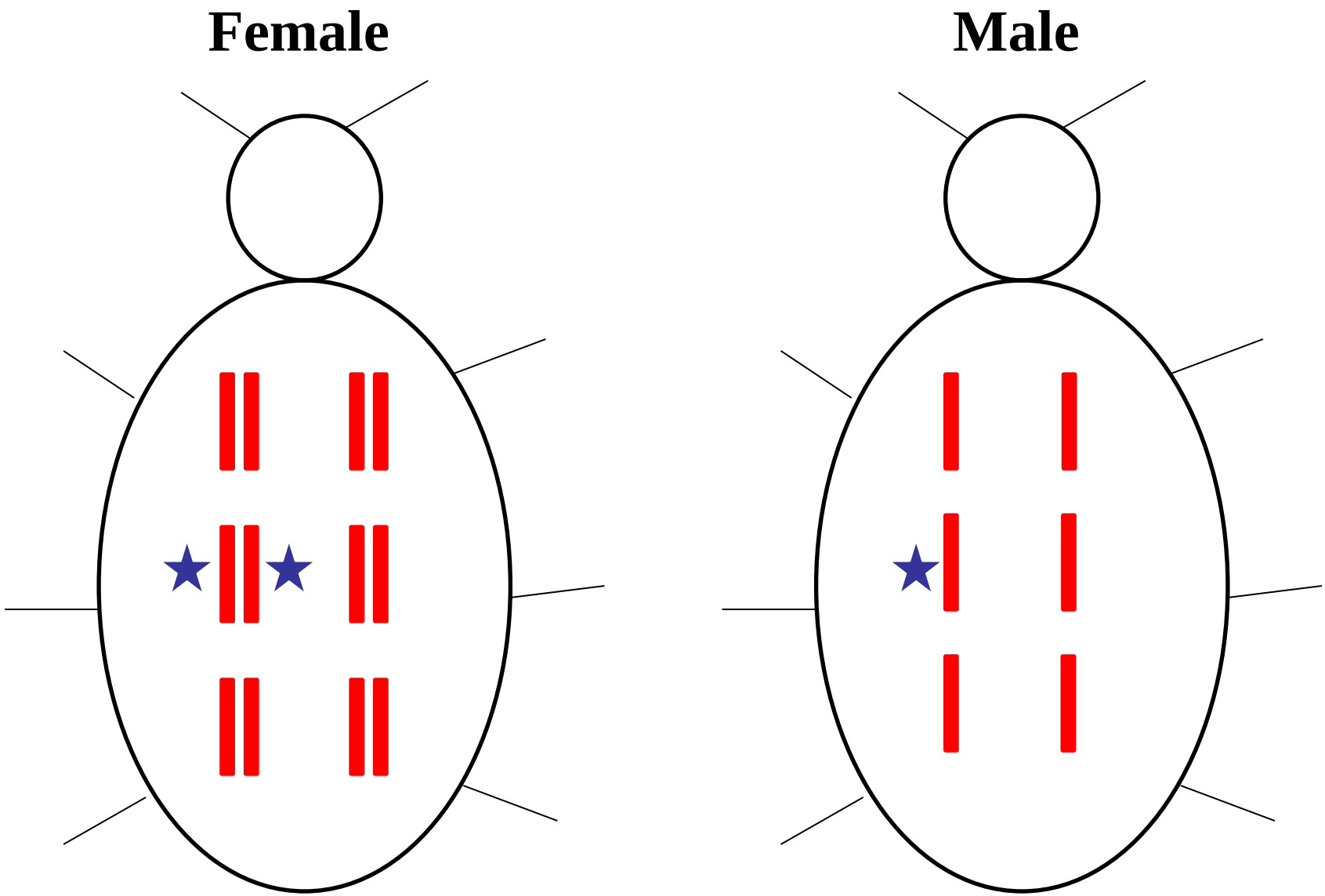
Haplodiploidy in arthropods

Bemisia tabaci

Frankliniella occidentalis

Tetranychus urticae

Dominance vs haplodiploidy



Haplodiploidy in arthropods

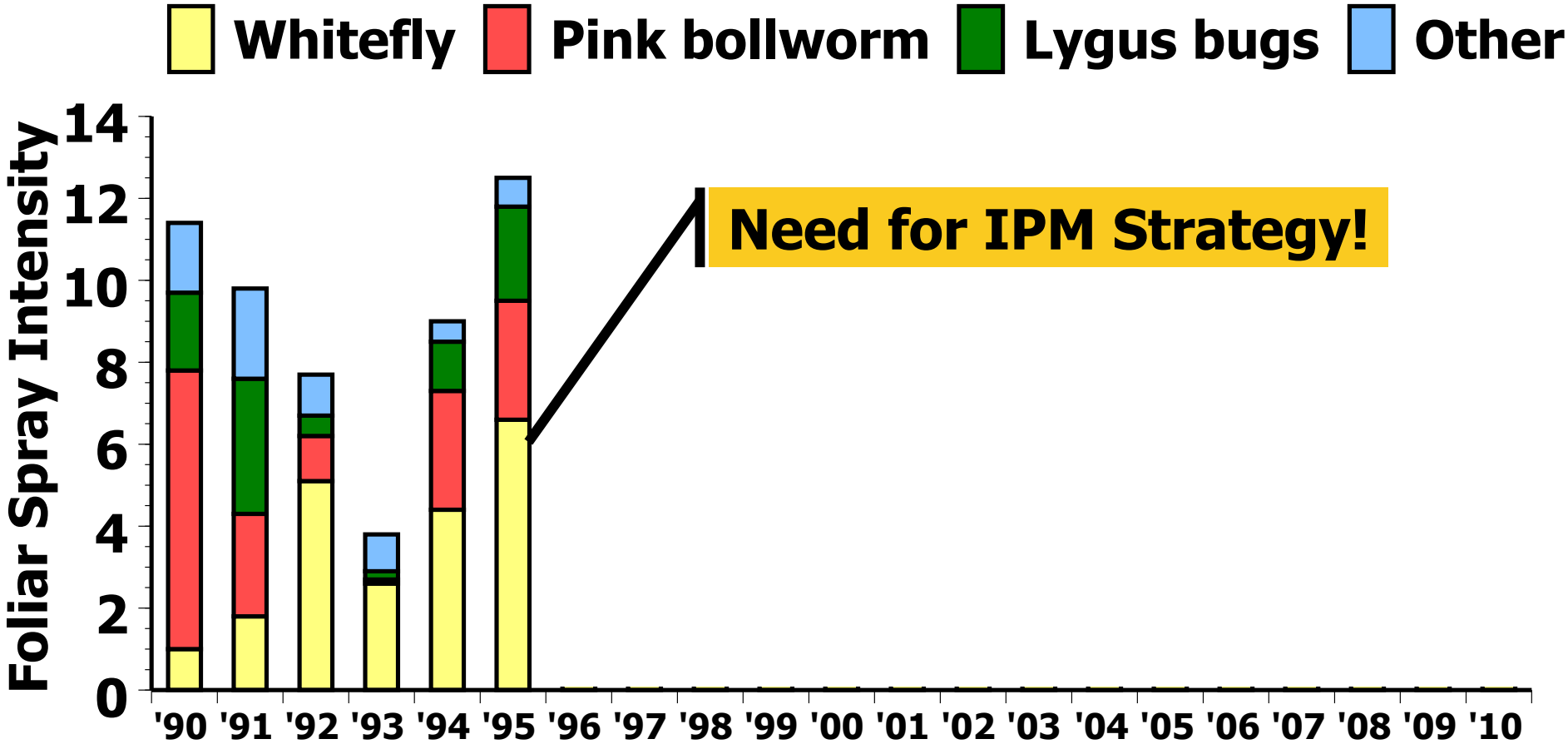
Bemisia tabaci

Frankliniella occidentalis

Tetranychus urticae

IRM in cotton in Arizona

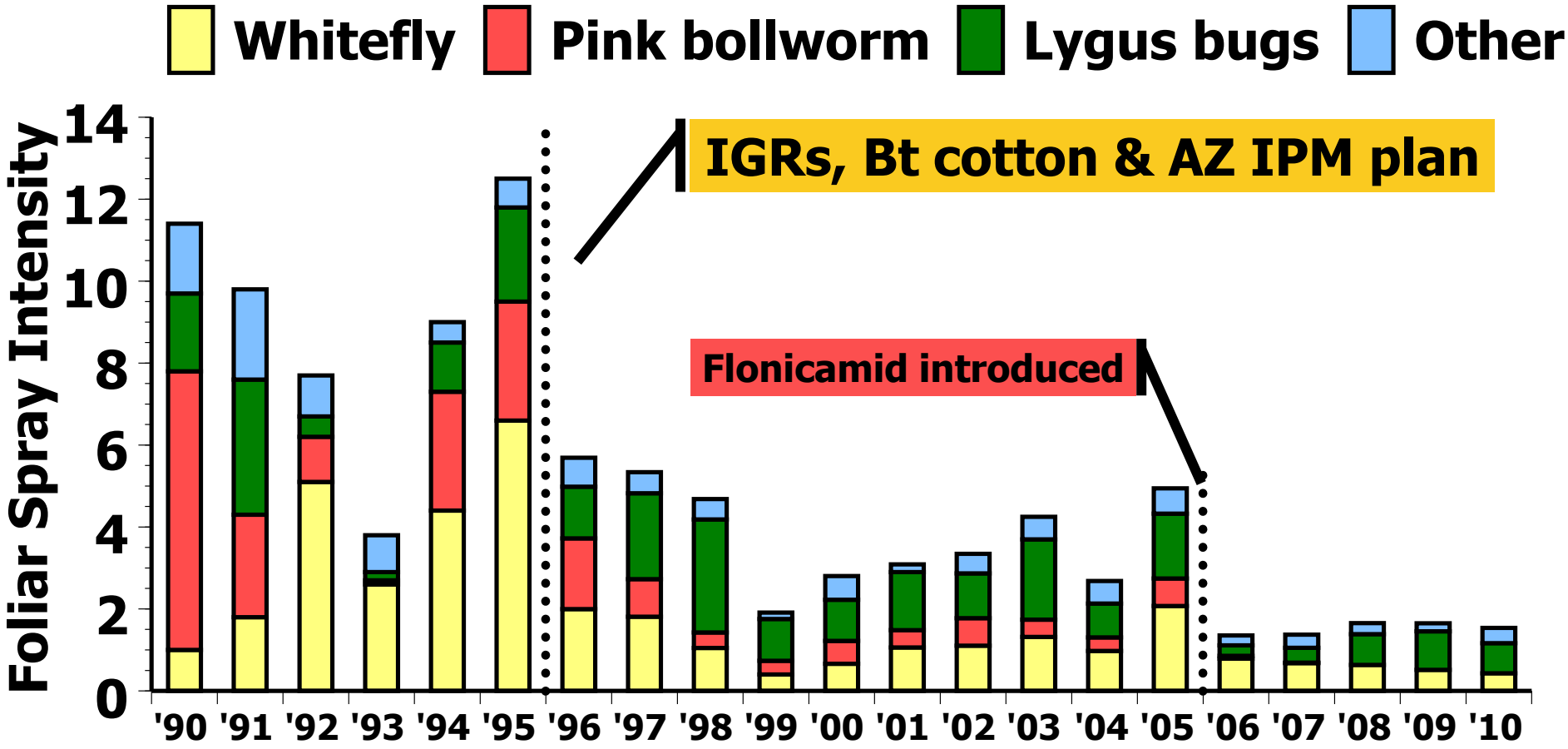
Statewide Cotton Sprays



Ellsworth/UA

IRM in cotton in Arizona

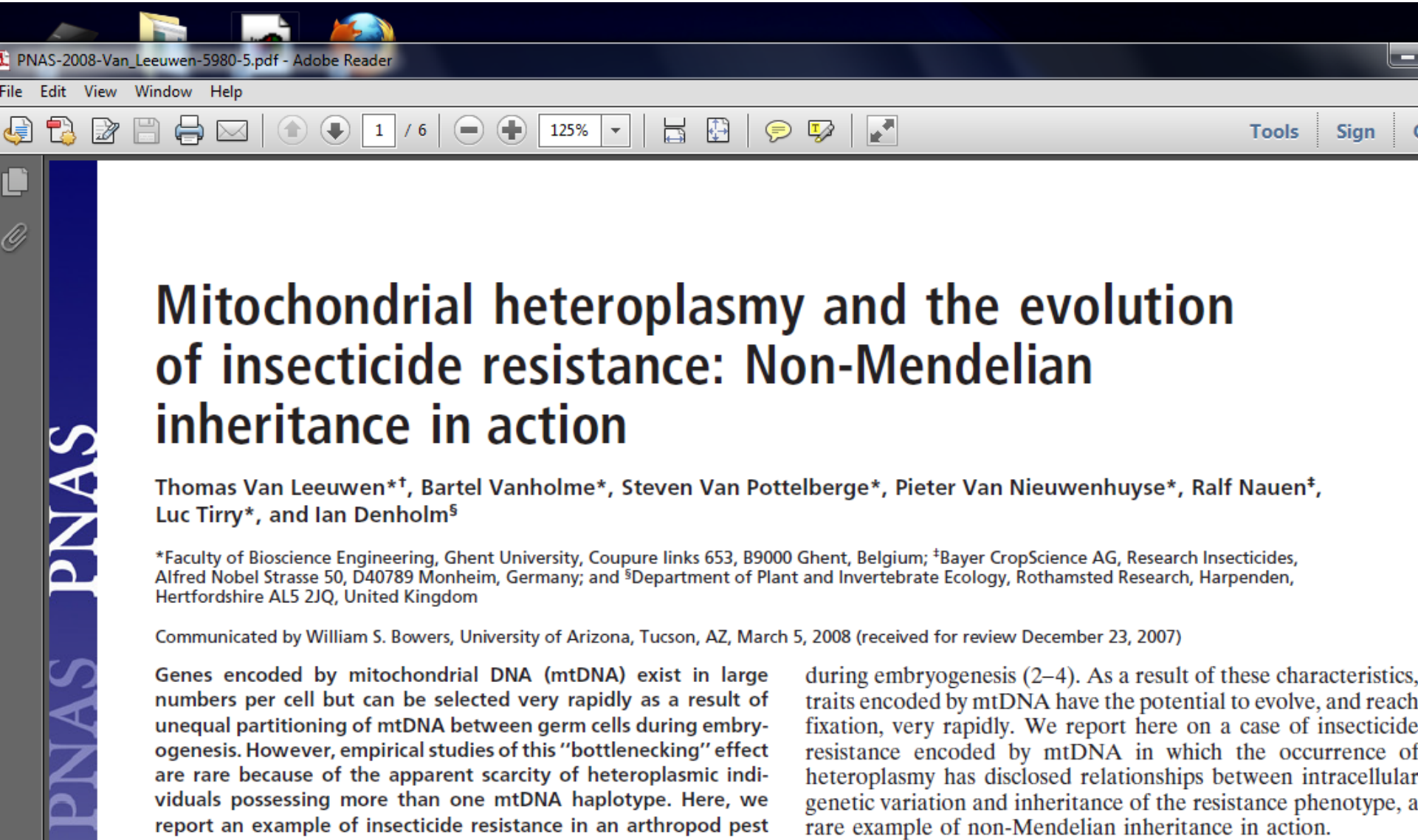
Selective approaches introduced



Ellsworth et al. 2011

Ellsworth/UA

Resistance conferred by a mitochondrial gene



Mitochondrial heteroplasmy and the evolution of insecticide resistance: Non-Mendelian inheritance in action

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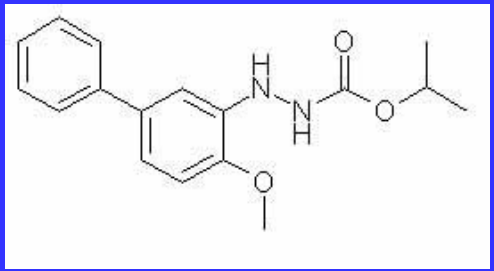
Genes encoded by mitochondrial DNA (mtDNA) exist in large numbers per cell but can be selected very rapidly as a result of unequal partitioning of mtDNA between germ cells during embryogenesis. However, empirical studies of this ''bottlenecking'' effect are rare because of the apparent scarcity of heteroplasmic individuals possessing more than one mtDNA haplotype. Here, we report an example of insecticide resistance in an arthropod pest

during embryogenesis (2–4). As a result of these characteristics, traits encoded by mtDNA have the potential to evolve, and reach fixation, very rapidly. We report here on a case of insecticide resistance encoded by mtDNA in which the occurrence of heteroplasmy has disclosed relationships between intracellular genetic variation and inheritance of the resistance phenotype, a rare example of non-Mendelian inheritance in action.

Bifenazate :

- new hydrazine carbazate
- selective miticide
- new chemical class
- unknown mode of action :

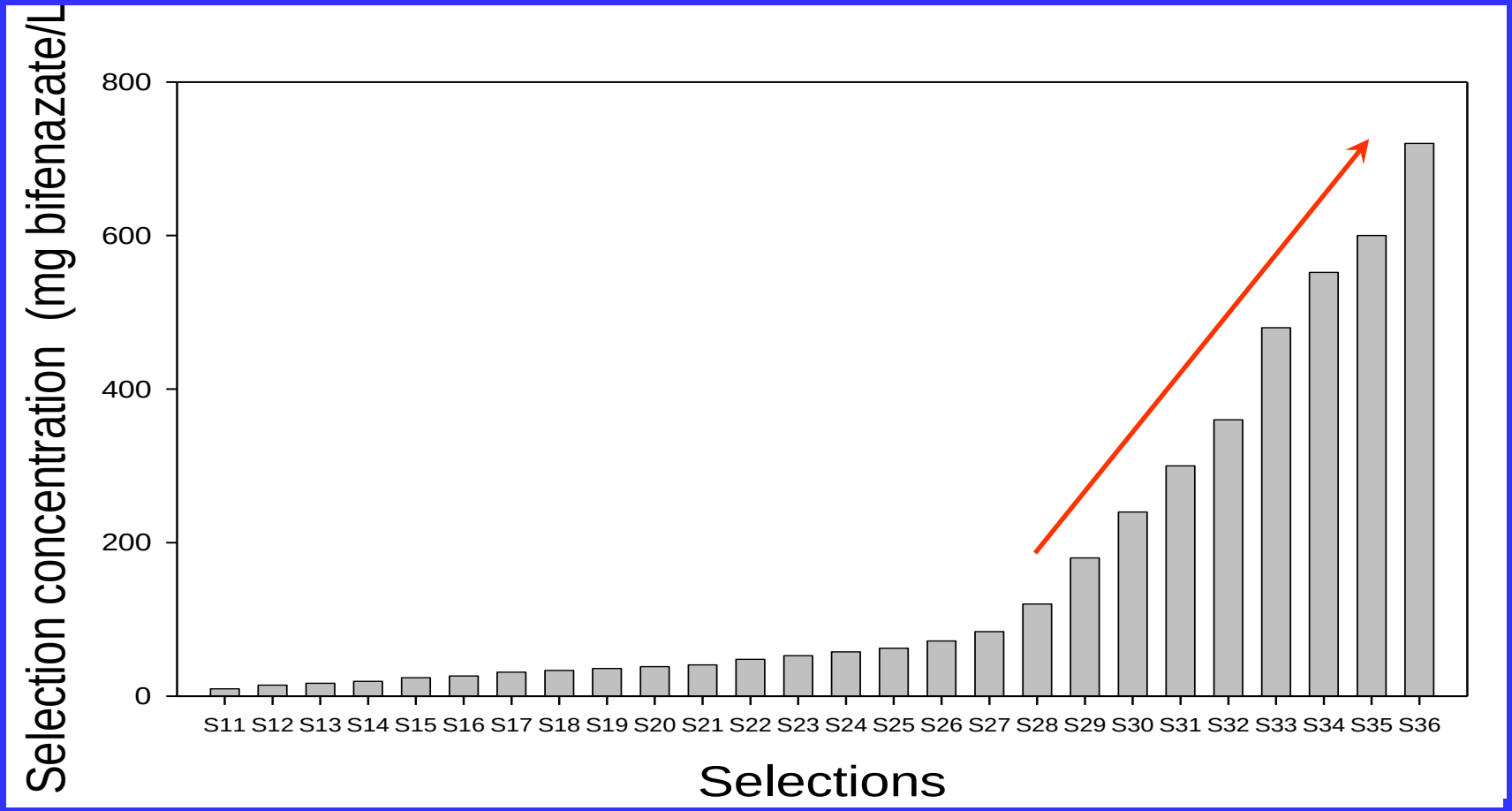


➔ acts as a gamma-amino butyric acid (GABA) agonist or antagonist at neuromuscular synapse

Selection with bifenazate

LS-VL → (selection: bifenazate) → BR-VL

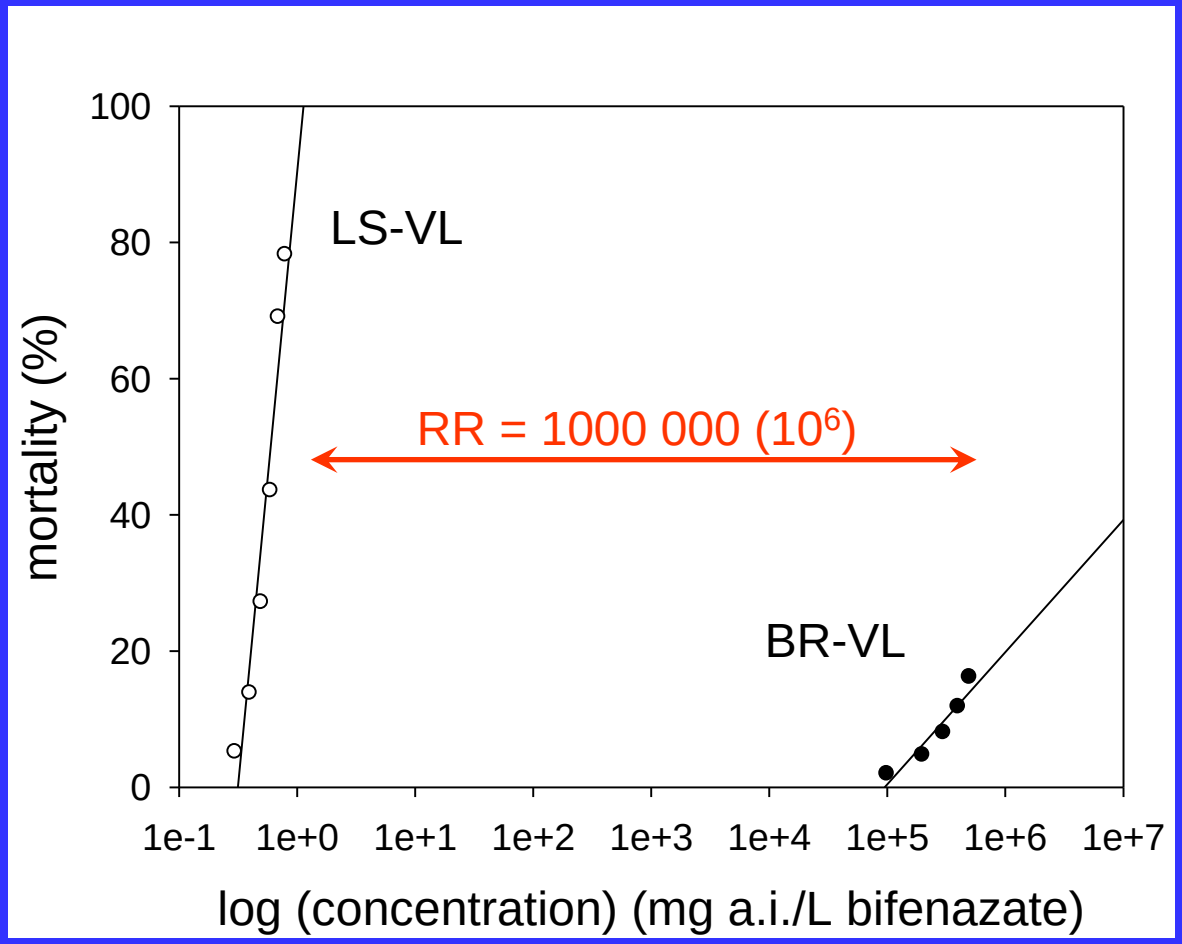
Start: 2000 mites, LC_{90} method



Toxicology

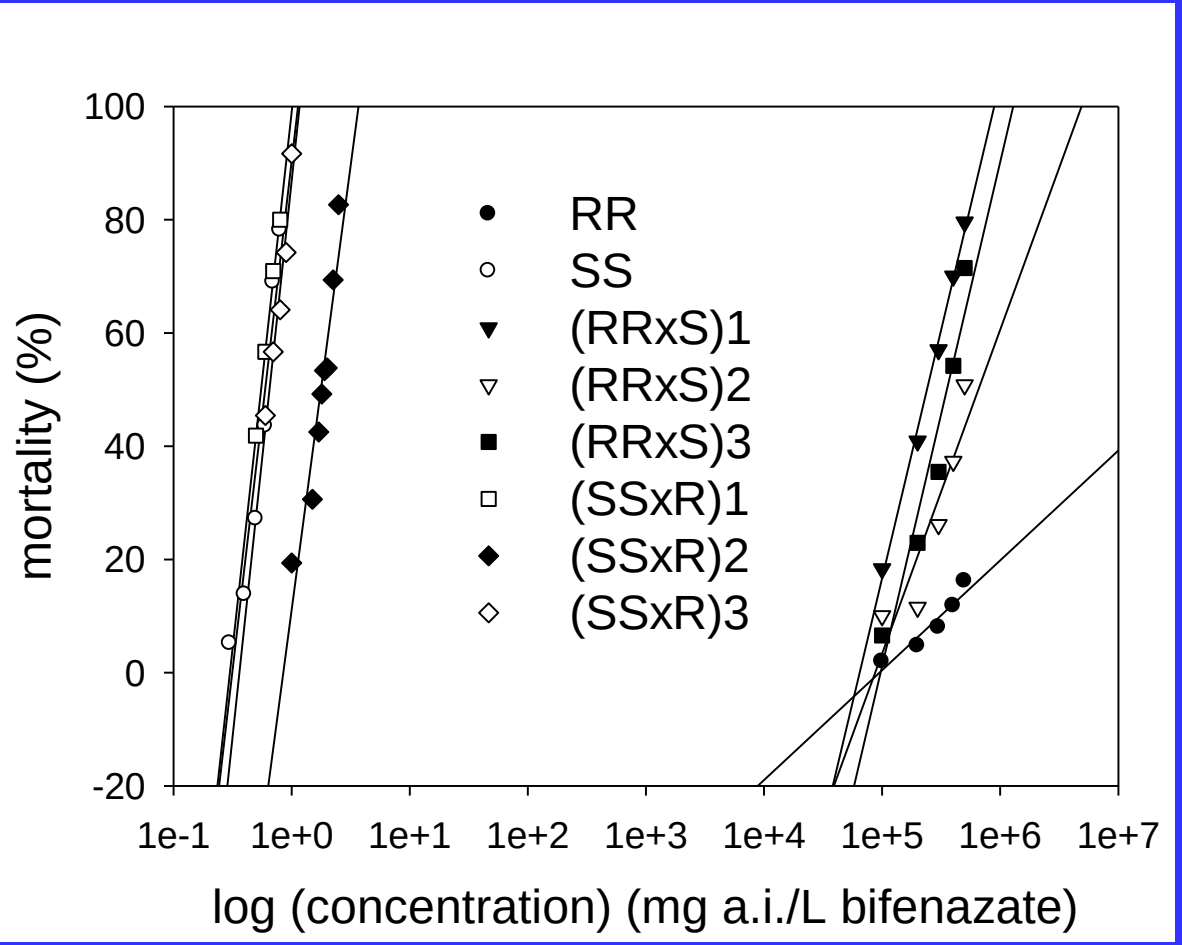
-extremely high RR (exceeding 10^6)

-no cross-resistance



Crossing experiments

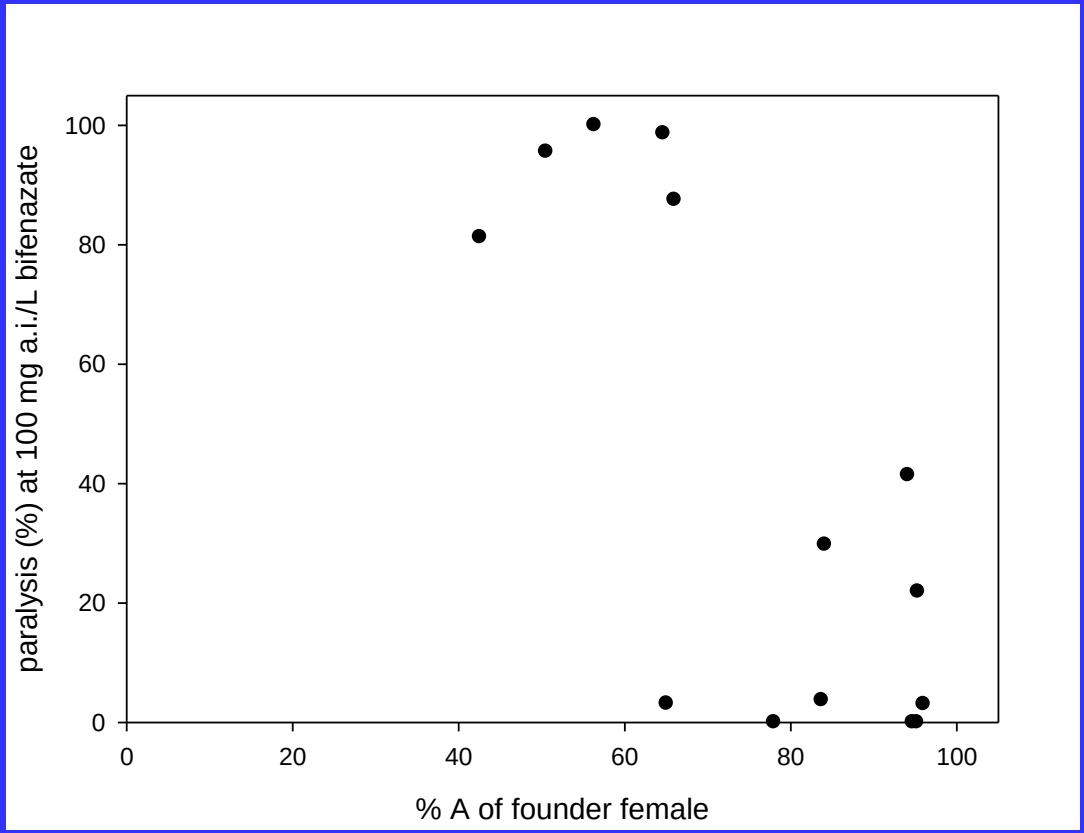
COMPLETE MATERNAL EFFECT



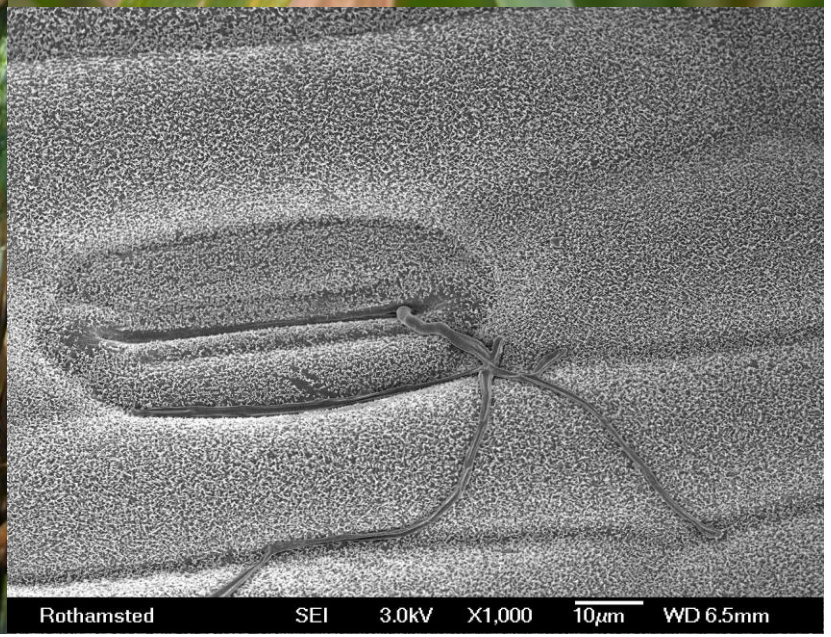
Link heteroplasmy and resistant phenotype ?

How high is the frequency of resistant haplotype to survive the field dose ?

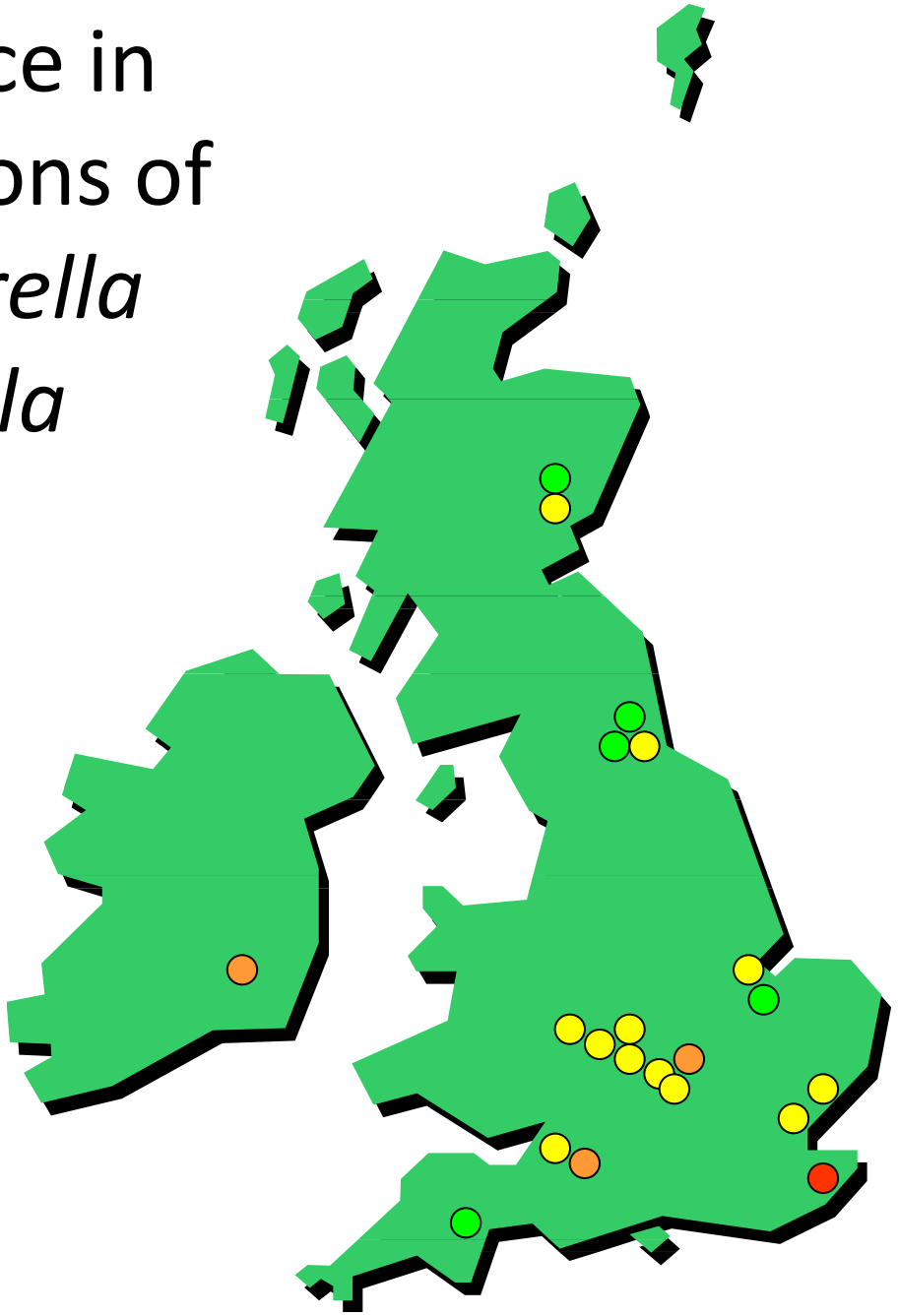
1. Sequencing mites treated with the fielddose: 60% threshold
2. Followed 3th generation offspring of females with know initial genotype



Mycosphaerella graminicola (Zymoseptoria tritici) on wheat



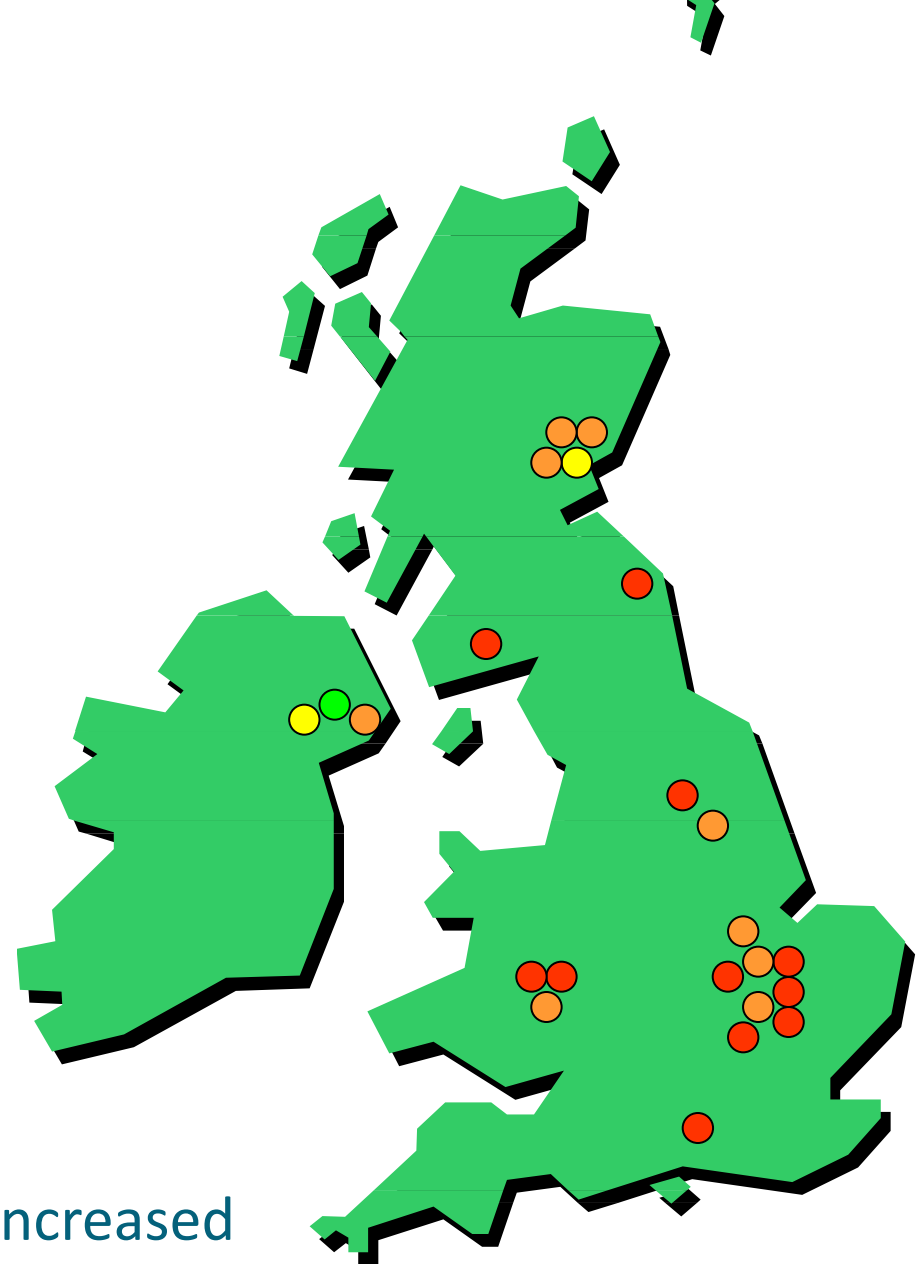
QoI resistance in field populations of *Mycosphaerella graminicola*



Spring 2003

Courtesy of Hans Cools

Spring 2004



- Average frequency increased from 39 to 80% in 2004.
- Frequency > 95 % since 2005

Courtesy of Hans Cools

Mitochondrial heteroplasmy and the evolution of insecticide resistance: Non-Mendelian inheritance in action

- **Highest resistance factor ever recorded in arthropods**
- **First case of complete maternal inheritance of resistance in arthropods**
- **First case of non-Mendelian inheritance of resistance in arthropods**

across fungi, protozoa, plants, and animals are mutated in resistant mite strains. Despite the unusual nature of these mutations, resistant mites showed no fitness costs in the absence of insecticide. Partially resistant strains, consisting of heteroplasmic indi-

teins (5). To date, however, there have been no reports of resistance encoded by mtDNA in arthropods. Several insecticides do target biochemical processes in mitochondria, but cases of resistance resolved so far have involved mutations in the