ICAR

Natural Resources Institute

Understanding the mechanisms of insecticide resistance in *Helicoverpa armigera*

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[7] *CIRAD, France*

Helicoverpa armigera damage

- **181 host plants - 69 crop species**
- **losses - >US $ 1 bill annually**

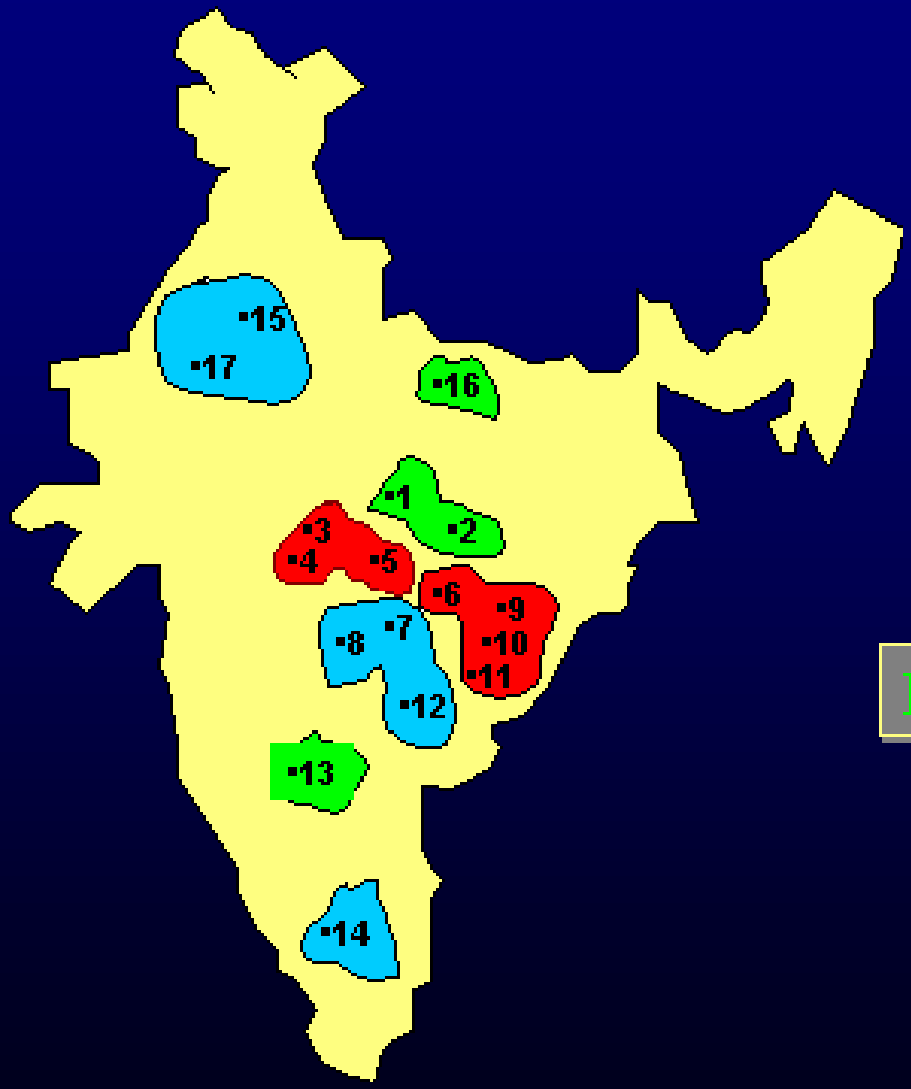
Technical Partners

• **India -**	Central Inst. for Cotton Res.	*K. Kranthi, S.Kranthi*
	- Tamil Nadu Agric. Univ.	*A.Regupathy*
	- Punjab Agricultural Univ.	*J.Singh, A.Sohi*
	- ICRISAT	*D.Jadhav*
• **China** -	Nanjing Agric.Univ.	*Wu Yidong*
		Yang Yehia, Song Shen
• **Pakistan** -	Central Cotton Res. Inst.	*Mushtaq Ahmad*
• **UK**	- Natural Resources Institute	*D. Russell, N.Armes*
	- Rothamsted Research	*G.Devine, G.Moores,*
		N.Javed
• **W.Africa** –	CIRAD	*Thibaud Martin*

PART 1

The Nature of the Problem

Pattern of insecticide use



Low insecticide use 2-6 sprays

1. Nagpur
2. Wardha
13. Bangalore
16. Varanasi

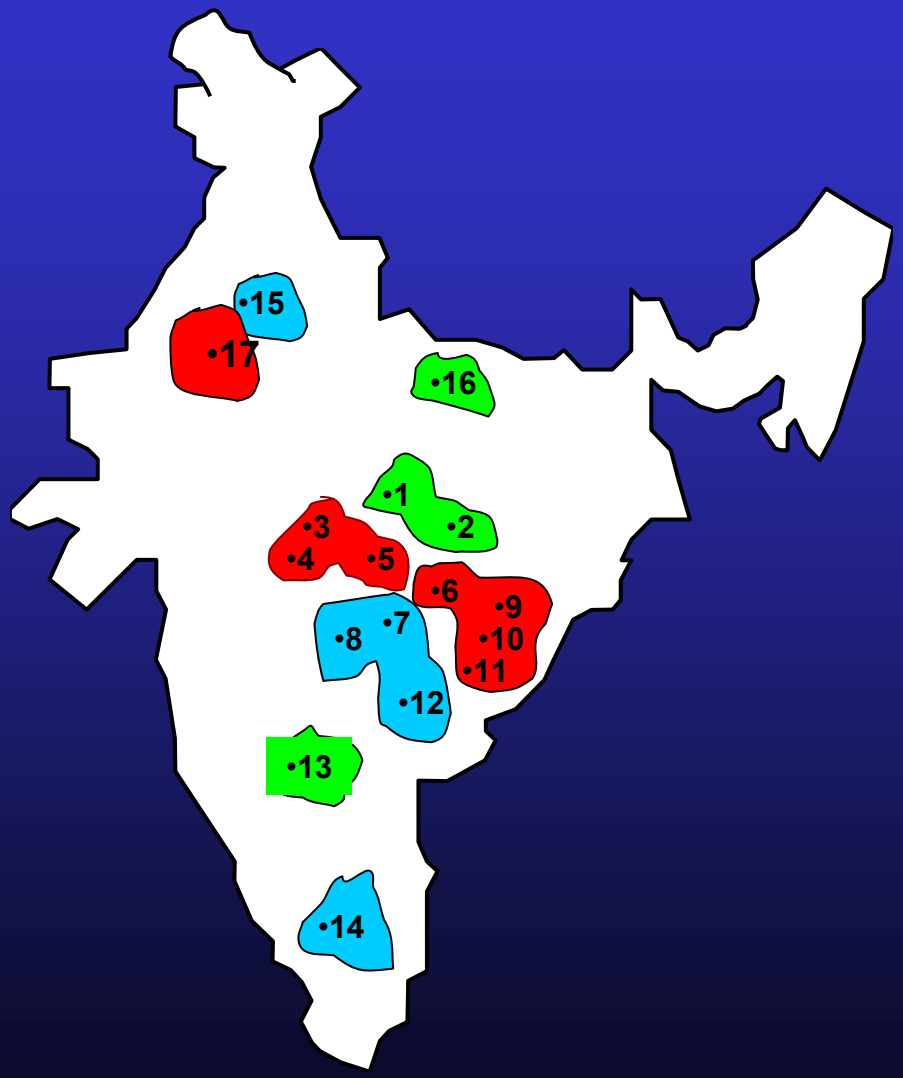
Moderate insecticide use 5-10 sprays

7. Siddipet
8. Rangareddy
12. Prakasam
14. Coimbatore
15. Sirsa
17. Bhatinda

High insecticide use 8-20 sprays

3. Amaravati
4. Akola
5. Yavatmal
6. Warangal
9. Karimnagar
10. Khammam
11. Guntur

Pyrethroid resistance 1993-2003



Low to moderate

1. Nagpur
2. Wardha
13. Bangalore
16. Varanasi

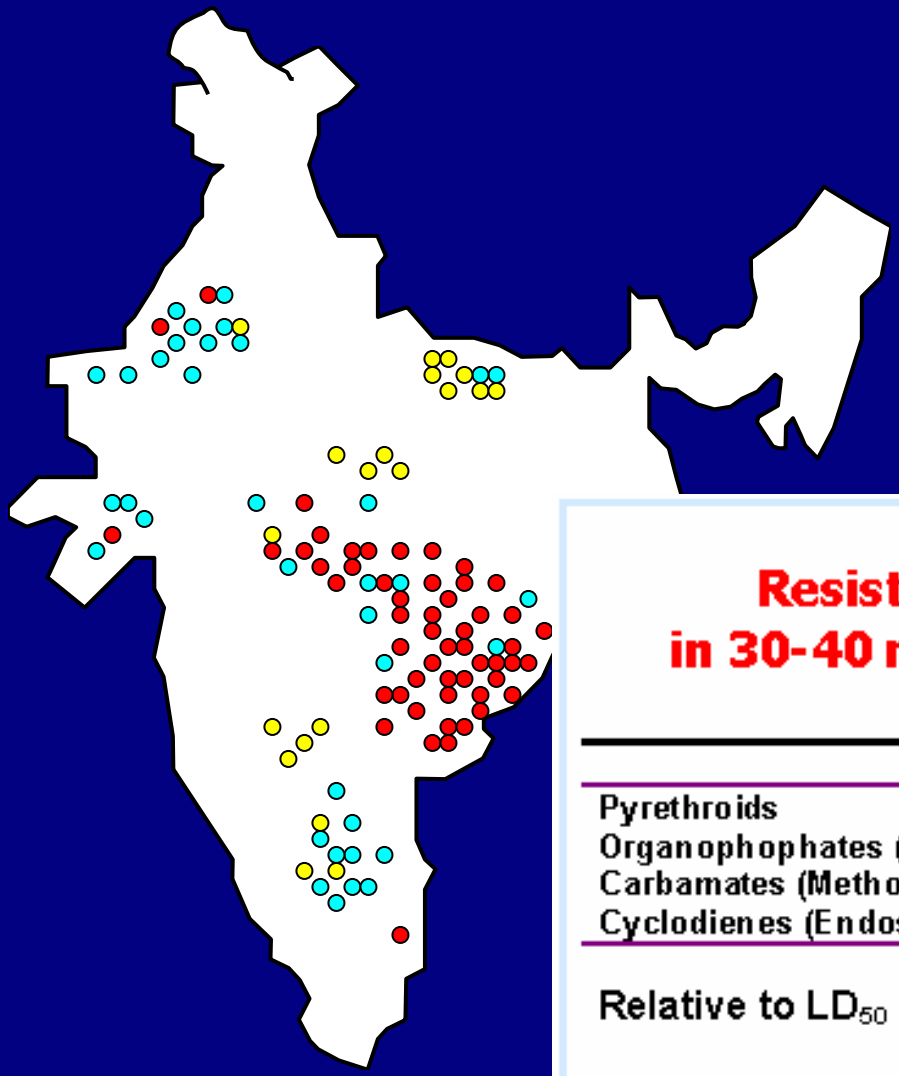
Moderate to high

7. Siddipet
8. Rangareddy
12. Prakasam
14.Coimbatore
15. Sirsa

Very High

3. Amaravati
4. Akola
5. Yavatmal
6. Warangal
9. Karimnagar
10. Khammam
11. Guntur

Insecticide resistance in *H. armigera* 1992-2001



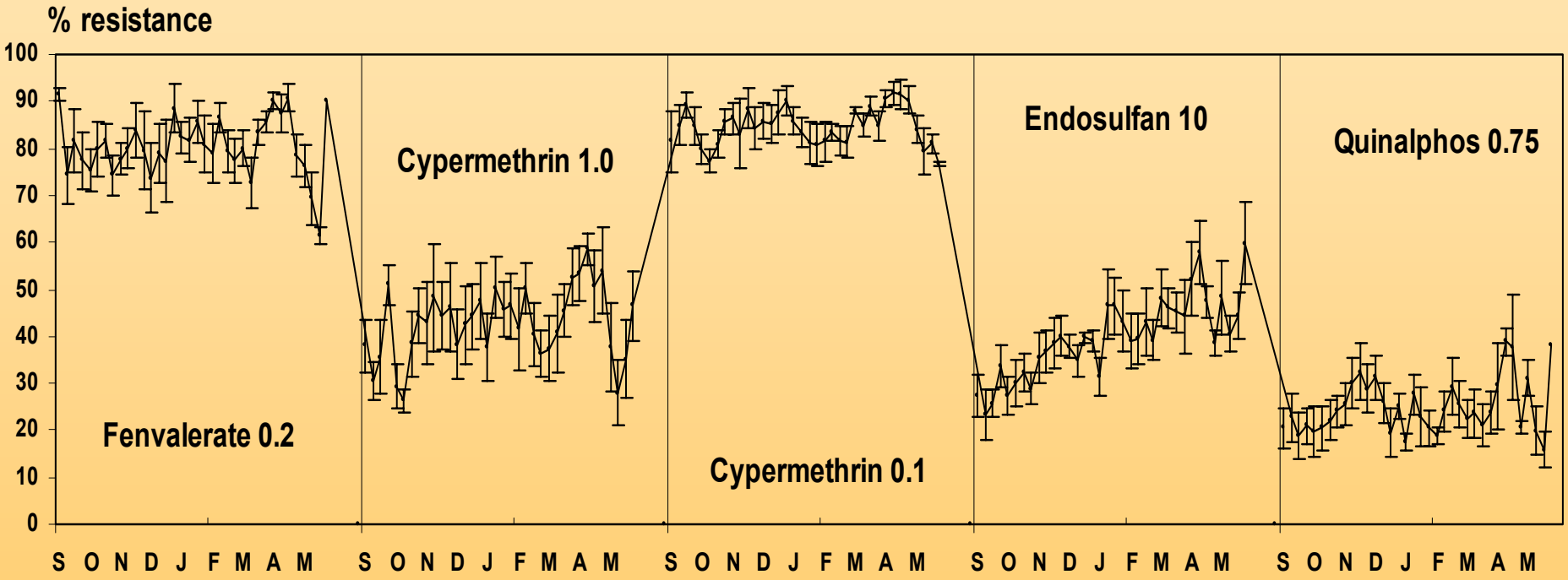
Resistance factors from LDP assays in 30-40 mg H. armigera larvae 1992-2001

	North India	Central India	South India
Pyrethroids	48 - 1340	54 - 6,000	73 - 21,000
Organophophates (Quinalphos)	6 - 33	4 - 41	6 - 67
Carbamates (Methomyl)	2 - 27	1 - 19	9 - 83
Cyclodienes (Endosulfan)	7 - 23	12 - 45	5 - 39

Relative to LD_{50} of lab reared field susceptible strain

Kranthi et al., J.Econ.Entomol., 2001, 2002

Average of six years (1993-99) discriminating dose monitoring data from Central India



H. armigera Resistance in India

Susceptible:	Thiodicarb Spinosad Indoxacarb Avermectin
Low:	Profenofos, Chlorpyrifos Methomyl
Medium:	Quinalphos Endosulfan Bifenthrin, Lambda Cyhalothrin, Deltamethrin
High:	Cyfluthrin, Cypermethrin, Lambda Cyhalothrin, Deltamethrin, Fenvalerate

Part II

Mechanisms

A.McCaffery (1999): Resistance to insecticides in heliothine Lepidoptera: a global view. Insecticide resistance from mechanisms to management. p59-74 . *Royal Society*

Applications of research into resistance mechanisms

- resistance diagnostics
- means of overpowering resistance mechanisms (e.g. synergists)
- support cross-resistance studies, and optimise insecticide rotations

- Ideally, resistance management strategies should be implemented before resistance has arisen, and therefore with no clear knowledge of the mechanisms that may appear

- But …. if resistance is already present, characterisation of the biochemical and molecular basis of resistance mechanisms can aid resistance management …..

Mechanisms of resistance

- increased detoxification of insecticides
- altered target sites
- reduced penetration/ enhanced excretion

Resistance mechanisms and cross-resistance

Approaches used in the CFC project

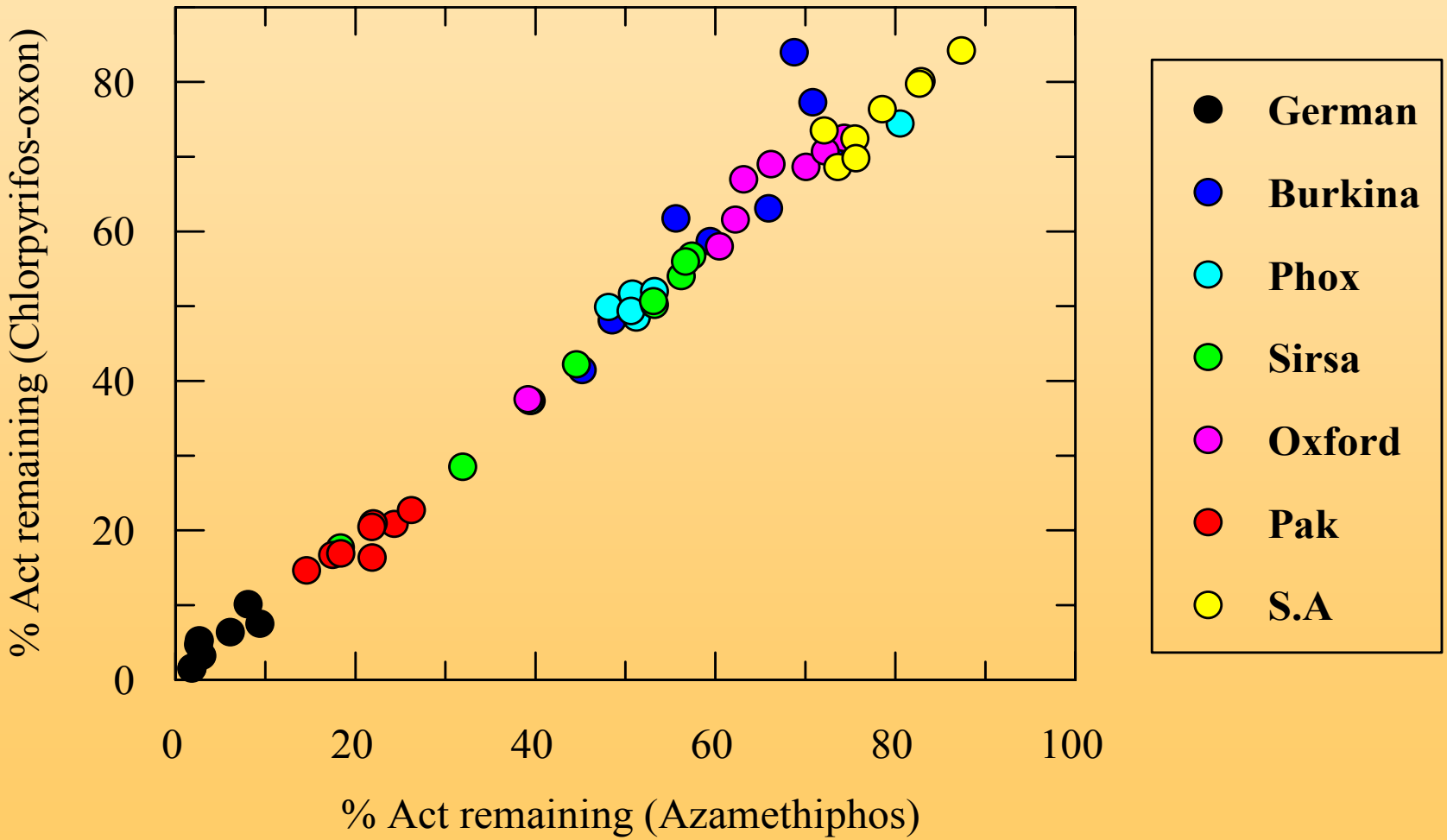
- Correlations in response to different insecticides
- Use of synergists to block enzymes potentially involved in insecticide detoxification
- Direct comparisons of the amounts of detoxifying enzymes present
- Comparisons of metabolism rates
- Look for DNA mutations in target-site proteins known to occur in other pest species
- Neurophysiology to detect evidence for nerve insensitivity
- Comparisons of penetration and excretion rates
- Reciprocal selection experiments (does selection with compound A also increase resistance to compound B?

Altered target sites

- acetylcholinesterase (AChE) (organophosphates, carbamates)
- sodium channels -knockdown resistance (kdr) (pyrethroids)
- GABA receptors- rdl mechanism (cyclodienes inc.endosulfan)

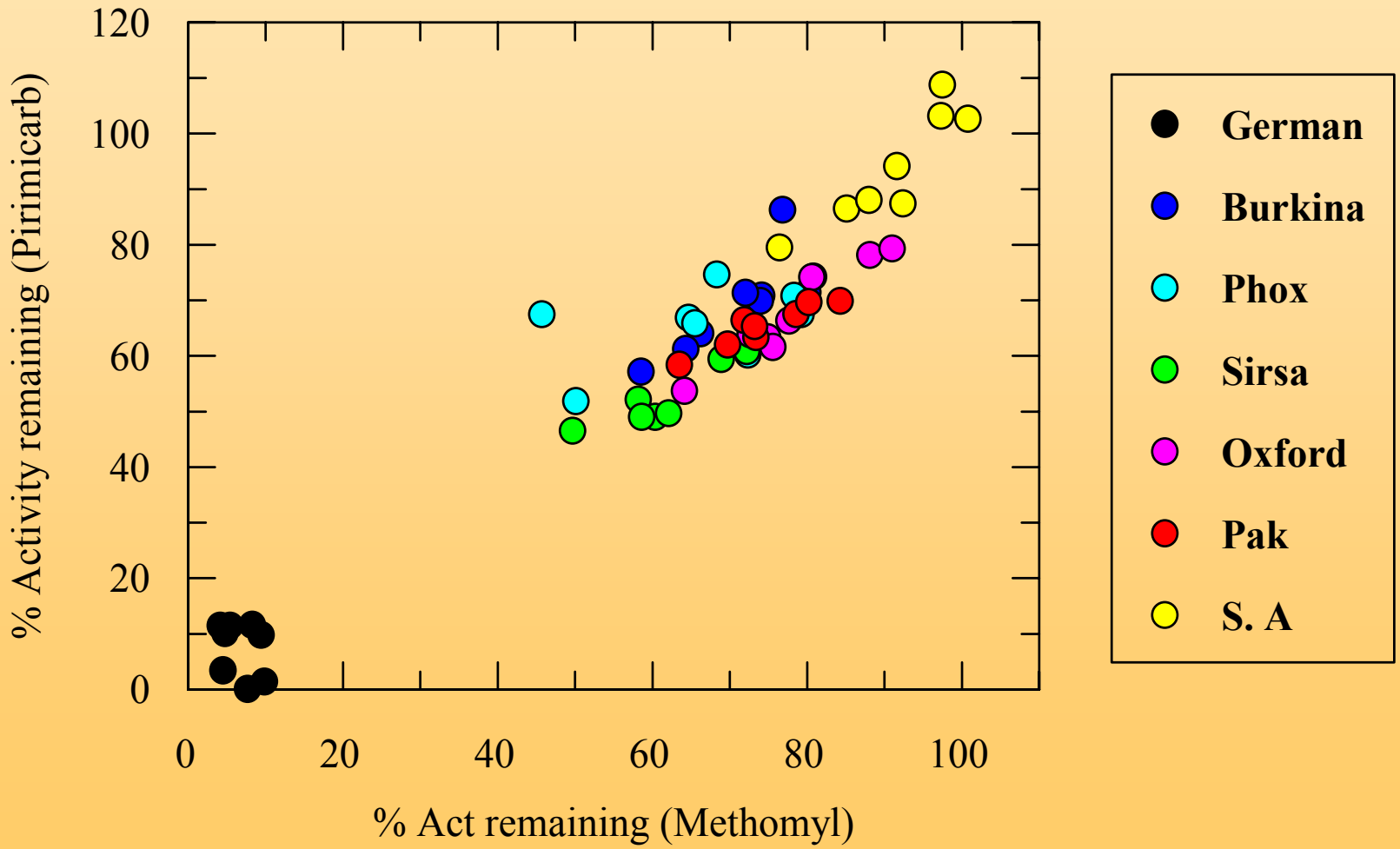
Acetylcholinesterase:

All field strains show insensitivity to organophosphates compared to the fully susceptible W.African (1978) strain

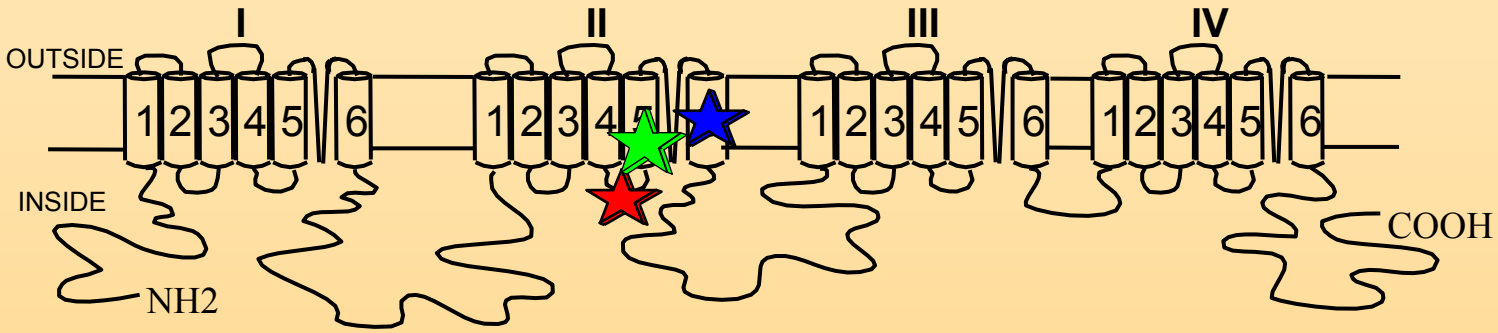


Acetylcholinesterase:

All field strains show insensitivity to carbamates compared to the fully susceptible W African (1978) strain



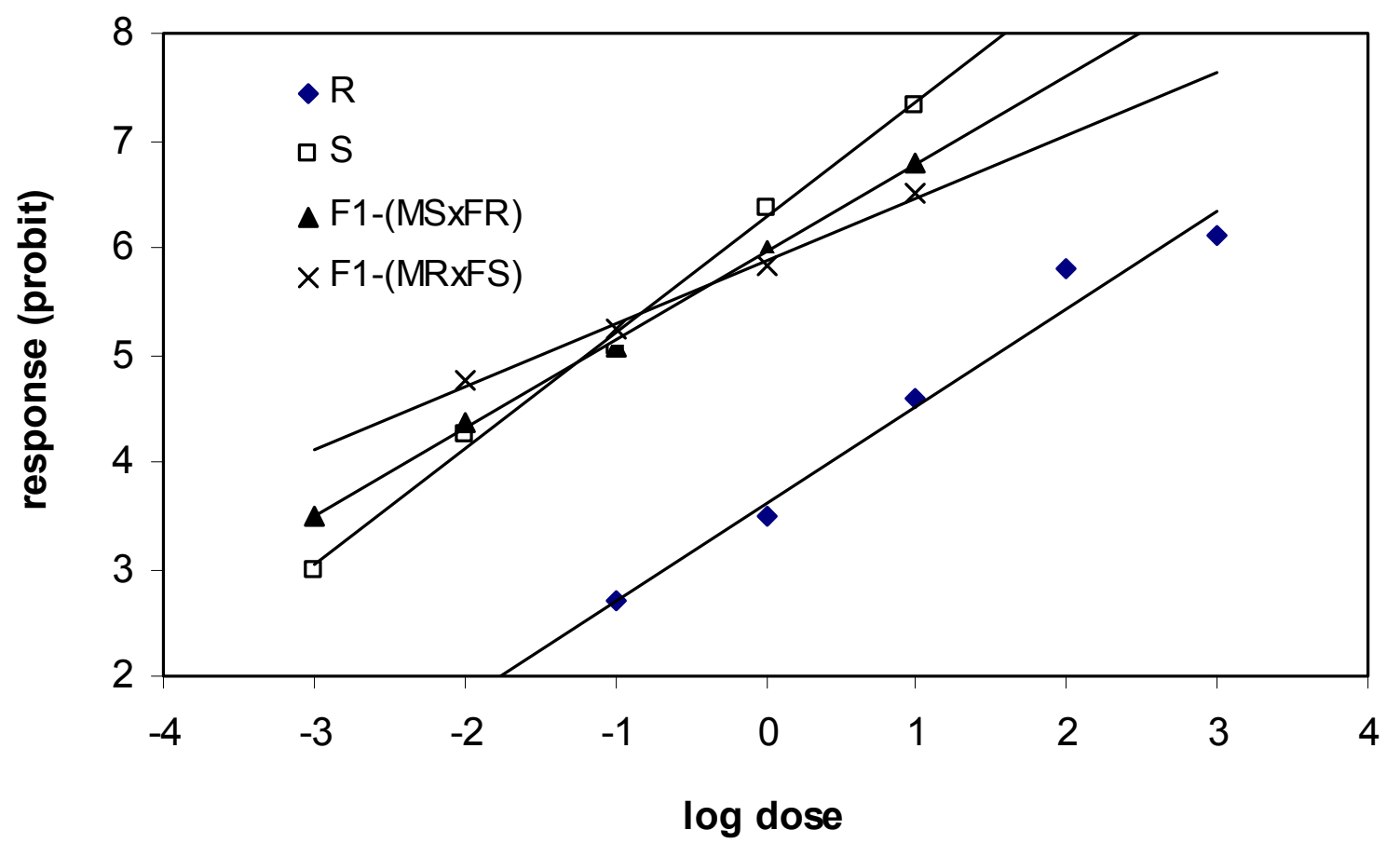
Sodium Channel Mutations in Resistant Insects



	★	★	★	
Musca domestica	Met/Thr	-	Leu/Phe	MGG (1996) 252, 51
Blattella germanica	-	-	Leu/Phe	MGG (1996) 252, 61
Anopheles gambiae	-	-	Leu/Phe	IMB (1998) 7, 179
Myzus persicae	-	-	Leu/Phe	IMB (1999) 8, 339
Haematobia irritans	Met/Thr	-	Leu/Phe	IBMB (1997) 27, 745
Plutella xylostella	-	Thr/Ile	Leu/Phe	PBP (1998) 59, 169
Heliothis virescens	-	-	Leu/His	IBMB (1997) 27, 9
Pediculus capitis	-	Thr/Ile	-	PBP (1999) 66, 130
Bemisia tabaci	Met/Val	Thr/Val	-	RES unpublished

Dose response curves in neurophysiological assays with alpha-cypermethrin

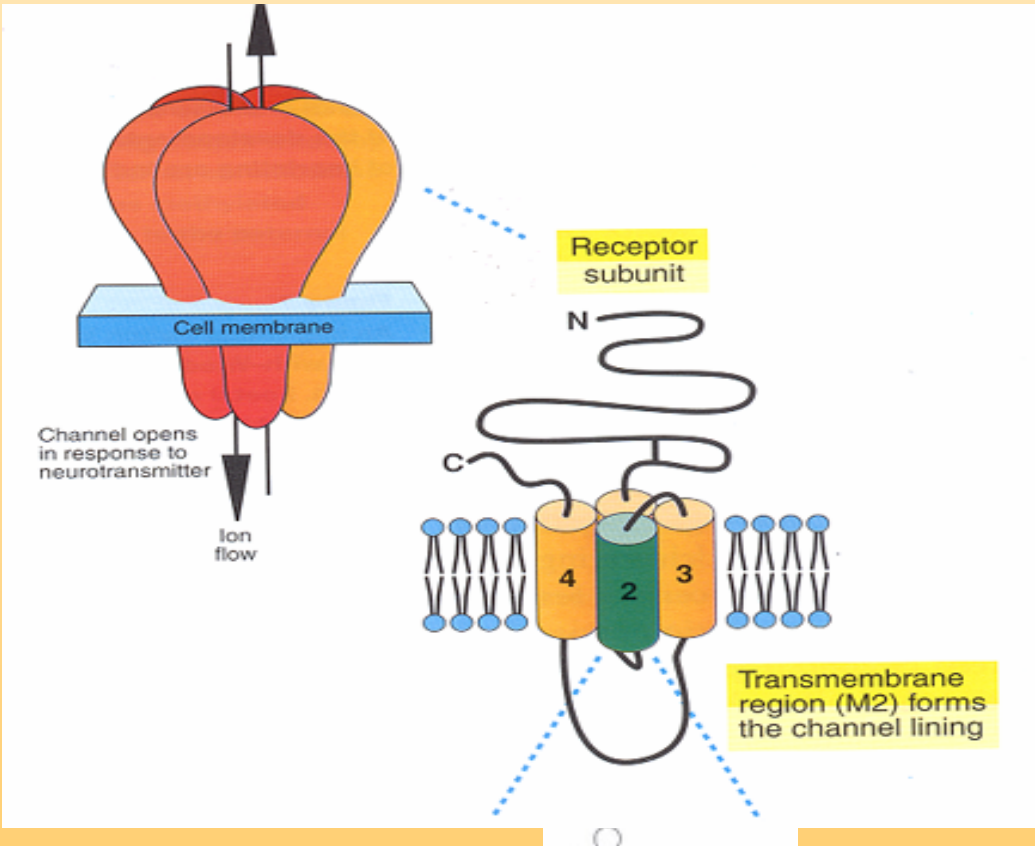
(evidence for kdr mechanism)



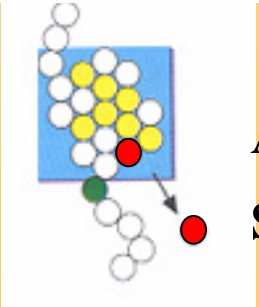
Nerve Insensitivity

- Demonstrated electrophysiologically in India and China and currently being explored in Pakistan strains.
- Sequencing the relevant domains has NOT found any of the classic mutations (all three countries)
- Is there a novel mutation somewhere else in the sodium channel gene?

Rdl mechanism – a point mutation in the GABA-gated chloride channel causes resistance to cyclodienes such as endosulfan in a wide range of insect species.



All *H. armigera* strains examined, including standard susceptible ones, contain the resistance mutation!



Ala

Ser/Gly

Target-site mechanisms - conclusions

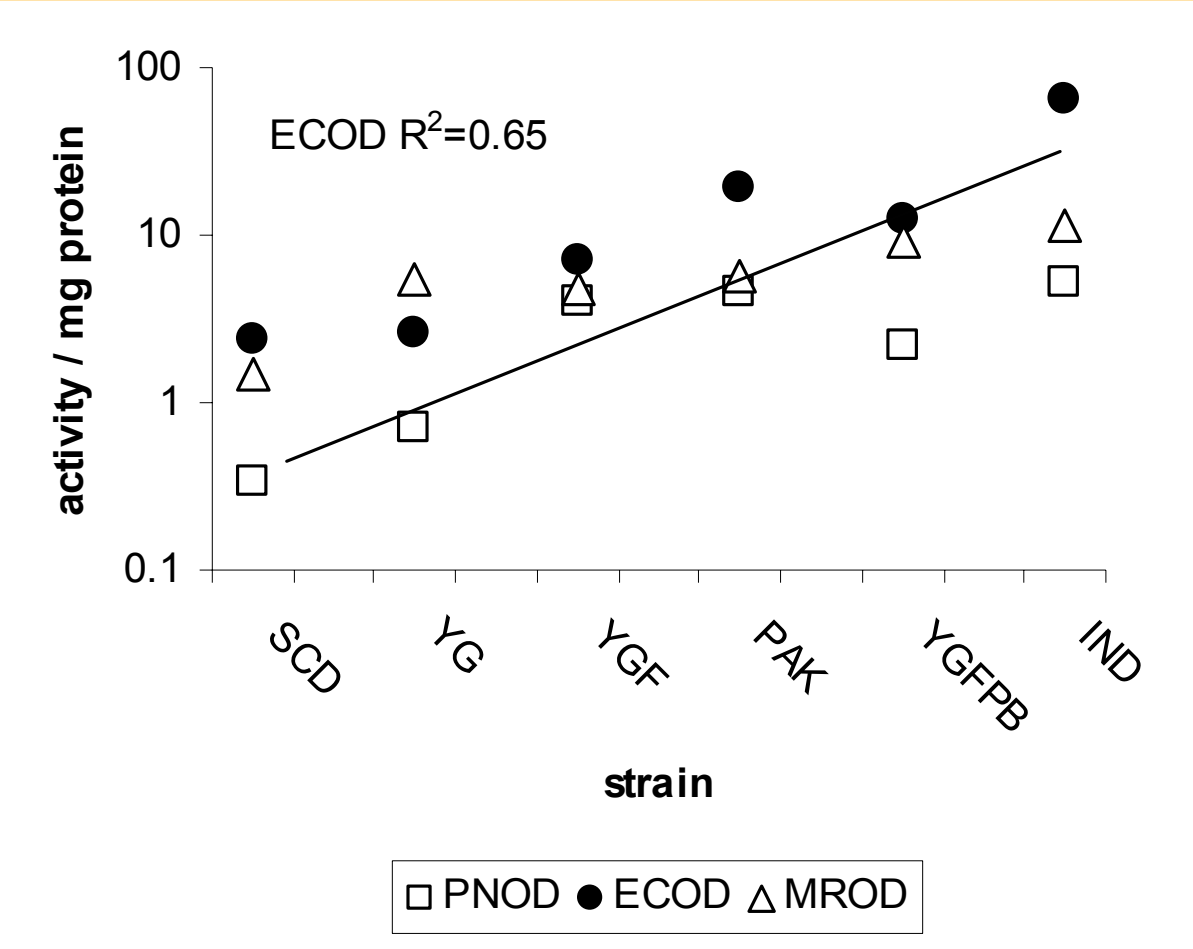
1. Rdl - ubiquitous in *H. armigera*. Likely to confer background resistance to endosulfan but doesn't account for variation in endosulfan resistance between field populations.
2. AChE – insensitive forms also appear ubiquitous, conferring basal resistance to OPs and carbamates but perhaps not the primary cause of variations in response between strains
3. Knockdown resistance to pyrethroids (kdr) – Evidence for its occurrence but not attributable to any of the known mutations.

Target-site mechanisms too ubiquitously present or absent to be a major consideration in resistance management? (Kdr needs further research)

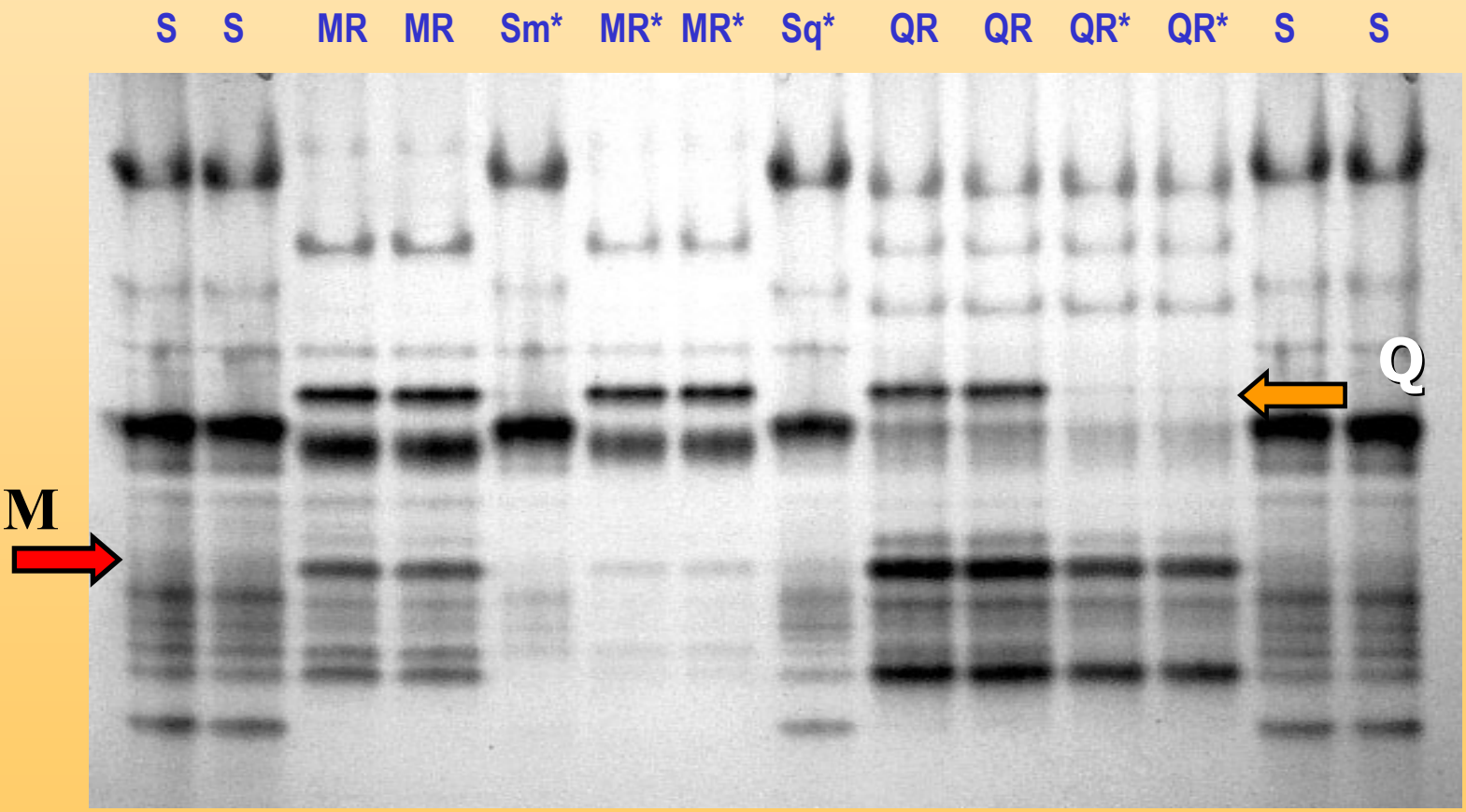
Metabolic Mechanisms

- mixed-function oxidases (mfo's)
- glutathione-dependent transferases (GST's)
- non-specific esterases (degradation and sequestration)

Correlation between monoxygenase activity and resistance factors to pyrethroids

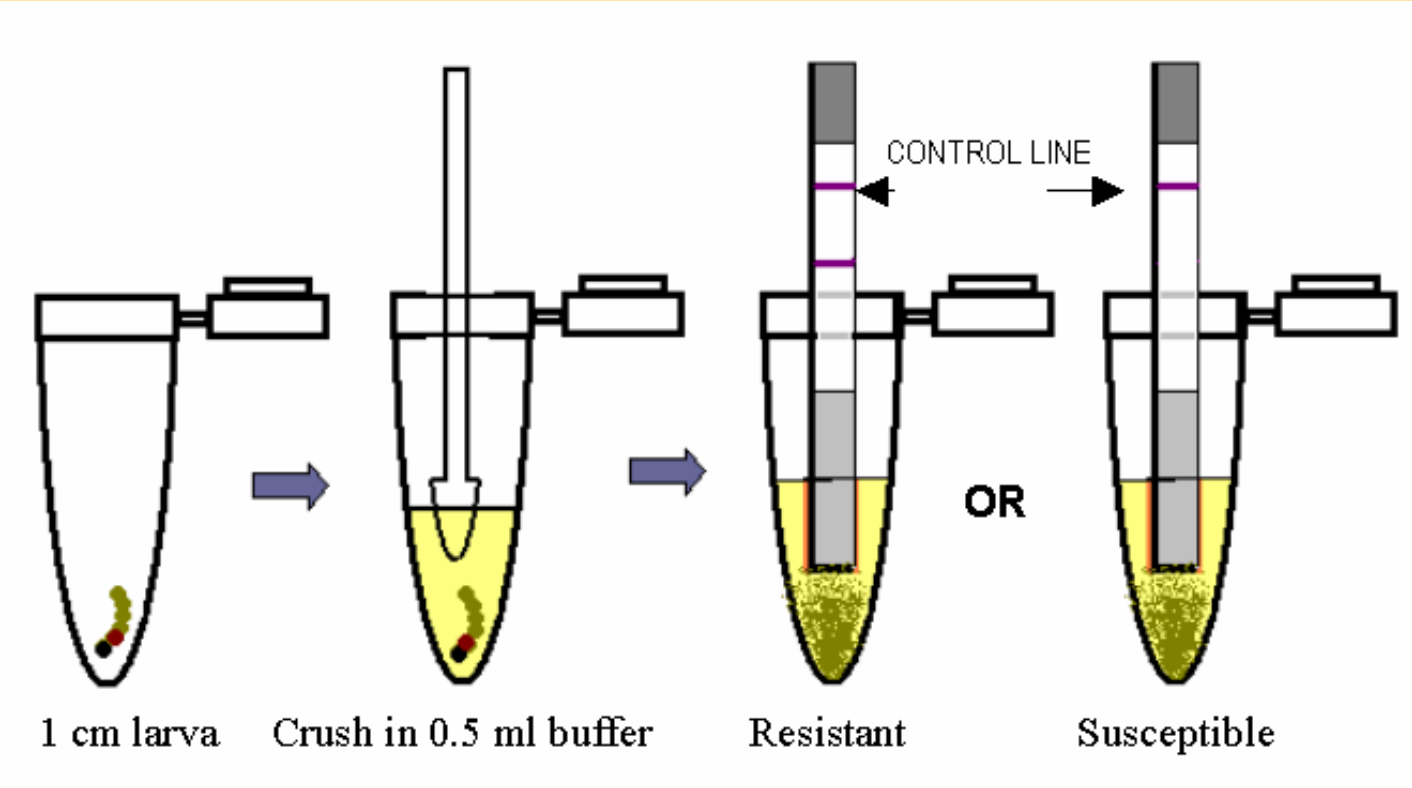


Resistance Associated Esterases



Insecticide Resistance detection kit

Immunochromatographic strip



Conclusions on metabolic mechanisms

- GSTs play only a minor role (RF<10)
- Oxidases are very important in pyrethroid resistance
- Esterases are less important in pyrethroid resistance (not true in Australia) but are involved with OP/carbamate/endosulfan resistance
- Different isozymes are involved in resistance to particular groups of chemicals (e.g. E9 for Methomyl)

Penetration resistance

(Radio-labelled deltamethrin)

Strain	Resistance Factor	Penetration in 1 hour
Susceptible	1	50%
Chinese	300	30%
Paksitan	600	20%

Conclusions on penetration/ excretion

- Significant for pyrethroids
- Multiplicative with metabolic mechanisms as the pesticide is delivered to them more slowly
- Excretion rates were much higher in resistant strains
- It is probable that these mechanisms are also important for other chemistries

Mechanisms and their importance

	Metabolic			Target Site			Penetration
	Oxidase Pyreth	Esterase OP/Car/ Pyr/Endo	GST Pyr	Ache OP/ Carb	Nerve Insen Pyreth.	rdl Endo	Pyrethroid (others?)
India	***	**	*	*	**	*	?
China	***	**	*	*	*	*	*
Pakistan	**	**	*	*	?	*	*
Australia		***		**		**	
W.Africa	***					*	

	Country	pyrethroids	organophosphates	carbamates	endosulfan
esterases	*India* *Pakistan* *China*	** ** **	** ** **	*** ? ?	* * *
oxidases	*India* *Pakistan* *China*	**** *** ****	* * *	? ? ?	* * *
GSTs	*India* *Pakistan* *China*				
AChE	*India* *Pakistan* *China*		** ** **	* * *	
kdr	*India* *Pakistan* *China*	* ? *			
rdl	*India* *Pakistan* *China*				* * *
penetration	*India* *Pakistan* *China*	? * *	? ? ?	? ? ?	? ? ?

Genetics of Resistance

	Estimate of dominance			
INSECTICIDE	SR	RS	Nature of the alleles	
Cyper	0.84	0.84	Inc-dom	Autosomal
Endo	0.58	0.64	Inc-dom	Autosomal
Quinal	0.59	0.57	Inc-dom	Autosomal
Methomyl	0.96	0.92	Dominant	Autosomal
Spinosad	0.13	0.11	Recessive	Autosomal

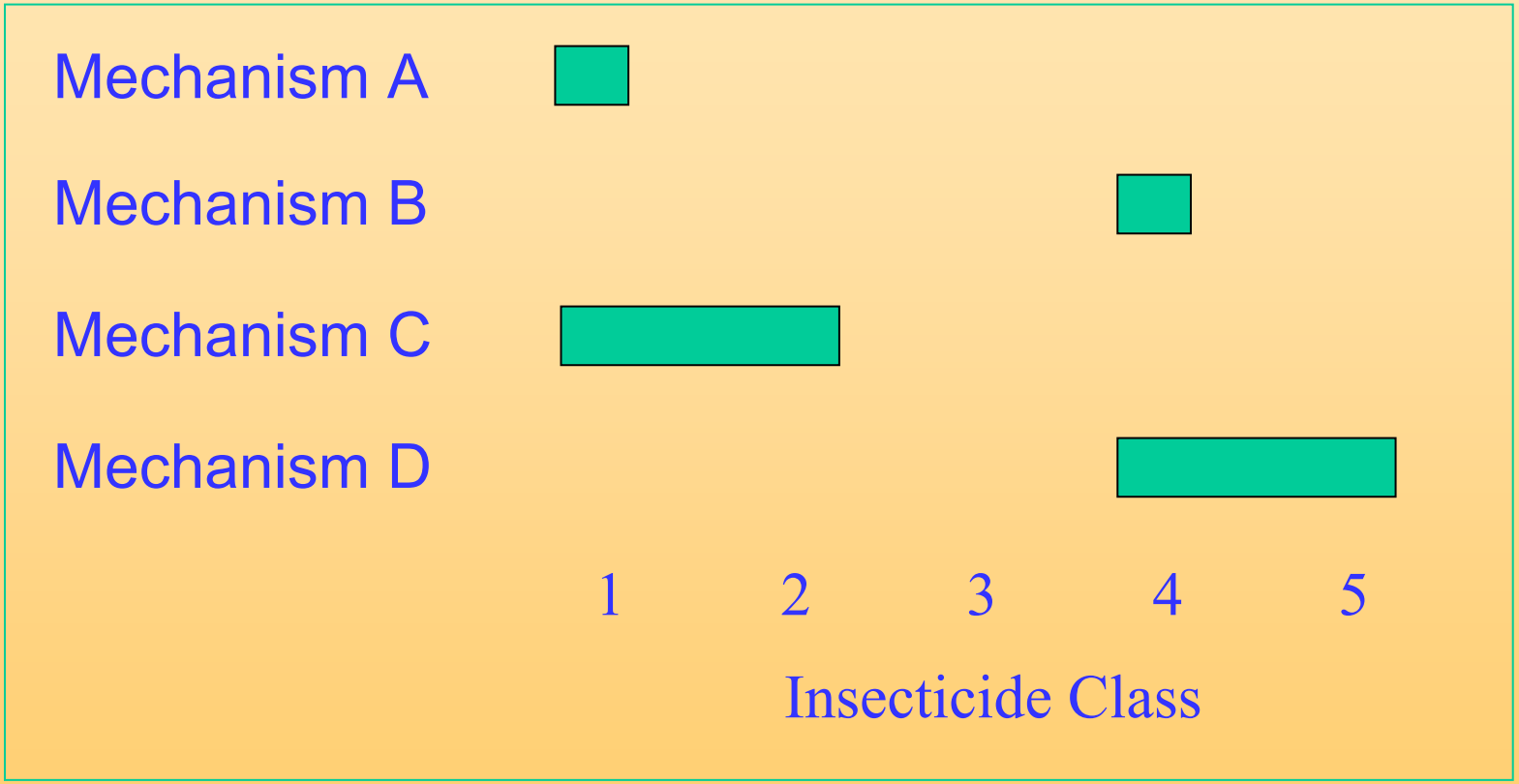
Genetics of Indian mechanisms

Insecticide	Mechanism	Nature	Frequency
Quinalphos	Esterase	Recessive	20%
	Insens-AchE	Inc-domi	80%
Methomyl	Esterase	dominant	90%
	Insens-AchE	Semi-dom	30%
Pyrethroid	Esterase	Inc-recessive	
	MFO	Inc-dominant	
	Nerve-Ins	Recessive	

Part III

Cross Resistance

Cross-resistance and insecticide rotations

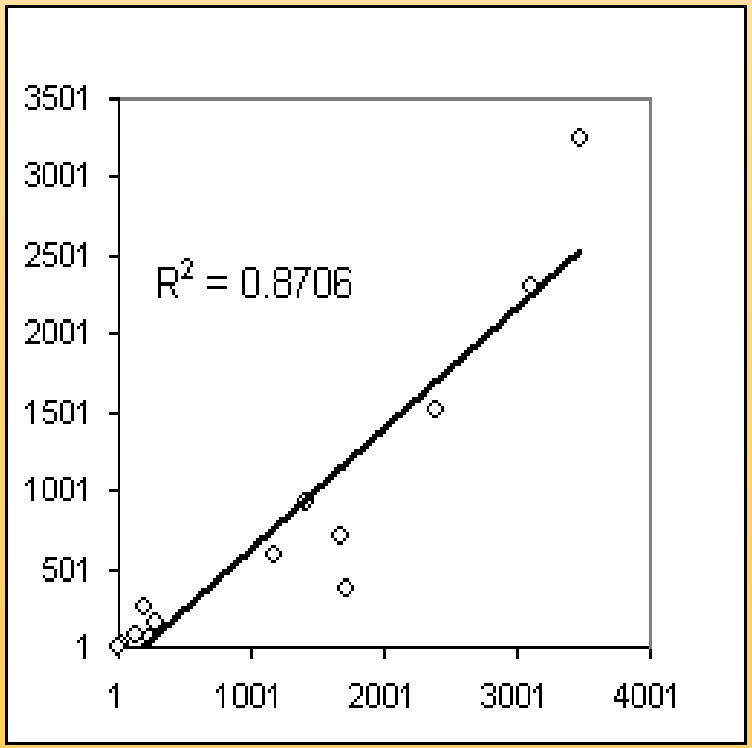


Three groups of insecticide can be rotated with confidence

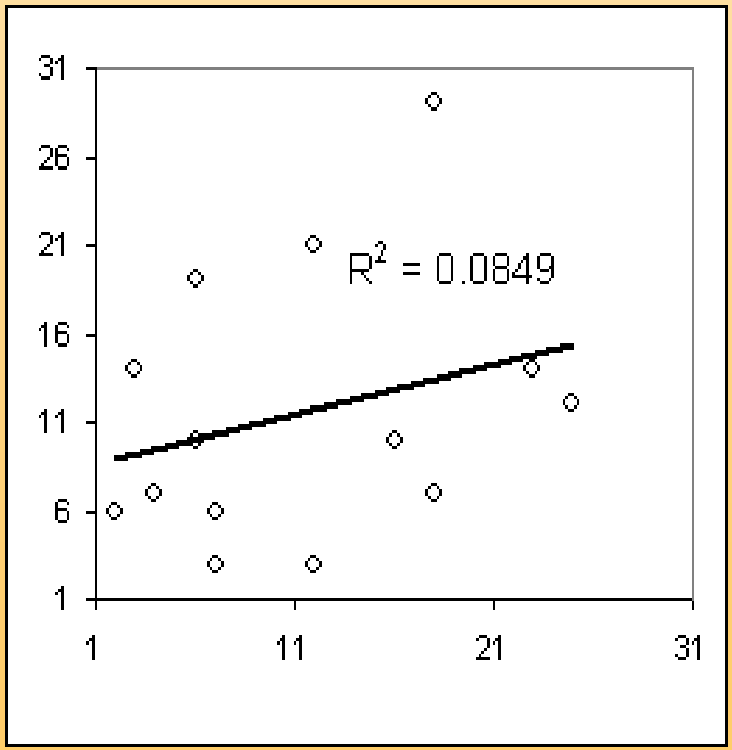
(1+2), 3, (4+5)

Correlations between resistance factors India

a) Cypermethrin vs. fenvalerate

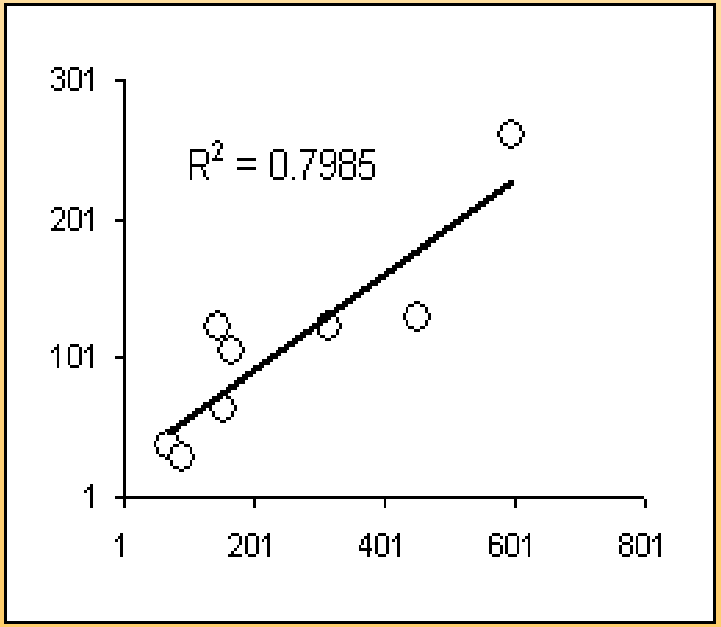


b) Cypermethrin vs quinalphos

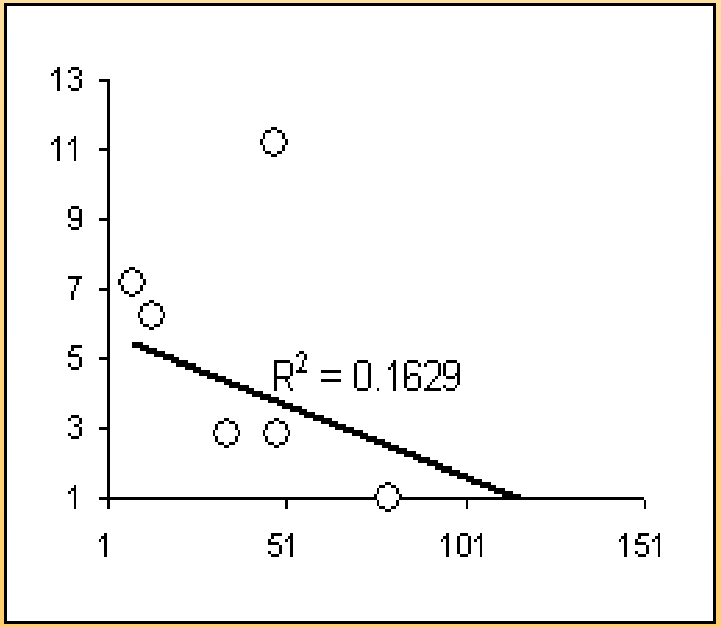


Correlations between resistance factors Pakistan

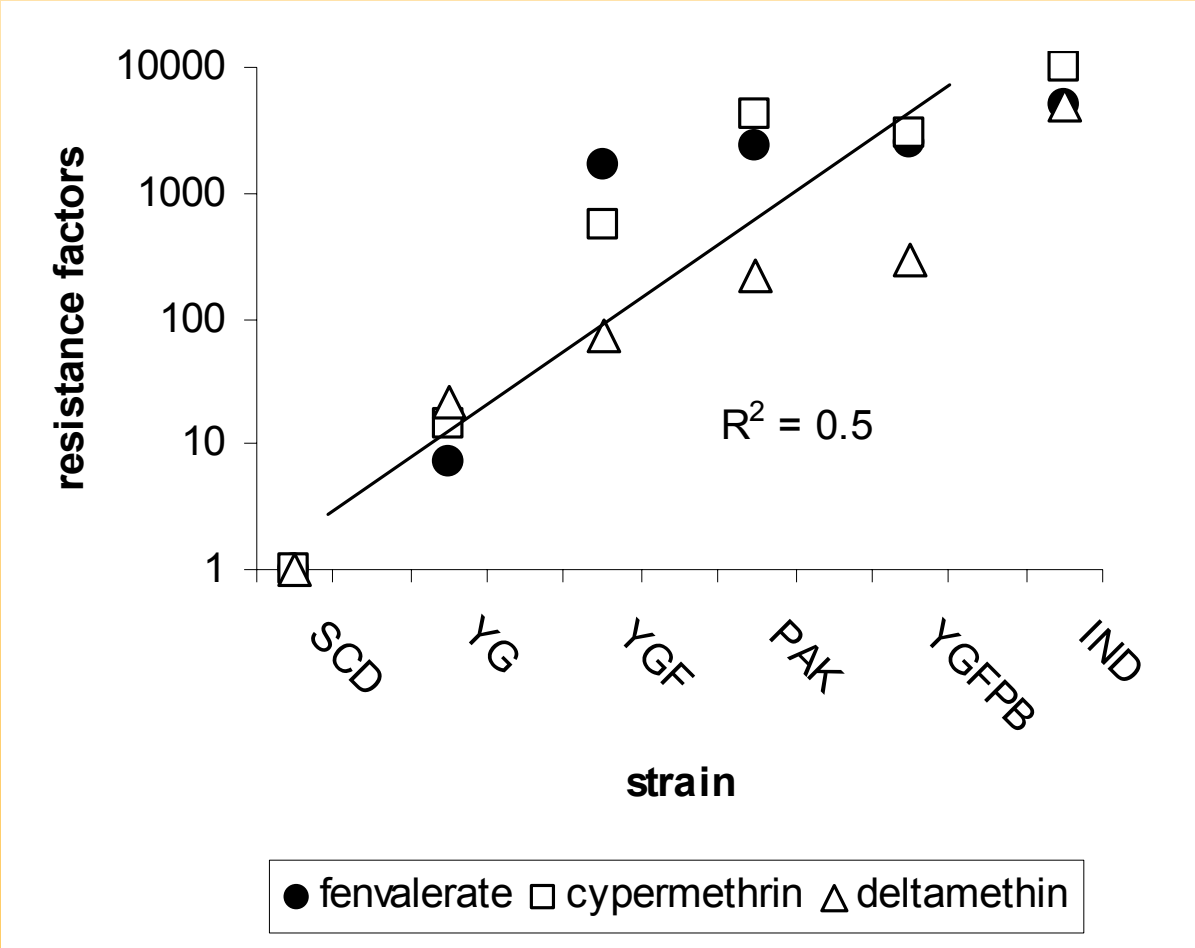
a) Cypermethrin vs. deltamethrin



b) Endosulfan vs methomyl



Correlation between resistance factors to pyrethroids



Use of Backcrossing to check the strength of cross resistance (linkages)

RR X SS

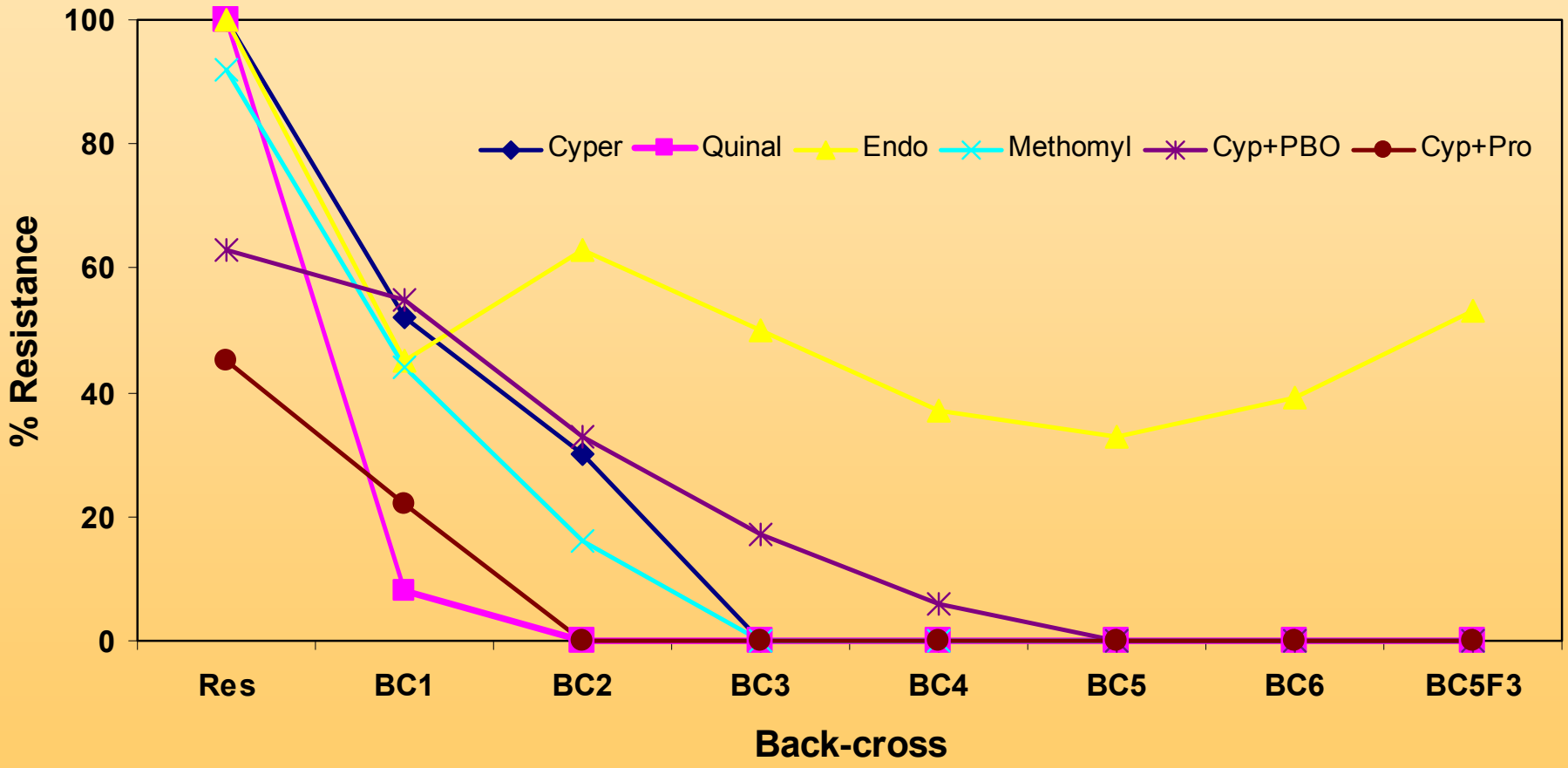
RS X <u>SS</u>

RS X <u>SS</u>

RS X <u>SS</u>

RS X SS

Introgression of resistance to Endosulfan

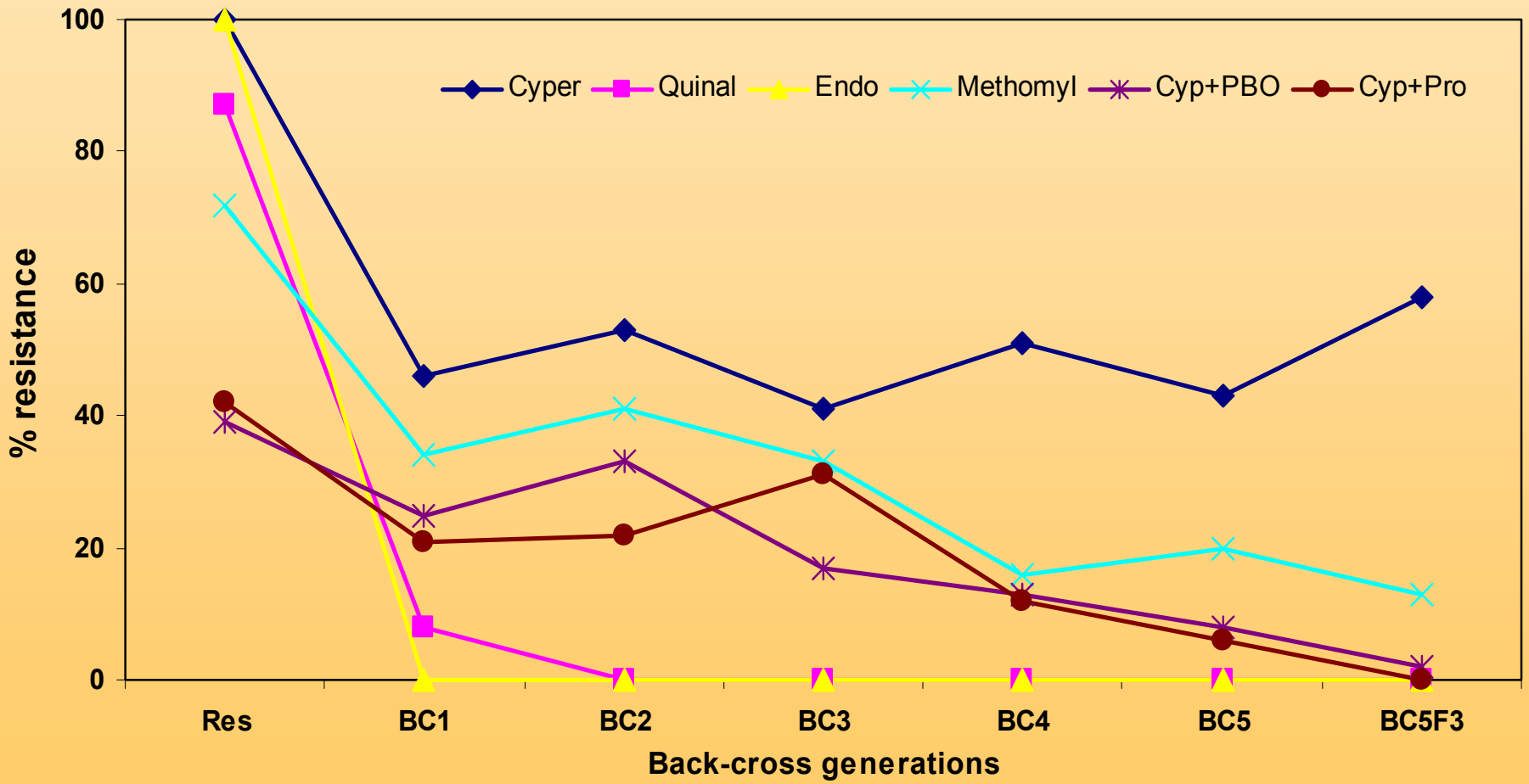


Endosulfan Cross Resistance

- None to Quinalphos
- None to Cypermethrin
- None to Methomyl

- Resistance to Cyp+PBO declined
- Resistance to Cyp+Profenofos declined

Introgression of resistance to Cypermethrin

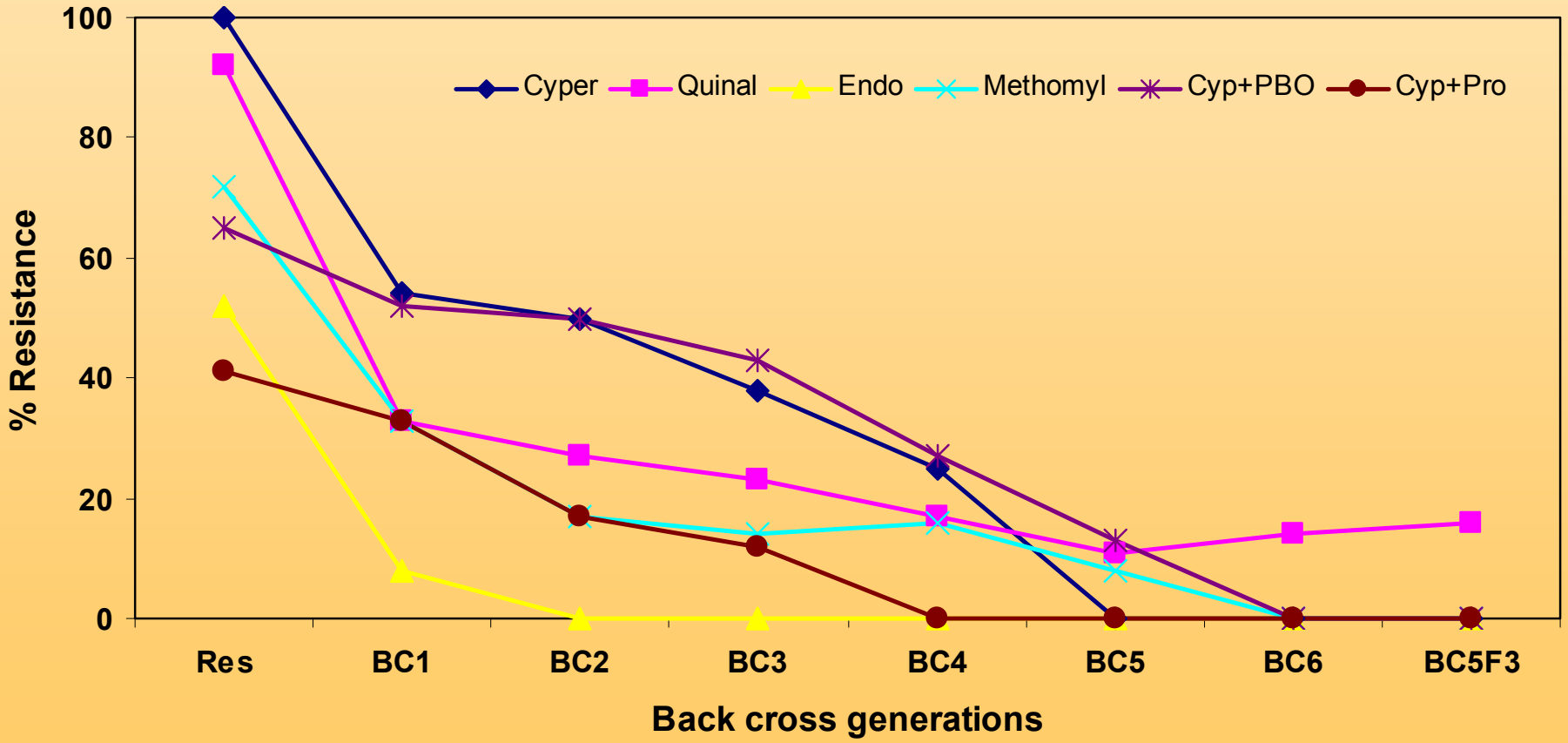


Cypermethrin cross resistance

- **None to Endosulfan**
- **None to Quinalphos**

- **Cross resistance to methomyl**
- **Resistance to Cyp+PBO declined**
- **Resistance to Cyp+Profenofos declined**

Introgression of Resistance to Quinalphos



Quinalphos Cross Resistance

- None to Endosulfan

- Cross resistance to Methomyl ??
- Cross resistance to Cypermethrin ??
- Resistance to Cyp+PBO declined
- Resistance to Cyp+Profenofos declined

Cross resistance *(H.armigera)*

	Pyrethroid	OP	Carbamate	Cyclodiene
Pyrethroid	Yes but some exceptions	No	No?	No
OP		Yes but not Monocrotophos	Yes variable	No
Carbamate			Yes Methomyl/ Carbaryl No Thiodicarb	No
Cyclodiene				Only one Endosulfan

Potential for rotations in India

	Major mech.	Minor mech.	Rotation groups
Pyrethroids	Oxidase	Esterase Nerve insensitivity Penetration	• **Most pyrethroids** • **Bifenthrin**
Organo-phosphates	Insensitive Ache	Esterase	• **Phosphatic –** **- monocrotophos** • **Phosphothioronate** **- profenophos** **- most others**
Carbamates	Esterase	Insensitive Ache	• **Methomyl/carbaryl** • **Thiodicarb**
Endosulfan	Esterase (sequestration)		• **Endosulfan**

Thank You for Your Attention

Genetics of Cytp450 in Cypermethrin Resistance