

Kits for Resistance Detection

Dr Keshav Kranthi

Central Institute for Cotton Research
Nagpur, India

Presented by
Dr. A. Regupathy,
Tamil Nadu Agricultural University,
Coimbatore
India

Components

1. Reviews of insecticide impact on pests and beneficials
2. Regional resistance monitoring
3. Cross resistance patterns
4. Principles of mixtures
5. Lab resistance v. field control
6. Spray practices
7. Insecticide quality kits
- 8. Resistance detection kits**
9. Field demonstration of control in Asia
10. Handbook of control of *H.armigera* in Asia

Why make Resistance detection kits?

- Identify resistance mechanisms
- Elucidate predominant field mechanisms
- Elucidate genetics of resistance mechanisms

Molecular

- SCAR marker (sequence characterised amplified regions)
- Co-Dominant markers –useful in recessive traits

Biochemical Mechanism based

- Unique esterase
- Unique cytochrome p450 properties
- Insensitive acetyl choline-esterase

Immunokits –Farmer empowerment

Quality detection kits; resistance detection kits; residue detection kits; disease diagnostics; insect diagnostics

- Highly sensitive – Can detect down to ppb
- Reliable – Extremely dependable results
- Rapid – Instant detection kits are designed
- Robust – Can be stored at room temperature
- Inexpensive – Cost: a few cents per test
- Bulk processing –Unlimited number of samples
- Easy to use – **Extremely farmer friendly**

Components 7&8: Immunodiagnostic kits

	ELISA	DIP STICK		
Insecticide	Quality	Quality	Residue	Resistance
Pyrethroid	Done	Done	Done	?????
Endosulfan	Done	Done	Done	Prototype
Cry1Ac	Comm.	Comm.	Done	Prototype
Cry1Ac+Cry2Ab	Done	Prototype	Done	Prototype
Carbamate	Done	Prototype	Prototype	Done
OP, Spinosad, Indoxacarb	Develop-ing	Develop-ing		Developing
HaNPV	Develop-ing			
Azadirachtin	Develop-ing			

Mechanisms and their importance

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

Mechanism A



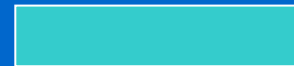
Mechanism B



Mechanism C



Mechanism D



1

2

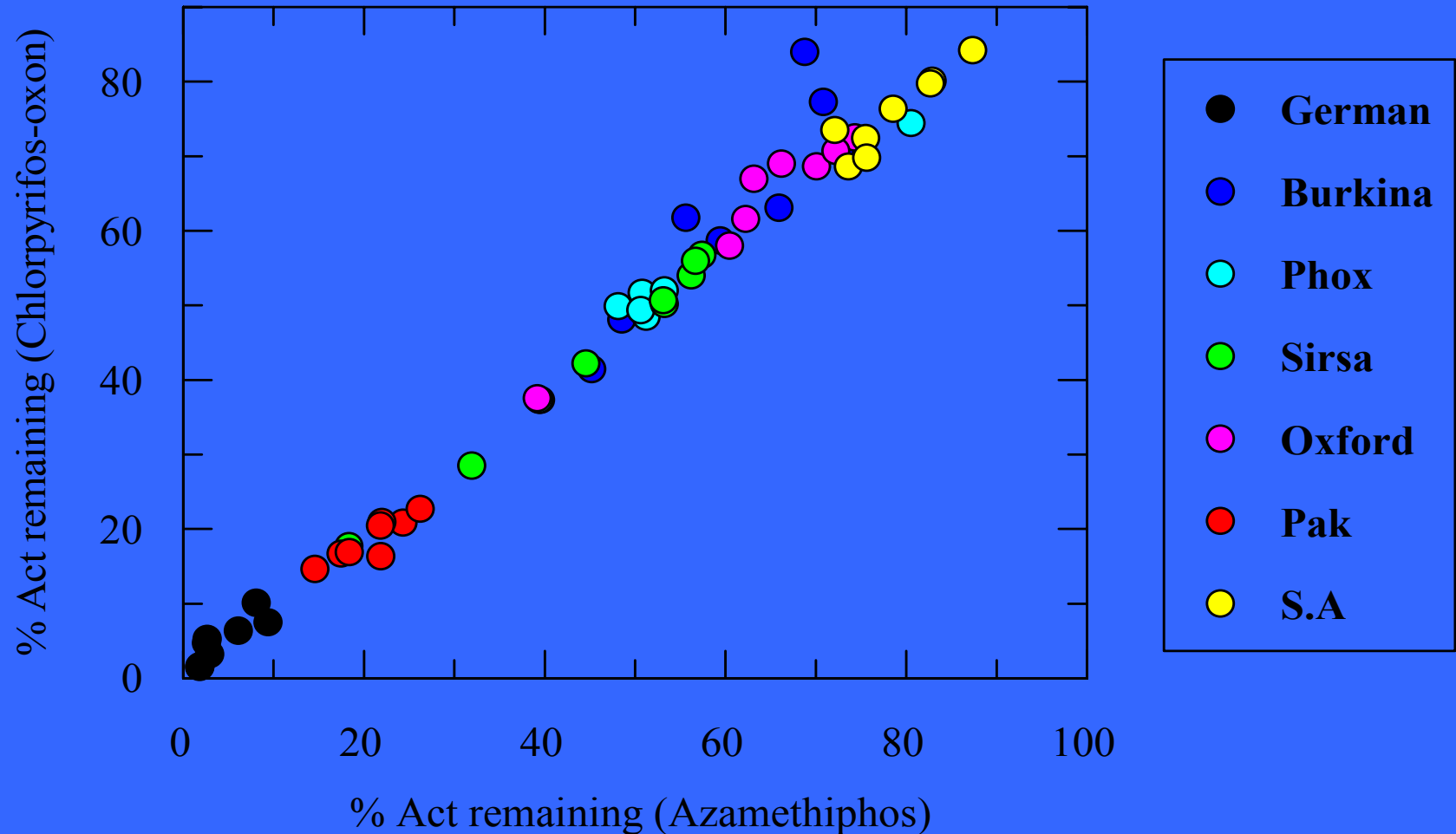
3

4

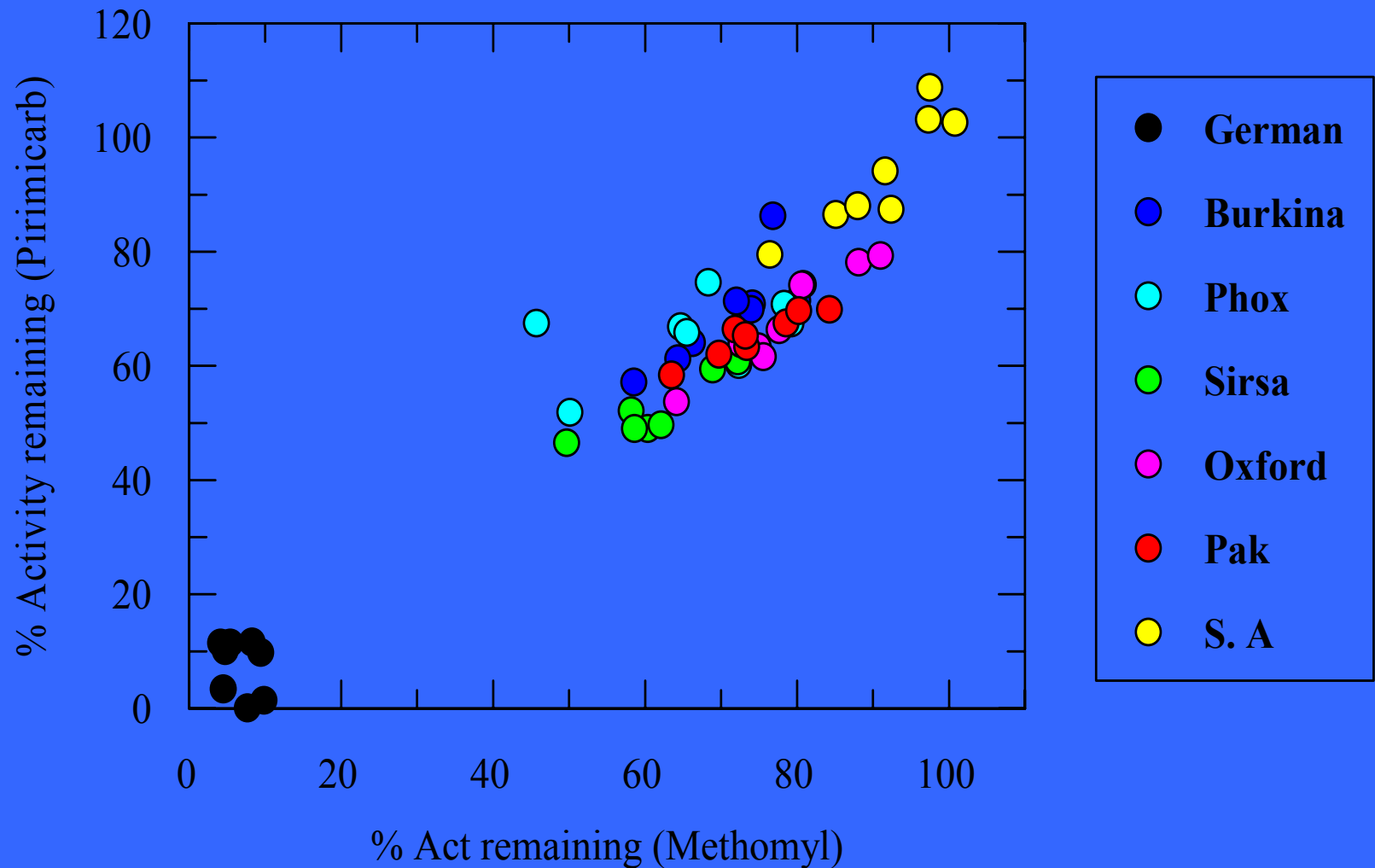
5

Insecticide Class

Acetylcholinesterase – all field strains show insensitivity to organophosphates compared to the fully susceptible German strain



Acetylcholinesterase – all field strains show insensitivity to carbamates and organophosphates compared to the fully susceptible German strain



Esterase-based* resistance to Methomyl

An example!

*esterases can sequester and/or detoxify methomyl

Genetics of mechanisms

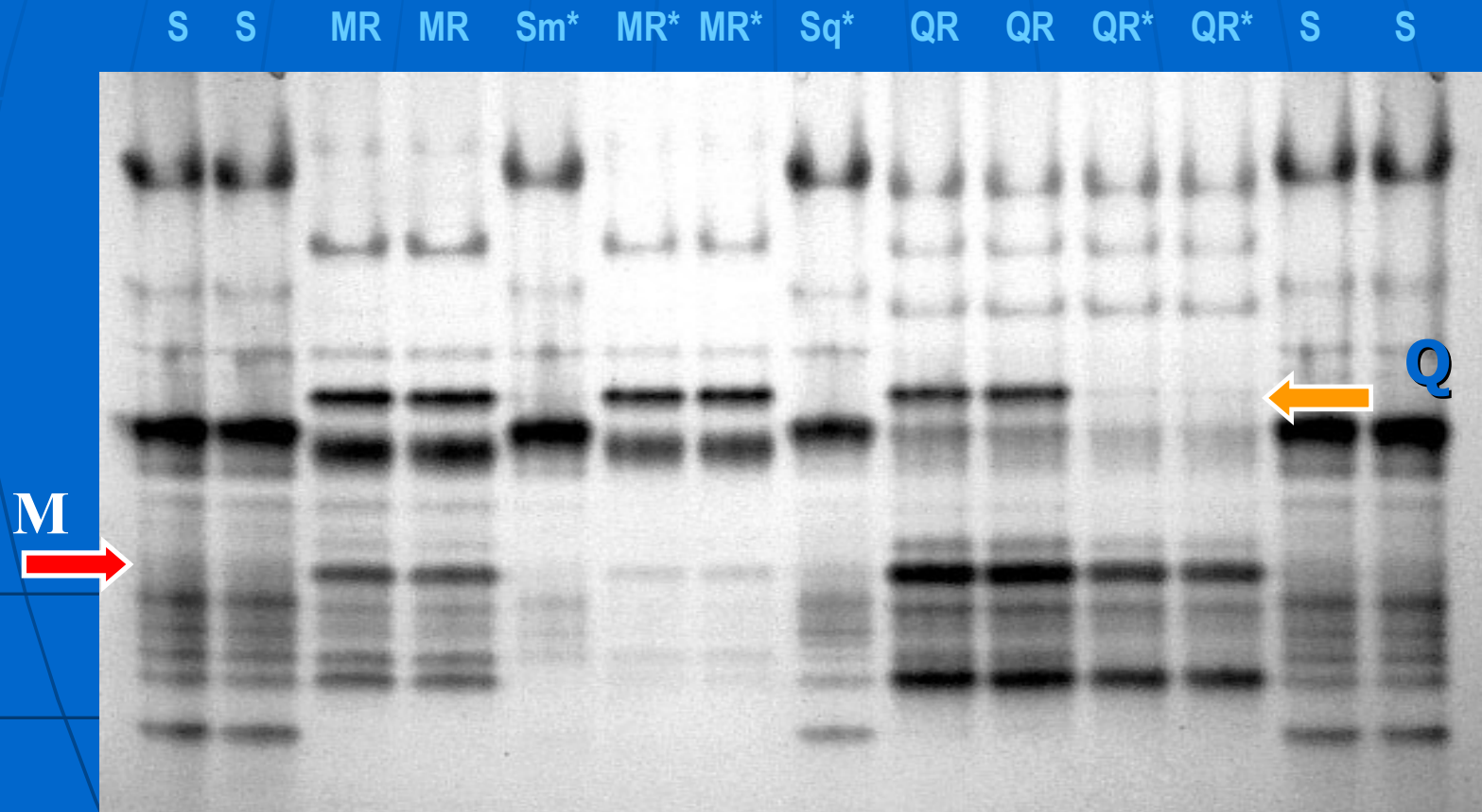
Insecticide	Mechanism	Nature	Frequency
Quinalphos	Esterase	recessive	20%
	Insens-AchE	inc-domi	80%
Methomyl	Esterase	dominant	90%
	Insens-AchE	semi-dom	30%
Pyrethroid	Esterase	inc-recessive	
	MFO	inc-dominant	
	Nerve-Ins	recessive	
Endosulfan	Esterase	inc-recessive	

Genetics of Resistance

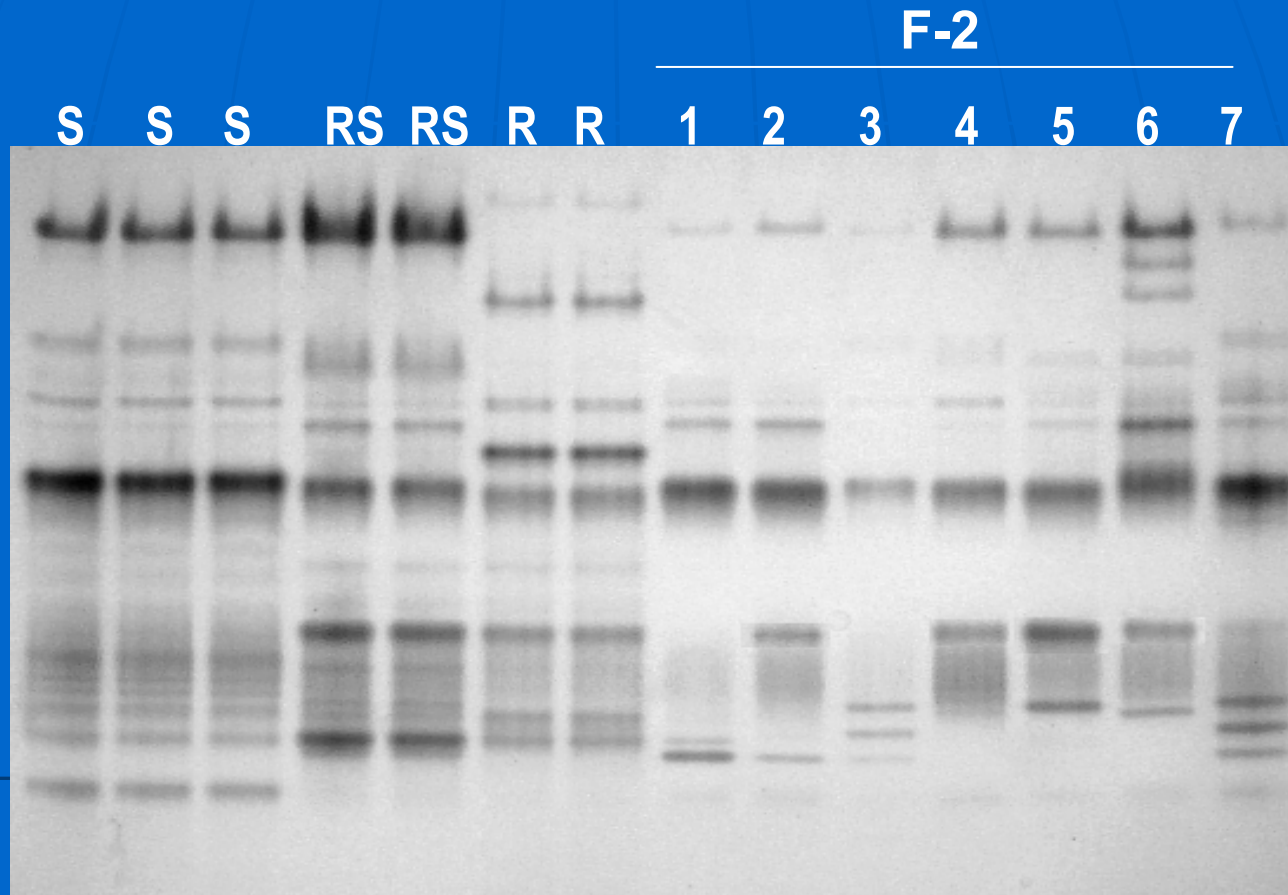
Estimate of dominance

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

Resistance Associated Esterases

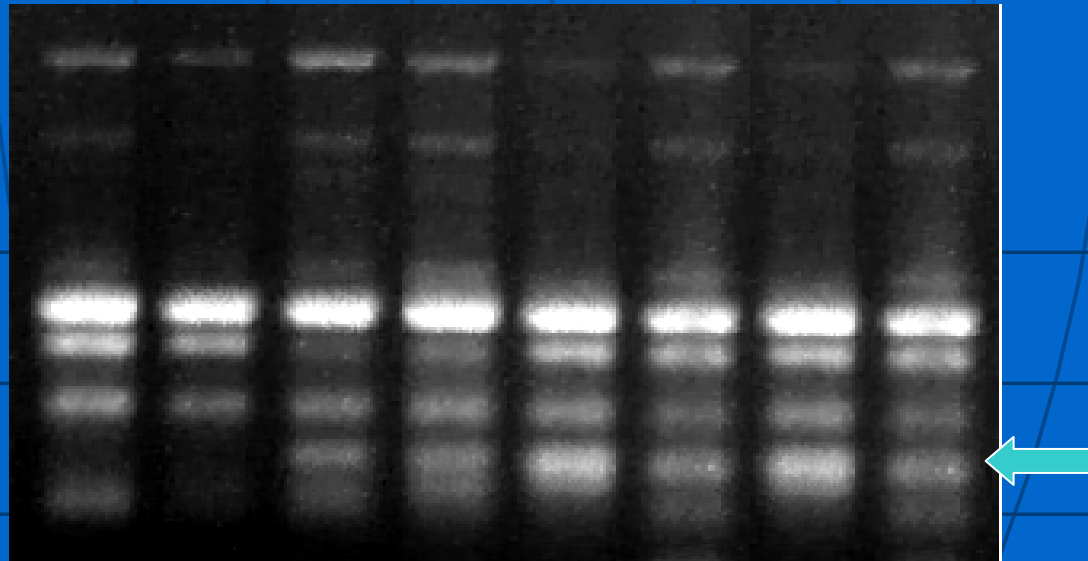
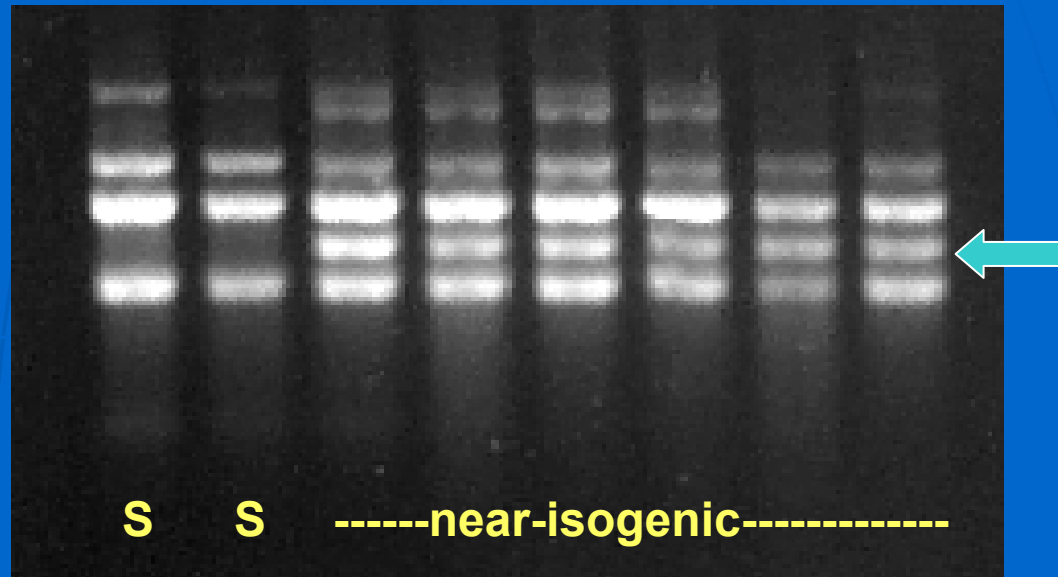


Esterases Genetics of Methomyl resistance



Dominant -autosomal

DNA polymorphism in Methomyl-R near-isogenic lines



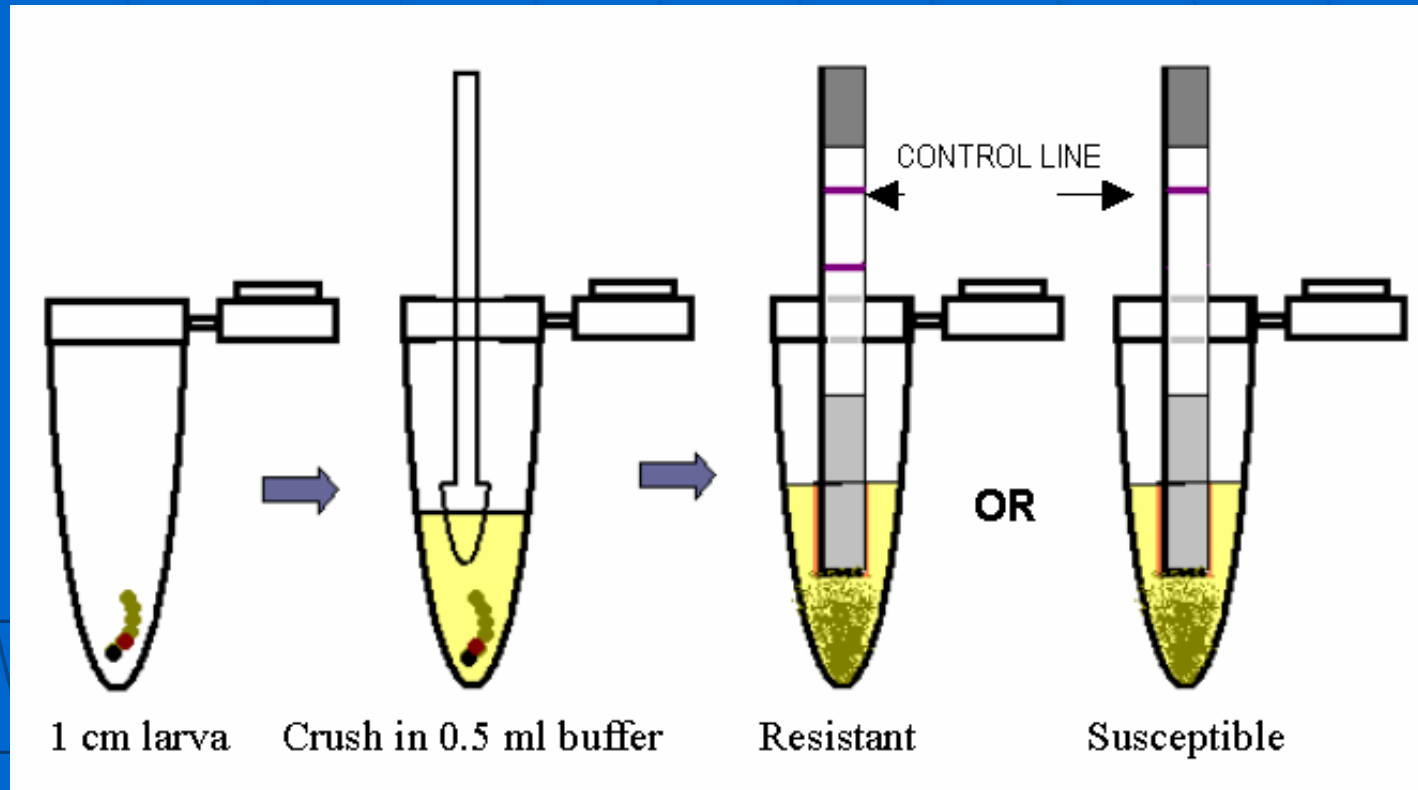
Making a 'dip-stick' kit for the methomyl-specific esterase

Steps:

- Inject the esterase (as **antigen**) into a rabbit
- Extract the **antibody** to the esterase from the rabbit's blood
- Use the **antibody** to make a 'stripe' across a nitrocellulose strip
- At the base of the strip add a 'pad' which will allow the esterase to pass through and move up the strip
- When the particular esterase (**antigen**) contacts the stripe (**antibody**) the stripe changes colour

Insecticide Resistance detection kit

Immunochromatographic strip



Pyrethroid resistance by metabolic mechanisms - dip-strip kit

- Farmer level kit should determine resistance by any mechanism.
- Pyrethroids – metabolic: Oxidases, Esterases
 - target site: Nerve insensitivity
 - penetration reduction
- Nerve insensitivity and penetration resistance are never present without metabolic resistance
- Developing a modified dip-strip system which relies on the sequestration of metabolism of a dilute insecticide solution by resistant larvae.

Other kits under development

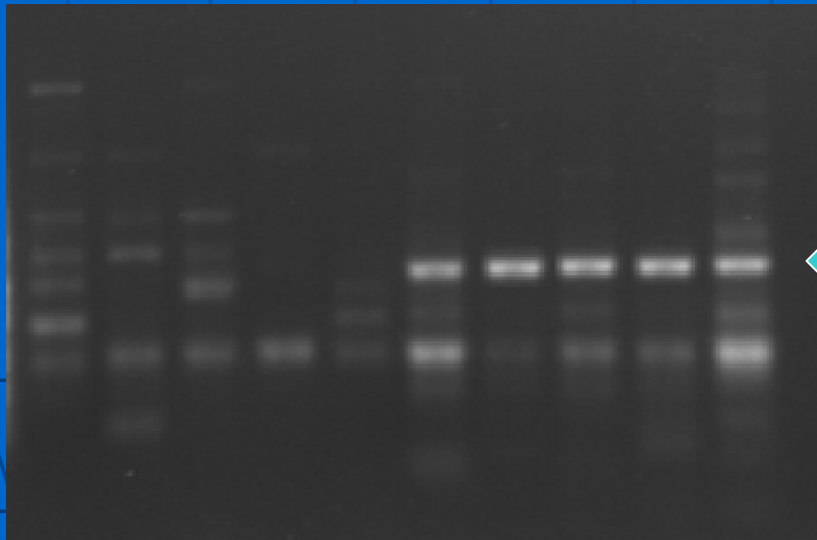
- SCAR-based pyrethroid nerve insensitivity resistance kit well underway based on primers to the four most polymorphic RAPD products.
- A dot-blot cypermethrin detection kit is being fine-tuned based on specific inhibition profiles to particular esterase and glutathione transferase iso-enzymes.

SCAR Marker for pyrethroid resistance

Forward : CTGTTGCTAC CTTATTATTC

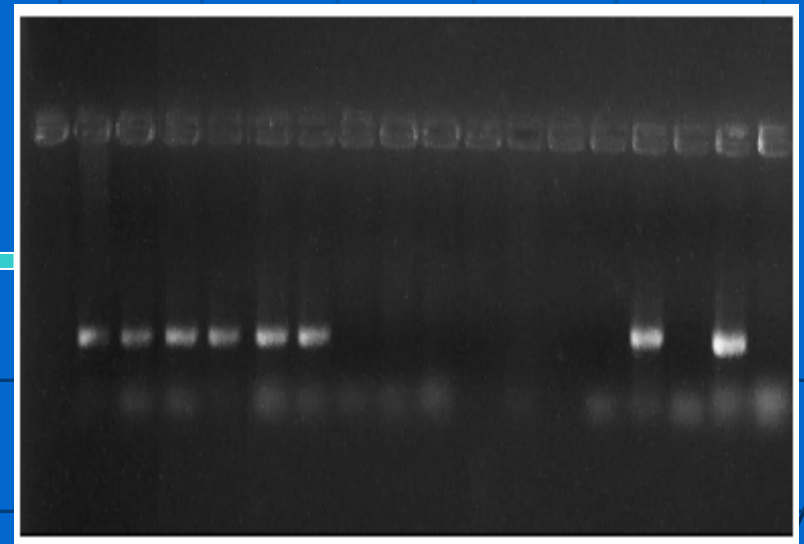
Reverse : ATCTTGCTACG AATGGTCCGC

RAPD of pyrethroid R & S strains



**The 0.6 kb band - sequenced &
SCAR marker was designed**

Marker validation

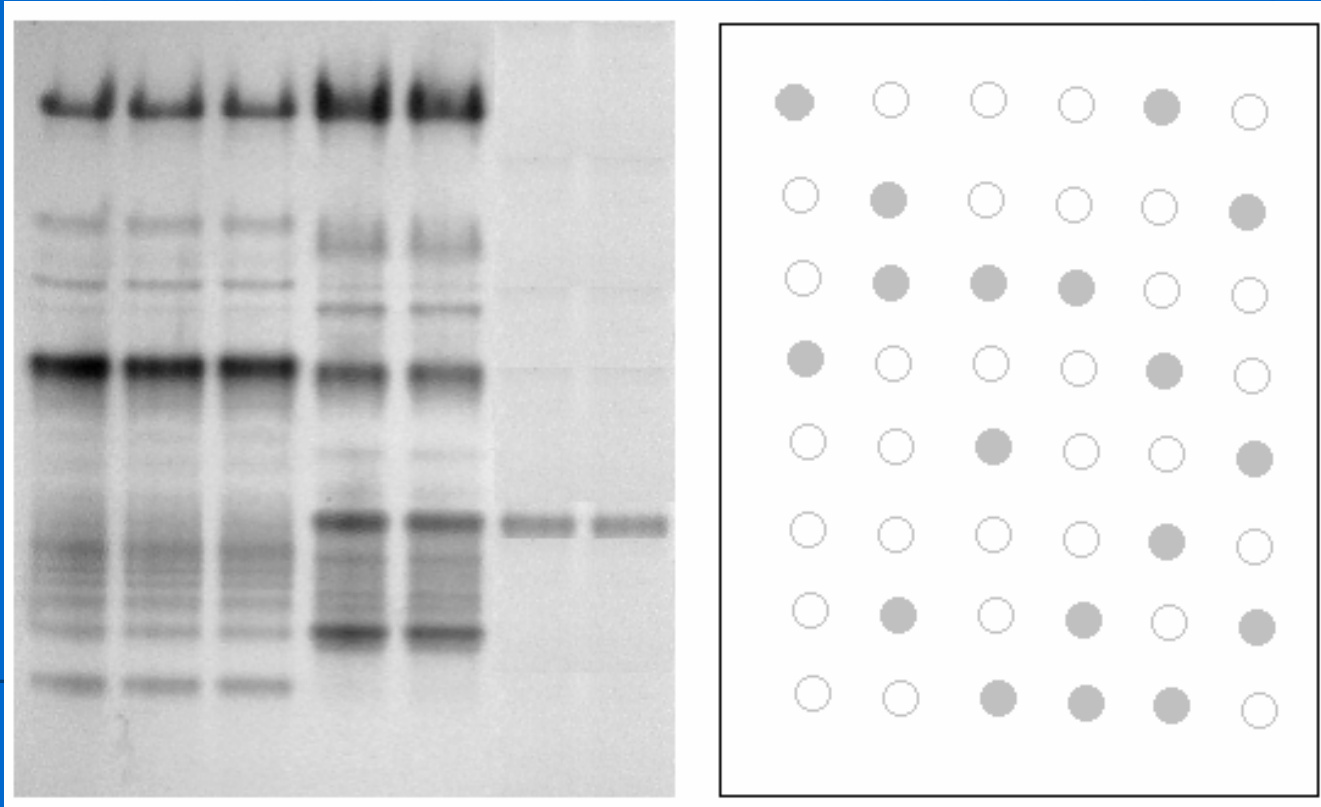


Resistant

Susceptible

F-2

Methomyl (carbamate) resistance detection kit

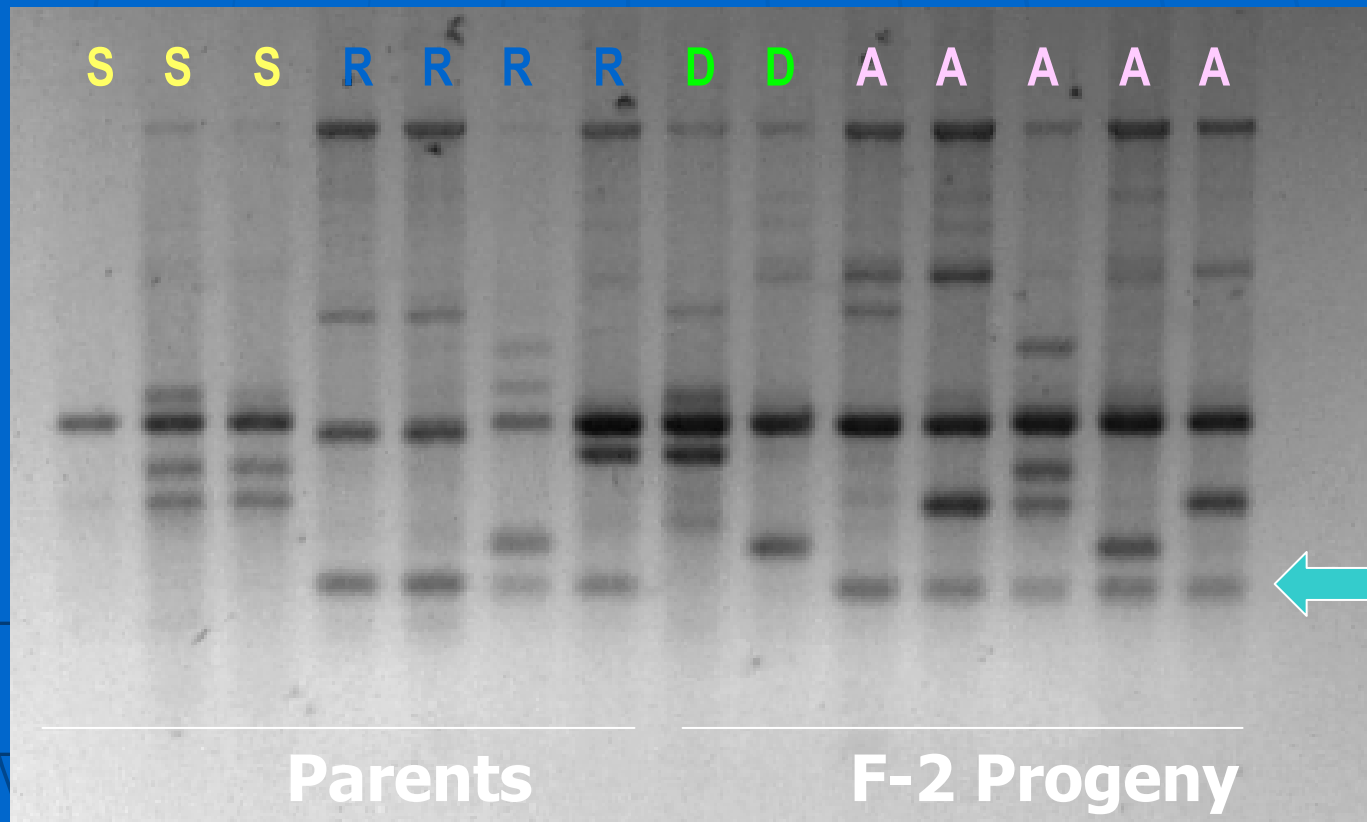


S S S R R * *

Dot-blot kit

RAPD FOR METHOMYL RESISTANCE

Primer- AB3-07



Component 8: Resistance detection kits - achievements

- A biochemical dip-strip kit for methomyl (dominant) resistance has been developed
- Other kits metabolic mechanisms are under development
- SCAR-based pyrethroid nerve insensitivity resistance kit underway based on primers to the four most polymorphic RAPD products.
- A dot-blot cypermethrin detection kit based on specific inhibition profiles to particular esterase and glutathione transferase iso-enzymes.

THANK YOU
for your attention

Strains developed

Pyrethroid -R	60,000 x
Endosulfan -R	500 X
Quinalphos -R	645 X
Methomyl -R	130 X
Cyper + pbo	50 x
Cyper + profenophos	5,700 x

Pyr-Esterase strain

Pyr-Oxidase strain

Pyr-Nerve insensitive strain

Quinalphos esterase strain

Quinalphos insensitive AChE strain

Methomyl esterase strain

Methomyl insensitive AChE strain

BC-6 (F2) near isogenic lines

Cypermethrin –R 180 X

Quinalphos –R 32 X

Methomyl –R 120 X

Endosulfan –R 45 X

Cyper + pbo 25 x

Cyper + profenophos 189 x

Cry1Ac 145 x

Pyr –Oxidase, Pyr-Esterase

Quantitative ELISA

Cry1Ac: Detection sensitivity: 10 ppb

Cry2Ab: Detection sensitivity: 20 ppb

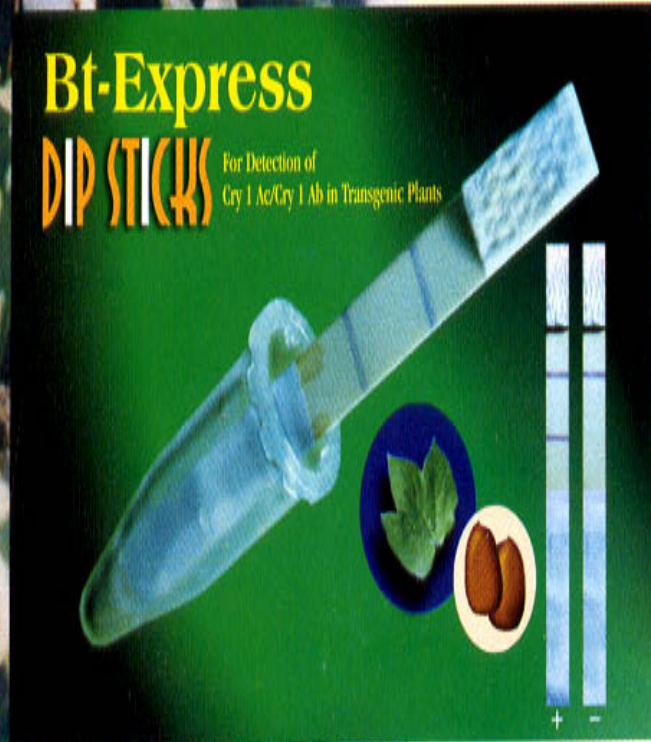
Pyrethroids: Detection sensitivity: 2 ppb

Endosulfan: Detection sensitivity: 5 ppb

Bt-Express

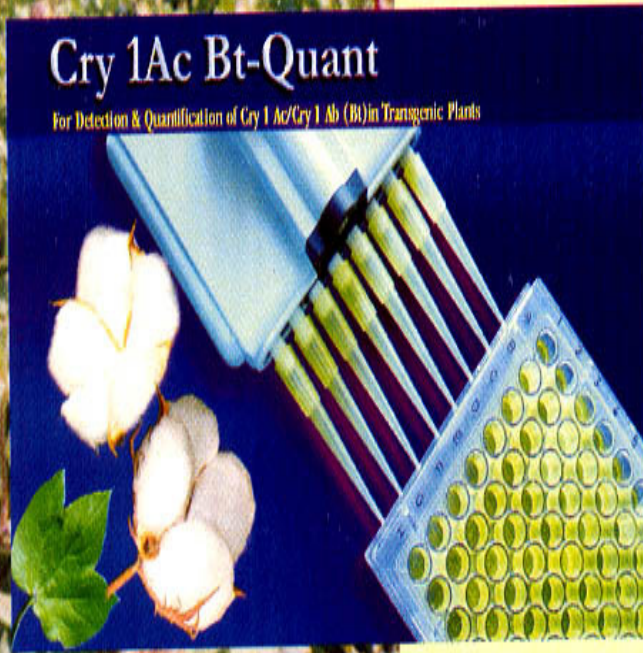
DIP STICKS

For Detection of
Cry I Ac/Cry I Ab in Transgenic Plants



Bt-Express

1. This is a dipstick format and can be used by even a layman.
2. Bt-Express has been designed for instantaneous detection of Bt- Toxin in either seeds or plant tissues.
3. It takes about 10 minutes for the test to be completed.
4. The test can be used in fields and does not require any additional facilities for use.
5. All material required for the testing is provided with the kit.
6. The kit is rapid, reliable and ready to use.



Cry 1Ac Bt-Quant

1. The Cry1AC Bt -Quant is an ELISA kit, which facilitates a precise quantification of Cry1Ab or Cry1Ac, expressed in transgenic plants.
2. The kit is simple, cost effective and very reliable.
3. It takes about 2hrs for completion of one set of ELISA assay.
4. Each ELISA plate can be used for 96 samples (including four wells for standards and two for blank). Depending on the capabilities of a laboratory, hundreds of samples can be processed in a single day.
5. ELISA plate reader is a requirement for use of the kit.
6. Additionally the ELISA kit can also be used for the quantification of Bt-toxins in Bt(*Bacillus thuringiensis*) insecticide formulations. Separate standards will be provided on request.



Component 7: Insecticide quality kits

www.indiaagronet.com/innovativebio

Bt-TEST KITS

RAPID - RELIABLE - ECONOMICAL

IMMUNODIAGNOSTIC KITS

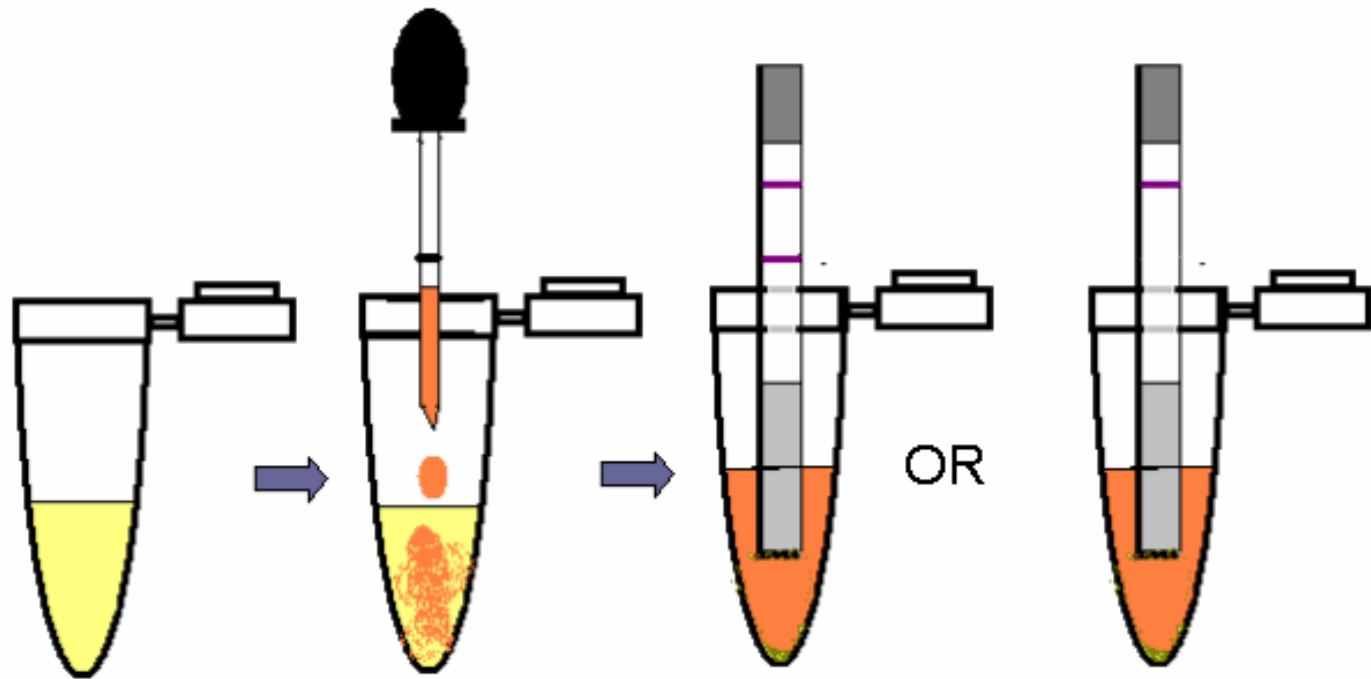
(Patent Pending)

Developed by :
CENTRAL INSTITUTE FOR COTTON RESEARCH
PANJARI, WARDHA ROAD, NAGPUR



Insecticide Quality Detection kit

Cypermethrin and Endosulfan



1. Buffer 0.5 ml

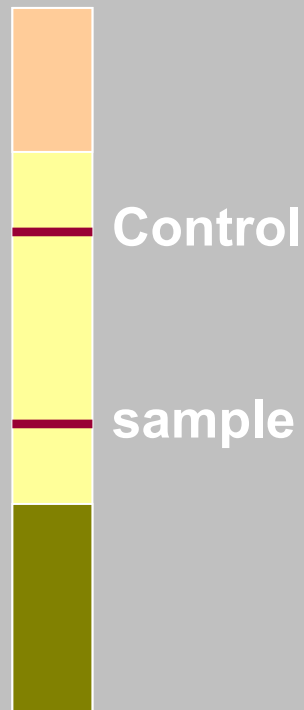
2. Add 0.01 ml insecticide

3. Poor quality

4. Good quality

The dip-stick strips (10 min)

The sensitive kit



Positive



negative

Quality detection



positive



negative

The ladder strips



Control line
Cypermethrin
Endosulfan
Carbamate
OP

A single strip can be used to detect more than one toxin simultaneously

- **Diagnostic**
- **Quality detection**
- **Residues**

Example: Cry1Ac & Cry2Ab (patent pending 600/del/2002)