Status of Insecticide Resistance in *Bemisia tabaci* to Commonly used Insecticides in Cotton from Punjab

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****CICR Regional Station, Sirsa, Haryana, India***

Whitefly

- Devastating pest of ~ 506 crop plants; sole vector of ~111 geminiviruses
- Serious on cotton, cassava, legumes, vegetables and many other crops causing yield losses between 20 and 100 percent
- **Outbreak on Bt cotton in North India caused huge losses due to pest and CLCuD in 2010 and 2013.**
- **In year 2015 the pest has appeared in endemic forms causing 60-70 % loses in North India**
- Management with insecticide is proving failure due to rapid resistance development & pest resurgence.

Whitefly attack: Tranquil Haryana, turbulent P

STARK CO[...]T High percentage of compensation paid for wheat, mustard damage keeps Haryana farmers hopeful

HT Correspondent

Hot, humid weather triggered whitefly attack, say experts

CHANDIGARH: The hot and humid weather in the northern region was instrumental in the huge build-up of whitefly that caused massive damage to the cotton crop in Punjab and Haryana.

Use of "cocktail of insecticides" by some "ignorant" cotton growers, which led to killing of "friendly insects" to keep a check on whitefly was another reason, experts said.

"It is hot and humid weather condition, which was the major factor," said HAU director of research, SS Siwach.

Farm scientists further pointed to farmers using a number of insecticides to contain the spread of whitefly that led to the flare-up of the problem. "Another factor is that worried farmers used cocktail of pesticides for spraying..."

The Tribune

MAINLY CLEAR SKY 34.0° 21.0°

T SCAN: NEELON-DORAHA ROAD A HTMARE

MEDICAL NEGLIGENCE: HIGH DRAMA AT CIVIL HOSPITAL

Commuters bear brunt of farmers' stir

■ Several trains cancelled, while others running 4 to 10 hours behind schedule

At the Ludhiana railway station.

The Tribune

THURSDAY, OCTOBER 8, 2015 ■ LATE CITY EDITION

RAIL ROKO Protest over agri crisis to continue today

25 trains cancelled, 1,500 held as farmers' stir derails Punjab

Sarbjit Dhaliwal

02 PUNJAB

Whitefly attack yet another blow to farmers in Malwa

After losses in paddy and wheat, cotton harvest goes for a toss

Sarbjit Dhaliwal

A farmer uproots cotton plants in his field.

Experts visit Muktsar, advise farmers

60% cotton crop lost, Punjab offers ₹600-cr relief package

Ruchika M Khanna

Faced with 60 per cent loss to cotton crop in the Malwa region, the Punjab Government has okayed a Rs 600-crore compensation package for the affected farmers.

The report of a special girdawri ordered by the Punjab Government shows that 6.75 lakh acres under cotton crop have been affected in a whitefly attack. Cotton was sown on 11.25 lakh acres in the Malwa region this year. Farmers have ploughed back crop on 11,800 acres because of the pest attack. The crop failure is not only because of the pest attack, but also because of spurious pesticides that flooded the state markets, and were unknowingly used by farmers to control the whitefly attack.

The failure of cotton crop in the state has acquired political overtones, which the "pro-farmer" Akali-BJP government is finding difficult to battle. With over 15 farmers in the Malwa region having committed suicide following crop failure, the ongoing agitation by cotton growers and the Opposition parties taking potshots at the government on the issue, the government is fire fighting on all fronts.

Though girdawri reports for two tehsils—Gidderbaha and Bathinda—are yet to come, the government has started releasing compensation to farmers to quell the disquiet among those affected.

CONTINUED ON P11

Damage in figures

- 11.25 lakh acres were under cotton crop in the Malwa region this year
- 6.75 lakh acres have been affected by the whitefly attack
- 11,800 acres is the area in which farmers have ploughed back their crop due to the pest attack

The compensation

- ₹600 crore package has been okayed for cotton growers
- ₹520 crore has been sanctioned for disbursal among affected farmers, of which ₹170 crore has been already disbursed

Farmers' grudge & govt claim

- Some farmers say they have received compensation cheques for just ₹80 to ₹150
- The government says the compensation offered is ₹8,000 per acre. Cheques for smaller amounts are being issued only where there are many khewatdars for an acre

PUNJAB

Whitefly attacks cotton in Moga too

Farmers allege even pest-resistant seeds have been damaged in the attack

WHITEFLY ATTACK

Dry spell precipitating damage to cotton crop in Haryana

Rajesh Moudgil

CHANDIGARH: As the ongoing dry spell is precipitating the menace of the whitefly on the cotton crop with every passing day in Haryana, at least four districts in the state are said to be badly hit.

FOUR DISTRICTS IN STATE BADLY HIT; EXPERTS SUGGEST NEEM-BASED OIL AS SOLUTION

...was in about 5,000 hectares in the district, more than 50% in about 10,000 hectare and over 25% damage in 25,000 hectare area while in remaining area, the damage was less than 25%. Besides holding meetings in each village, the officials are also spreading awareness about methods such as use of neem-based oil or spray of ... through media and ...

...hectare crop cultivation, the whitefly had caused damage to an extent of more than 75% ... to an extent of about 50%. He too said that Neem-based oil was the time-tested remedy for the menace.

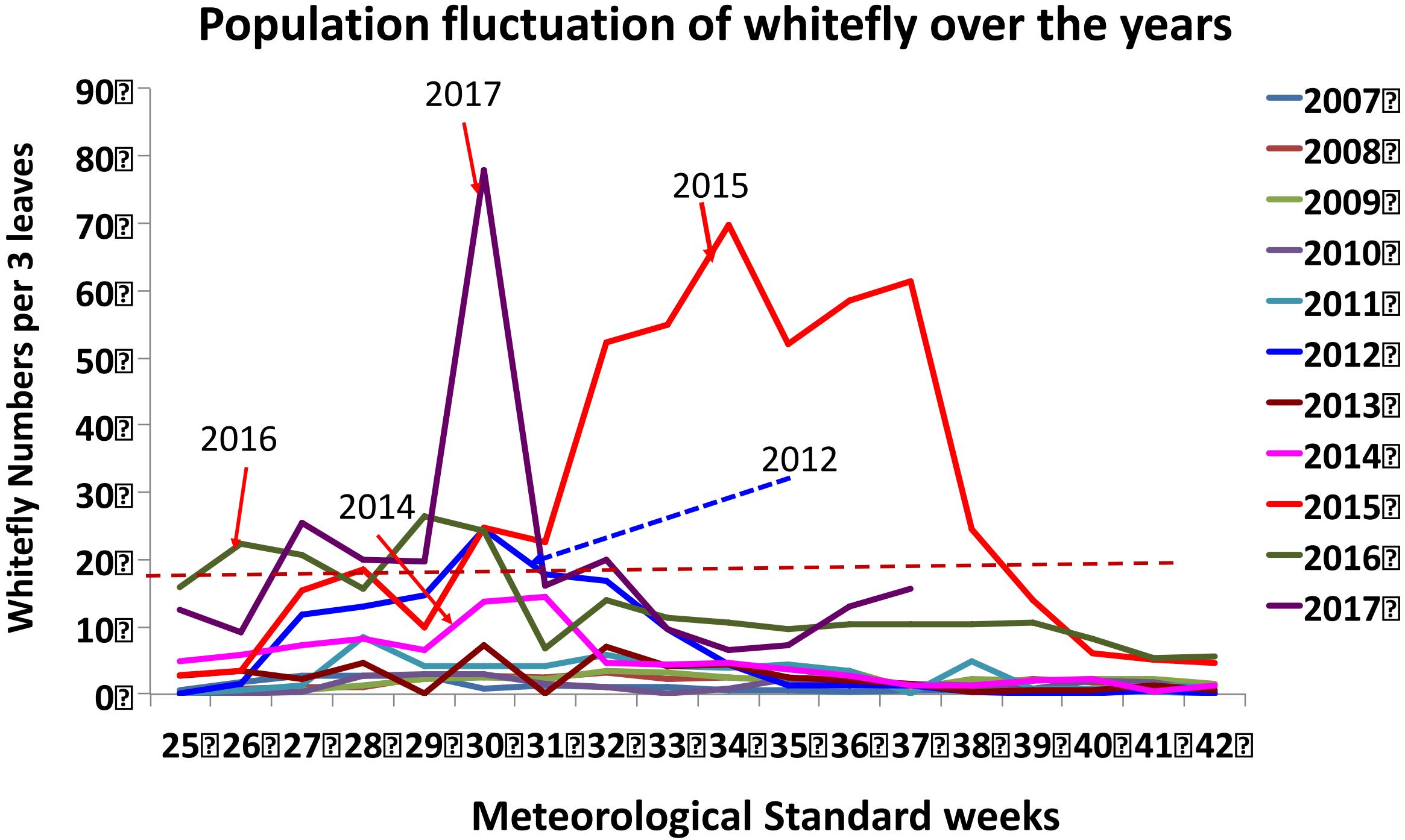
Agriculture department additional director Suresh Gehlawat

ICAL launches 'help the cotton farmer' campaign

HT Correspondent

BATHINDA: With farmers already feeling the brunt of damage to their cotton crop following the whitefly attack, the textile industrialists have also expressed concern over the quality of cotton yield during ...

4 - 5 HARYANA


Population fluctuation of whitefly over the years
Whitefly Numbers per 3 leaves
90
80
70
60
50
40
30
20
10
0
2017
2015
2016
2014
2012
2007
2008
2009
2010
2011
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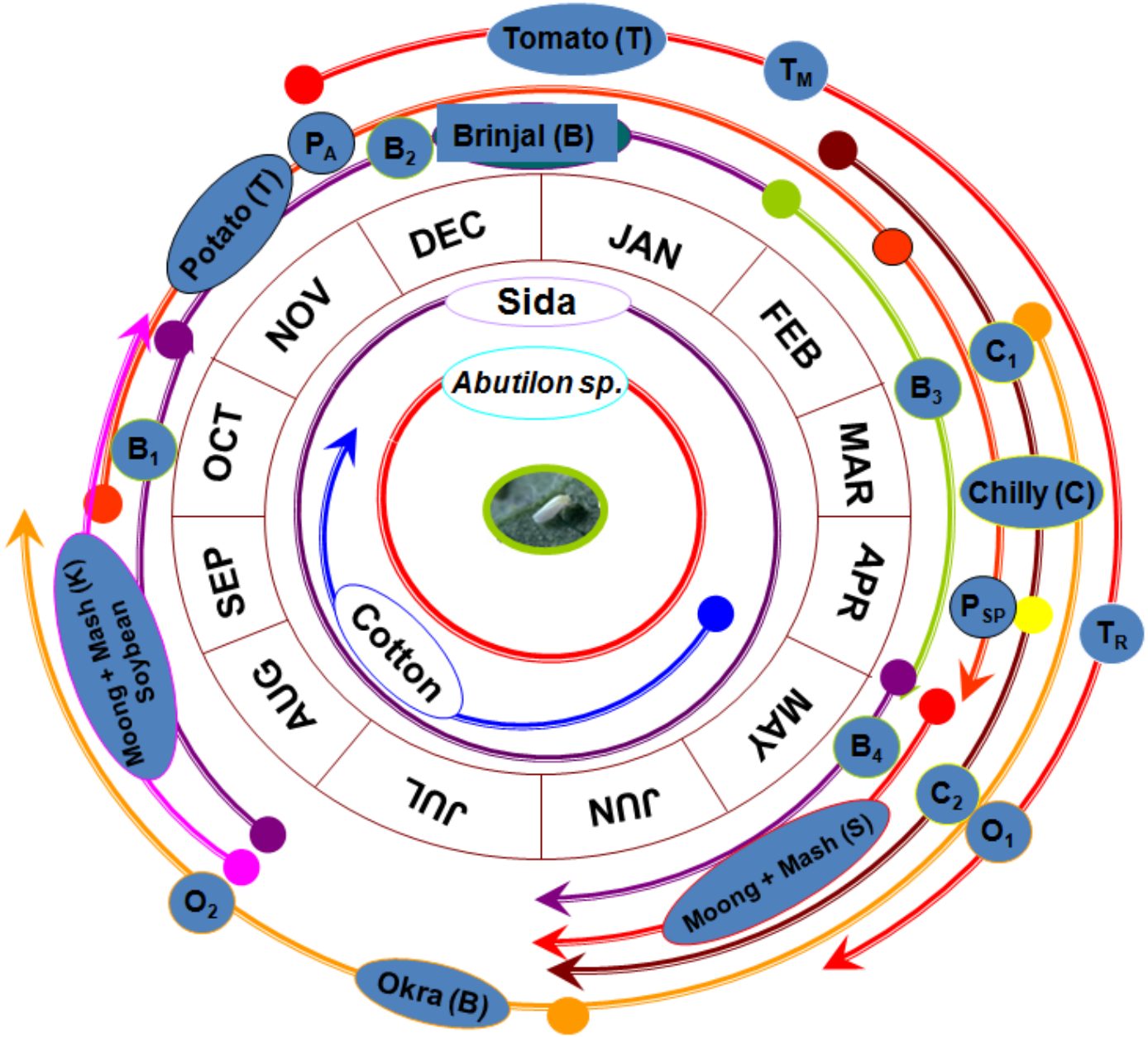
Meteorological Standard weeks

Diverse agro ecosystem and Host availability

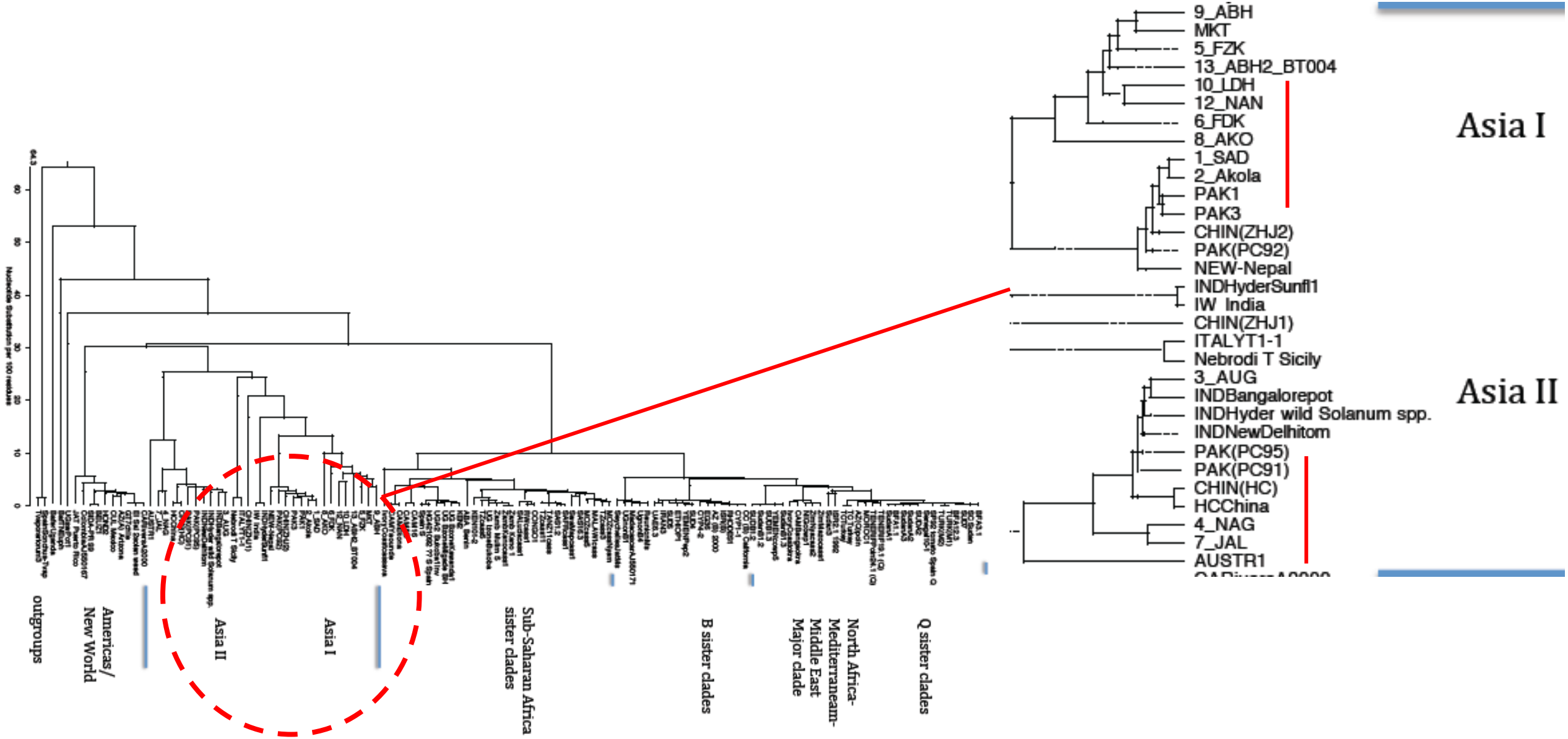


Whitefly calendar by Satnam Singh and V K Gupta

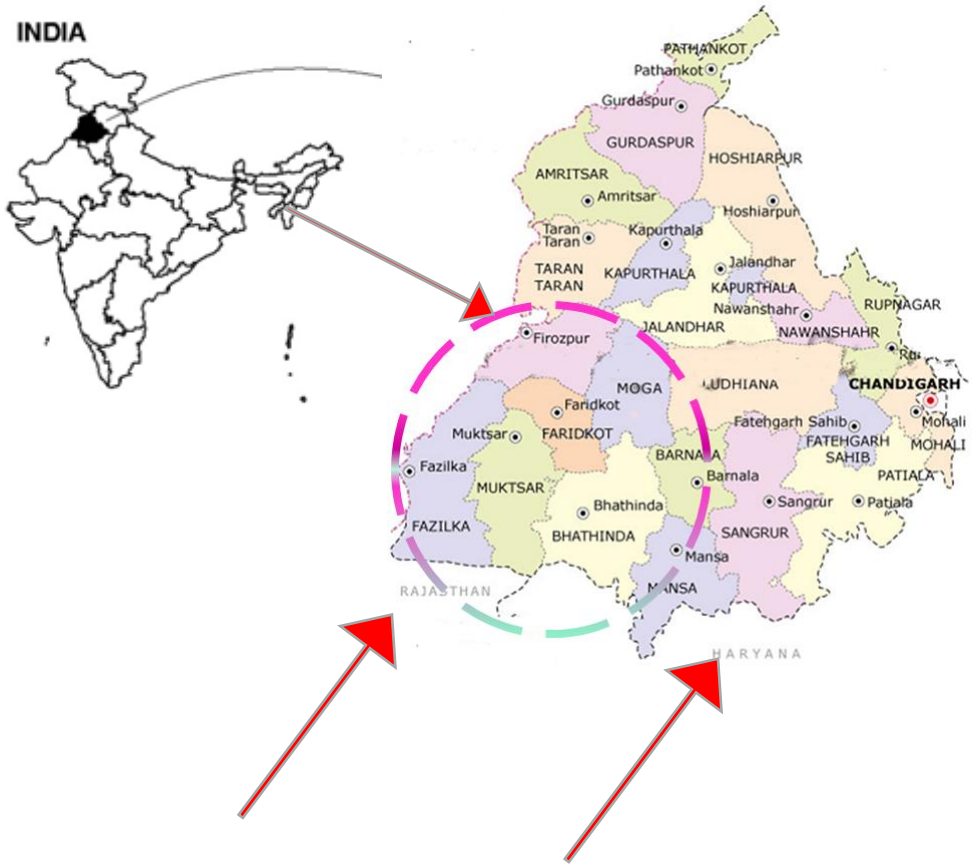
What we speculate for the upsurge

- High temperature and high humidity
- Inflow of susceptible germplasm vis-a vis multiplicity of the hybrids:
- The number of Bt cotton hybrids have increased substantially to more than 1000 compared to just 3 hybrids in 2002, the year of introduction.
- This number for North zone has raised to ~450 till April 2017.
- State of Confusion
  - Farmers what to grow? Extension Scientist what to recommend?
- Decrease in insecticide usage due to less number of sprays in Bt cotton
- Seed treatment of Gaucho as the crop is also sprayed with Imidacloprid (50 to 5450-fold R in 75% populations tested).
- Is it a change in biotype?
- Excess use of insecticides such a fipronil, acephate, acetamaprid
- **Insecticide resistance** or quality of insecticides?

Phylogenetic Analysis of *B.tabaci* populations across Punjab



Methodologies



- Population collected from different districts cotton, some individuals of same location were collected in RNA stabilization buffer for RNA isolation
- Leaf dip bioassay with serial concentrations of insecticide
- Calculation of LC 50 using Polo plus
- cDNA was synthesized from total RNA
- Relative expression analysis done using Cyp genes specific primers with the help of qPCR using ΔCt method with actin as internal control

Resistance monitoring on whitefly collected from hot spot areas of North Zone (2016-17)

Treatment	Susceptible (Nagpur) LC50	Sirsa			Hisar			Abohar			SGN		
		Season(LC50)		RR	Season(LC50)		RR	Season(LC50)		RR	Season(LC50)		RR
		Initial	End		Initial	End		Initial	End		Initial	End	
Neonicotinoids													
Acetamprid	0.43	1.2	1.2	2.79	2.1	3.4	7.91	2.4	5.0	11.63	0.99	0.43	1
Imidacloprid	0.35	0.51	0.71	2.03	0.10	0.08	0.23	0.25	0.93	2.6	0.24	1.1	3.14
Thiacloprid	-	2.5	4.8		0.68	0.82		0.10	0.39		1.4	0.282	
Thiamethoxam	0.25	0.96	13.07	52.2	1.1	7.3	29.20	0.36	14.74	58.96	0.36	3.2	12.8
Clothianidin	1.09	0.58	0.39	-	0.8	0.85	-	0.64	0.68	-	0.21	0.182	-
Dinotefuron	0.05	0.27	0.89	17.8	0.05	0.21	4.20	0.05	0.097	1.94	0.01	0.048	0.96
IGR,'s and insecticides with new mode of action													
Buprofenzin	2.58	11.62	32.67	12.66	4.0	12.46	4.83	6.6	73.74	2.58	1.0	79.93	30.98
Spiromesifen	0.49	9.3	31.6	64.4	0.16	3.4	6.94	0.7	116.6	238.6	2.0	4.46	9.10
Pyriproxyfen	40.12	9.5	56.82	1.42	3.0	1.7	0.04	0.7	3.6	0.09	1.0	3.4	0.08
Flonicamid	0.30	0.26	4.40	14.67	0.05	0.30	1.00	0.05	0.56	1.87	0.08	0.38	1.27
Fipronil 5SC	1.29	3.0	27.78	21.53	5.9	11.26	8.73	5.8	21.84	16.93	2.9	11.27	8.74
Diafenthiuron	0.03	0.3	2.45	81.6	0.38	2.12	70.67	0.44	2.30	76.67	0.23	1.45	48.34

Resistance monitoring on whitefly collected from hot spot areas of North Zone (2016-17)

Treatment	Susceptible Nagpur (LC50)	Sirsa			Hisar			Abohar			SGN		
		Season(LC50)		RR	Season(LC50)		RR	Season(LC50)		RR	Season(LC50)		RR
		Initial	End		Initial	End		Initial	End		Initial	End	
Organophosphates													
Chlorpyriphos 20EC	1.51	7.0	12.6	8.34	13.9	5.5	3.64	10.5	17.0	11.26	3.4	14.41	9.54
Ethion 50EC	1.64	1.6	9.8	5.98	1.7	12.10	7.38	1.6	6.8	4.14	1.8	4.3	2.62
Monocrotophos 36 SL	0.40	1.4	1.2	3.00	0.32	0.91	2.28	0.63	2.6	6.5	2.2	2.44	6.10
Profenophos 50EC	-	0.09	0.1		0.1	0.16		0.2	0.19		0.06	0.050	
Triazophos 40EC	0.19	2.8	7.1	37.37	4.0	4.45	23.42	1.0	8.2	43.15	2.8	5.5	28.94
Synthetic Pyrethroids and mixtures													
Fenpropathrin 30EC	0.45	0.4	1.2	2.67	0.34	1.71	3.80	0.26	1.6	3.56	0.26	0.46	1.02
Bifenthrin 10EC	0.08	0.08	0.52	6.50	0.02	0.16	2.00	0.01	10.66	133.3	0.03	0.05	0.63
Chloro+Cyper	-	1.8	2.9		0.28	1.5		0.8	1.1		0.83	1.33	
Deltamethrin+Triazophos	-	2.1	3.1		2.0	2.3		1.3	1.8		0.81	1.06	
Indoxacarb+Acetamprid	-	2.5	3.0		2.4	1.7		1.4	2.1		0.97	0.971	
Pyriproxyfen+Fanopropathrin	0.75	5.1	5.5	7.33	4.7	15.1	20.13	1.9	3.3	4.40	2.3	2.380	3.17

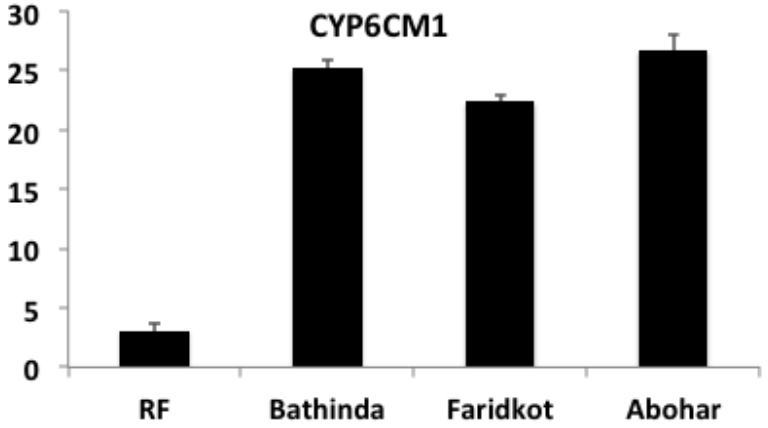
Resistance monitoring on whitefly collected from hot spot areas of Punjab (2017-18)

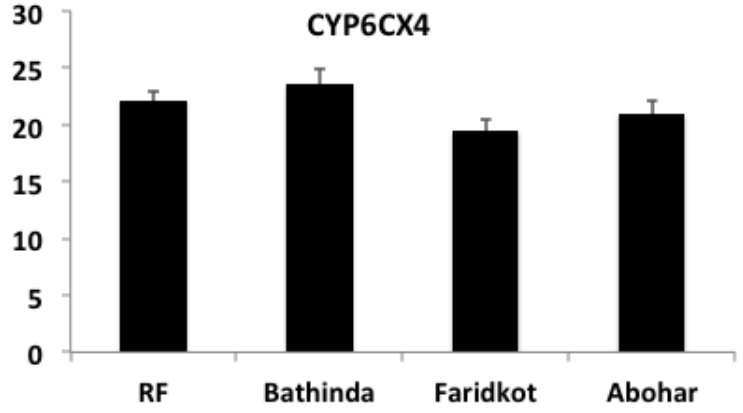
Treatment	Susceptible (Hos) LC50			Bathinda		Abohar	
			RR		RR		RR
Acetamprid 20 SP	1.60	12.32	7.70	16.23	10.14	18.24	11.40
Ethion 50EC	6.32	11.34	1.79	13.54	2.14	16.14	2.55
Fipronil 5SC	2.34	21.50	9.19	36.31	15.52	29.24	12.50
Flonicamid 50WG	0.03	0.67	22.33	0.05	1.67	1.25	41.67
Imidacloprid17.8SL	0.23	1.35	5.87	0.34	1.48	0.03	0.13
Monocrotophos 36SL	0.16	12.23	76.44	0.67	4.19	18.71	116.94
Triazophos 40EC	1.00	6.73	6.73	3.67	3.67	6.50	6.50
Diafenthiuron 50WDG	0.43	0.53	1.23	1.12	2.60	0.44	1.02
Spiromesifen 22.9SC	1.34	6.32	4.72	2.36	1.76	9.34	6.97
Thiamethoxam 25WG	0.65	11.63	17.89	16.53	25.43	14.90	22.92

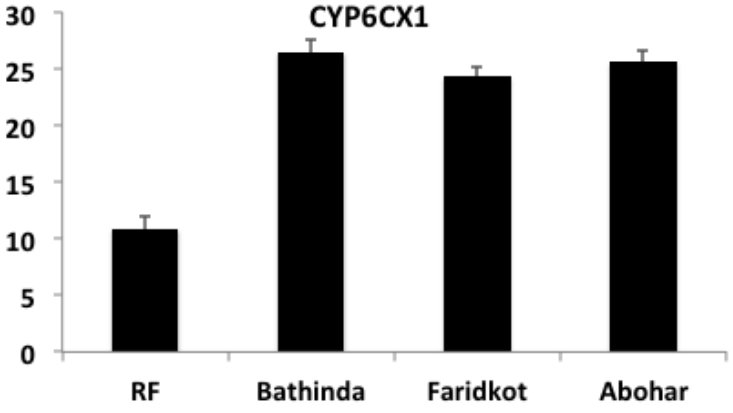
Genes and primers for neonecotinoid resistance

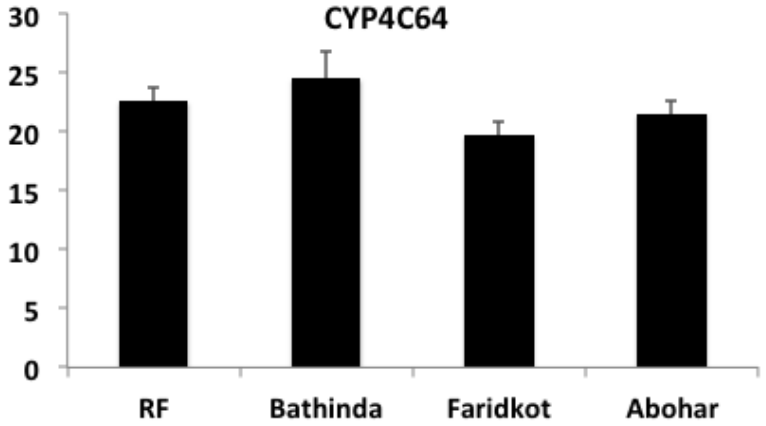
Gene Name	Forward Primer	Reverse Primer	Product (bp)
Cyp4g61	5' TGG CTC CAG TGA ACG CGG '3	5' GACGAAGGTGCTGGGTAGAT '3	144
Cyp6cx1	5' GTGCCCTACATCTCGCCTATC '3	5'CATTTCTTTCGTCGTCTCCAAC'3	90
Cyp6cm1	5' CACTCTTTTGGATTTACTGC '3	5'GTGAAGCTGCCTCTTTAATG'3	110
Cyp4c64	5'TCGGATTACGTCAGAGCTATTTAC'3	5'GTGGAGCACGCTTAGACA'3	138
Cyp6cx4	TTGACAAACTTGCGGGGAACCTC'3 5'	5'CACAGTCTTTCAGCGTCTCGT'3	126
Actin	'3 ACCGCAAGATTCCATACCC 5'	CGCTGCCTCCACCTCATT'3 5'	174

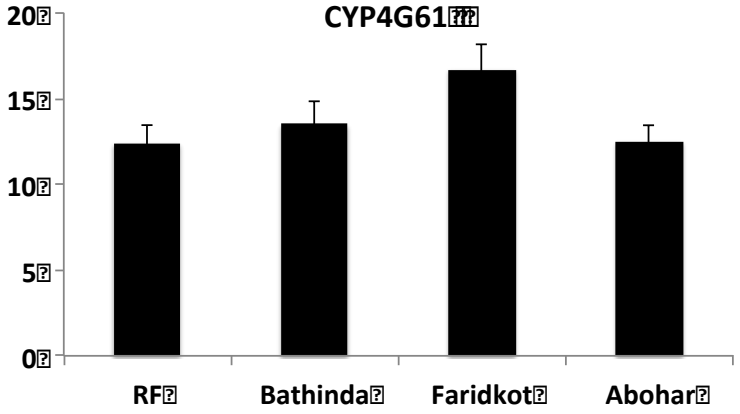
Relative expression profiles of CYP genes in the three different populations of *B. tabaci*











Present chemistries for whitefly

Neem oil at initial stages of crop growth
Spiromesifen, Pyriproxyfen
Trizophos and Ethion
Floicamid
Diafenthiuron

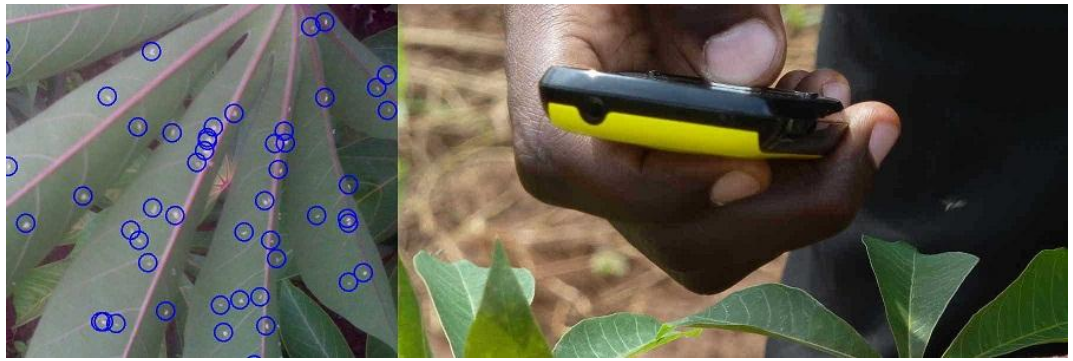
Summary

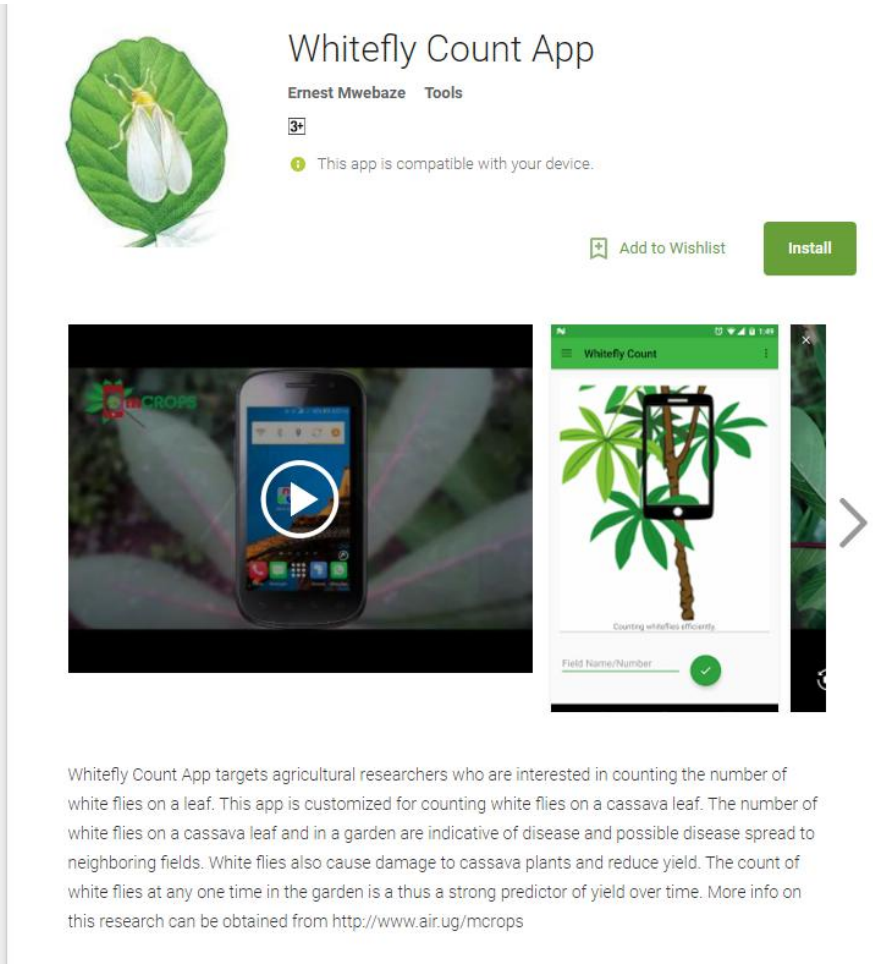
- B.tabaci populations exhibit resistance to neonecotinoids (Acetamprid, thiamethoxam) organophosphate (trizophos, monocrotophos)
- The new molecule Spiromesifen is at risk of resistance development due to excessive usage
- High level of resistance has been observed in fipronil
- The resistance to neonecotinoids may be associated with overexpression of **Cyp6cm1 and Cyp6cx1**

Android app for counting whitefly

Solomon Nsumba
Software Developer mCROPS, Uganda

Anna Neuman
Massachusetts Institute of Technology (MIT) – Boston Campus, USA





I will appreciate your suggestions for whitefly management……..

Thank