Identification of trichomes, loci and chemical compounds derived from *Solanum habrochaites* accession LA1777 that are associated with resistance to the sweetpotato whitefly, *Bemisia tabaci* in tomato, *S. lycopersicum*.

By
Mohamed Rakha, Jay Scott, Samuel Hutton, Hugh Smith

43rd Tomato Breeders Roundtable Meeting
October 9 -11, 2011

TOMATO PRODUCTION

- Tomato (*Solanum lycopersicum* L.) is the second most important vegetable crop.

- Total world production 152.9 million ton
  with a value $74.1 billion (FAOSTAT Database, 2009).

- Total U.S.A. production 14.1 million ton
  with a value $10.86 billion (USDA, 2010).

- Florida contributed 13.8% of that production in 2010, all in fresh market tomatoes, $622, 251 and $520,205 million in 2009 and 2010, respectively (USDA 2010)

Whitefly Damage

- Direct damage through feeding
  - Leaf and fruit spotting
  - Plant debilitation
  - Irregular ripening
  - Growth of sooty molds

- Transmission of viruses
  - TYLCV
  - ToMoV
  - ….etc

Whitefly control

- Whitefly control is based on pesticide applications.
- Pesticides can be harmful to the environment, and exterminate beneficial insects.
- The whitefly has been difficult to control with insecticides because it feeds and oviposits mainly on the abaxial leaf surfaces and it has developed resistance to most insecticides

- Whitefly resistance has been reported in tomato wild species, *S. pennellii*, *S. habrochaites and S. pimpinellifolium*

Problems of breeding tomatoes for resistance to whitefly

- Breeding efforts have been hampered by:

  1- Polygenic inheritance

  2- Linkage drag

  3- Limited resistance screening information

  4- Limited information on markers linked to quantitative trait loci(QTL) for SPWF resistance from LA1777

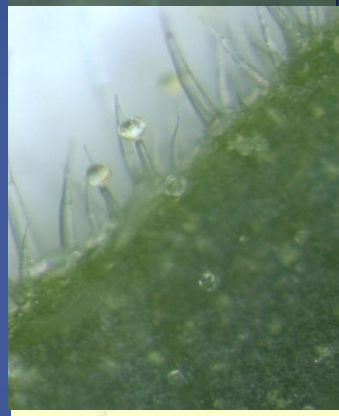
Trichomes

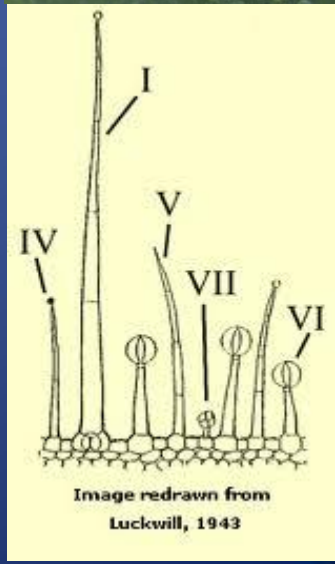
Solanum genus has eight trichomes types, which vary by length, presence or absence of glands in the apical extremity

Mechanisms of resistance:

1- Physical defense: interfere with whitefly feeding and oviposition

2- Biochemical defense: based on glandular trichomes (acyl sugar, methyl ketones, sesquiterpenes and ……?)





Molecular markers linked to whitefly resistance

- Some QTLs have already been reported for whitefly resistance in *S. habrochaites* f. *glabratum* accession CGN.1561 (Maliepaard et al.,1995) and *S. pennellii* (Mutschler et al., 1996; Lawson et al., 1997; Blauth et al.,1999).

- Later Momtaz et al. (2010) identified four QTL associated with SPWF resistance derived from *Solanum habrochaites* Accession LA1777

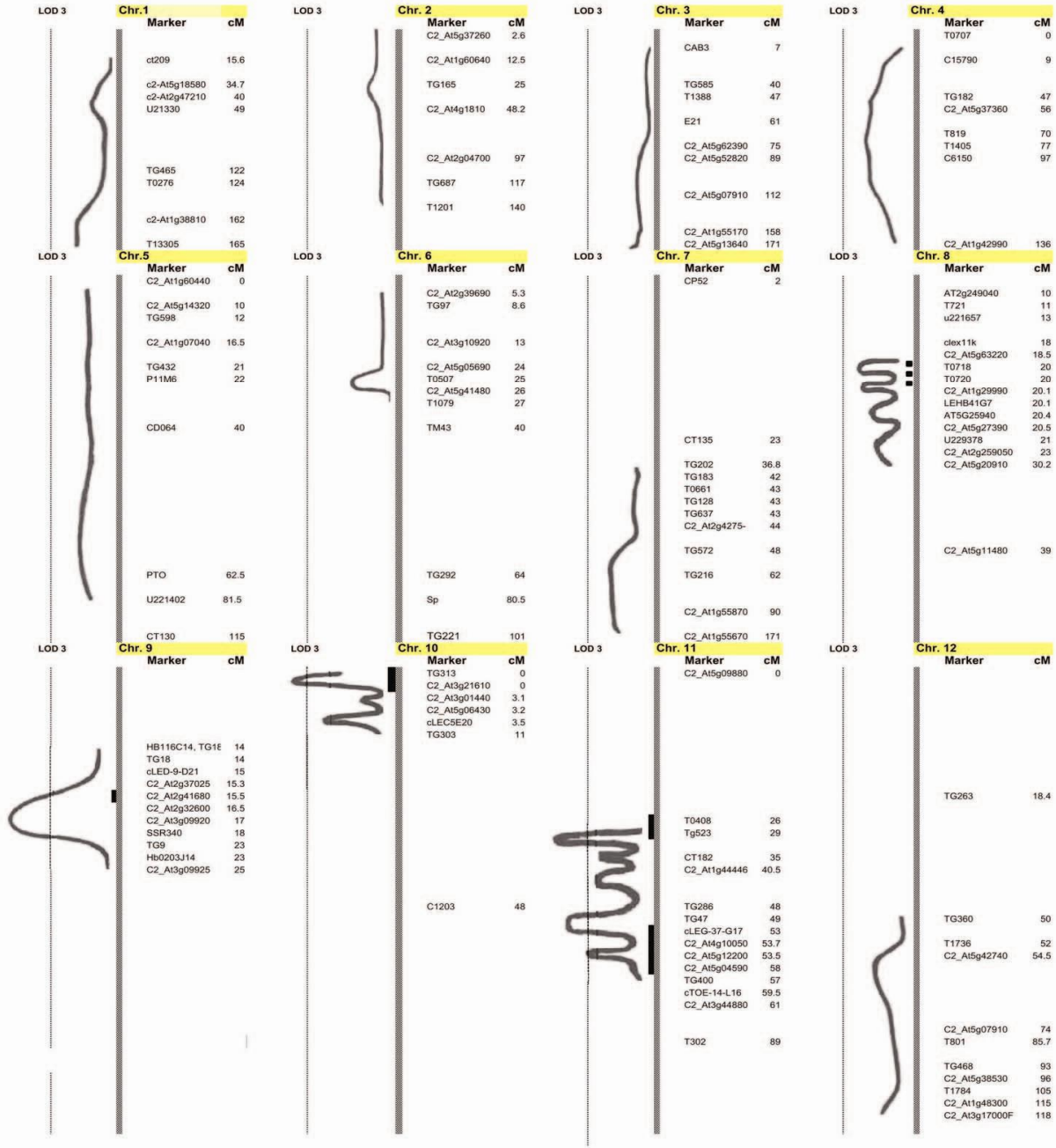


Fig. 3. QTL associated with low sweetpotato whitefly egg deposition from an F_2 population derived from susceptible tomato lines crossed with *S. habrochaites* accession LA1777. Four regions with LOD scores >3 were considered significant and are indicated with a solid line. A fifth region on chromosome 8 was associated with type IV trichomes, but not egg deposition in the selective genotyping analysis, and is indicated by the hatched line. The distances between markers are from Fulton et al., (2000).

Research Objectives: Resistance from LA1777

1- Validate previously identified QTLs

2- Investigate alternative high-throughput bioassay

3- Further investigate the role of trichome types

4- Identification of chemical compounds associated with SPWF resistance

Tomato populations used to study SPWF resistance in Florida

***S. lycopersicum* x LA 1777**

↓

F_1

↓ Si b-mated

F_2

↓

Genetic study (Momotaz et al. 2010)
3 R F_2 plants selected and sib mated

8059 x F_3

↓

$F_3BC_1F_1$

↓ Self pollinated

$F_3BC_1F_2$

↓

Selective Phenotyping Bioassay: 3 R plants selected and self pollinated

$F_3BC_1F_3$

↙ ↘

8059 x (8059 x $F_3BC_1F_3$)

Selective phenotyping Bioassay: 3 plants (each het. at 4 loci) backcrossed 2x

↓

$F_3BC_3F_1$

↓ Sib-mated

$F_3BC_3F_2$

8059 x $F_3BC_1F_3$

Selective Phenotyping Bioassay: 2 R plants (each hom. at 4 loci) backcrossed 1x

↓

$F_3BC_2F_1$

↓ Sib-mated

$F_3BC_2F_2$

Procedure

2400 plants

Genotyped using four QTL

200 plants

At various combinations of 4 QTL

Phenotyped using no-choice assay

No combinations of the 4 QTL uniformly resistance

???

2-Investigate alternative high-throughput bioassay

Why?

- ✓ Markers did not work
- ✓ Problems of no-choice bioassay:
  - – Labor intensive
  - – Difficult with large plant populations

Bioassay Procedure

2400 plants

$F_3BC_2F_2$

- Choice test counting number of WF per leaf
- Growth chamber

500 plants

- 250 plants R (0-5)
- 250 plants S (≥17)
- Repeated choice test 2x

No-choice test

- Greenhouse
- Classified the tomato lines to five groups of resistance

Requires large numbers of adult whiteflies

Screening result

- 2400 plants

Choice test 1x

500 plants

Choice test 2x

N0-choice test 2x

 # 7 CR

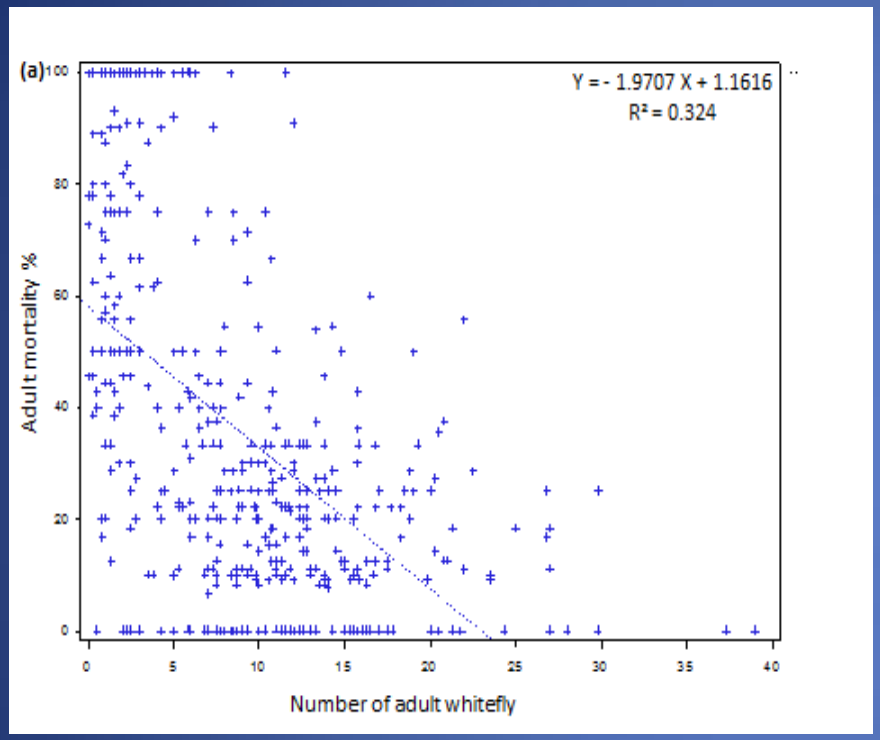
 # 60 HR

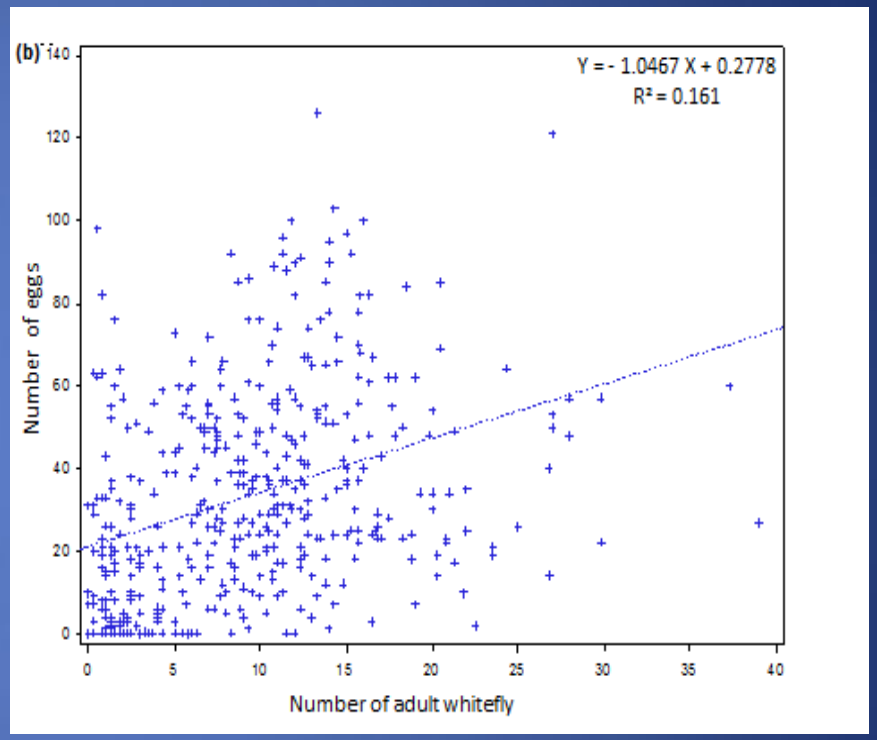
 # 147 MR

 # 213 LR

 # 73 S

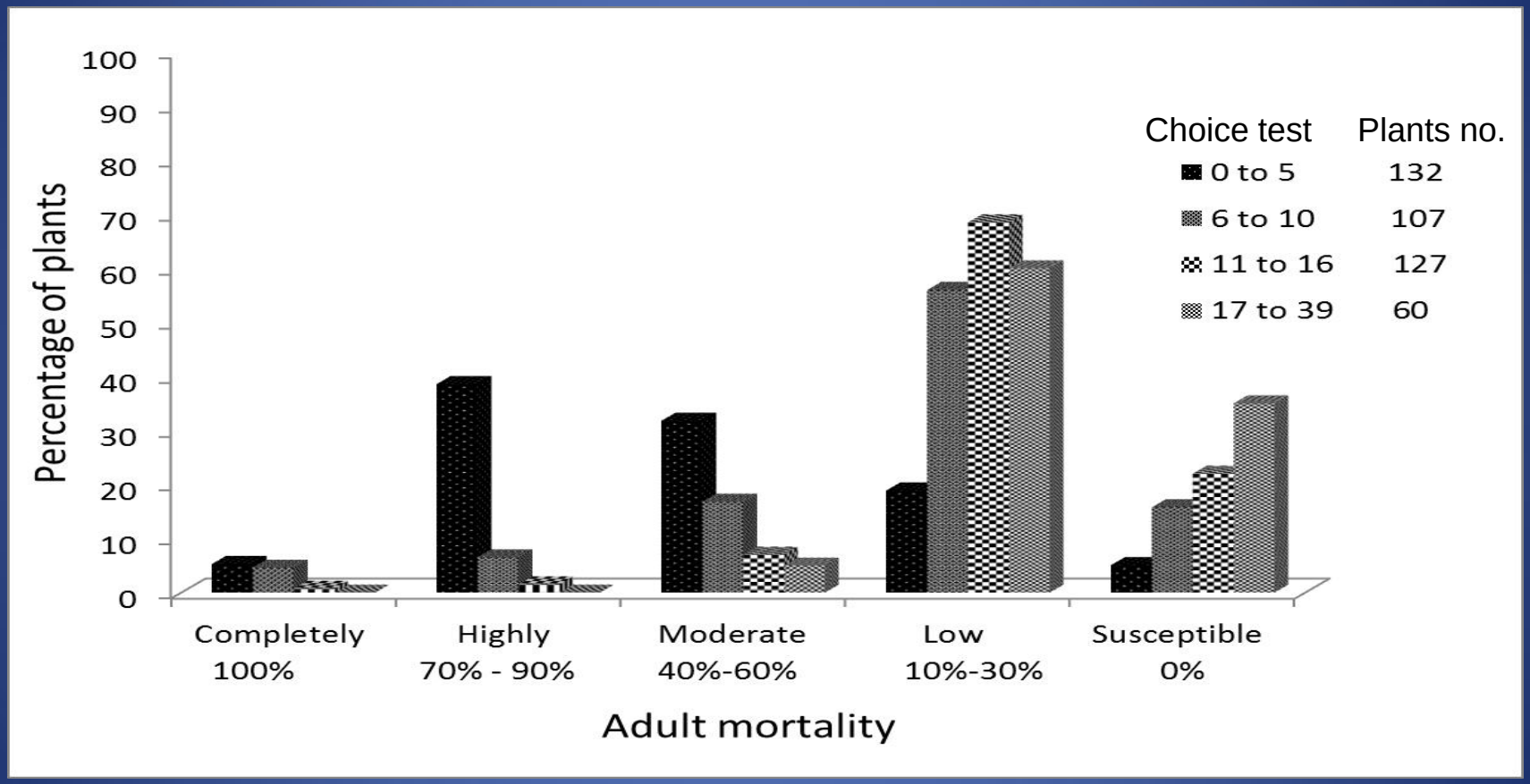
Relationship between choice and no-choice bioassays





Regression (R^2) between adult whitefly number in the no-choice test and adult mortality and egg number in the choice test on the 3th leaf node of $F_3BC_2F_2$ generation derived from susceptible tomato x LA1777, Spring 2011

Choice and no-choice bioassay



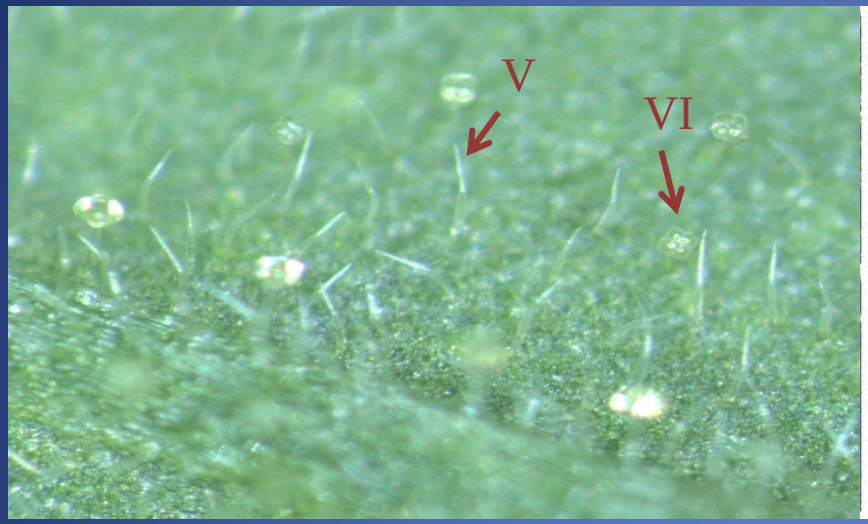
Segregation for resistance based on adult mortality in the no-choice bioassy for groups differing in whitefly numbers from the choice bioassay. Spring 2011

3- Further investigate the role of trichome types associated with SPWF resistance

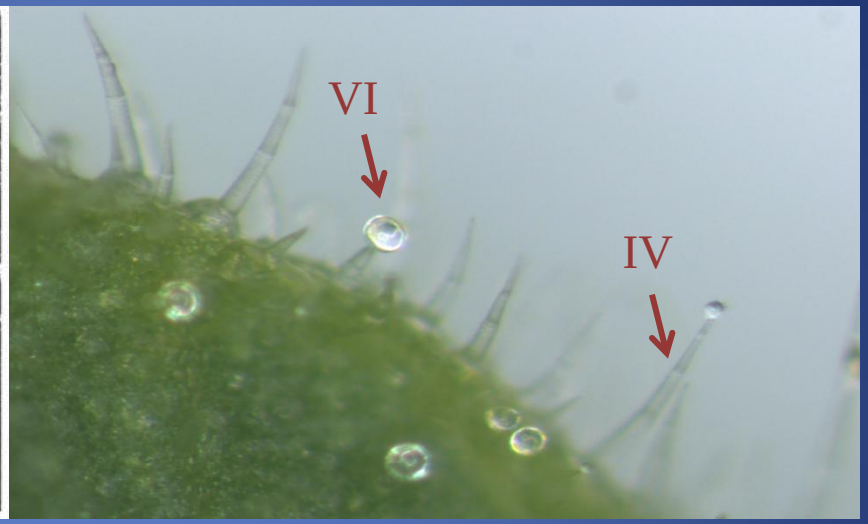
Scale	Type IV no.[y]	Type VI no.
0	None	None
1	1- 19	1-19
2	20- 59	20 - 40
3	60- 119	41 – 80
4	120- 199	81 – 120
5	≥200	≥121

[y]Number per 50x microscope field.

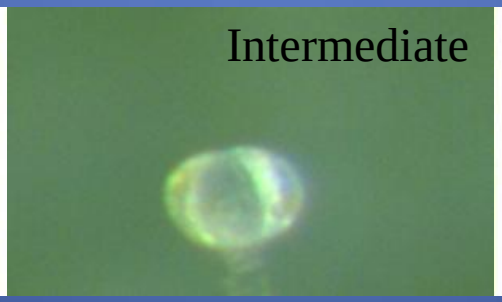
Shape of type VI

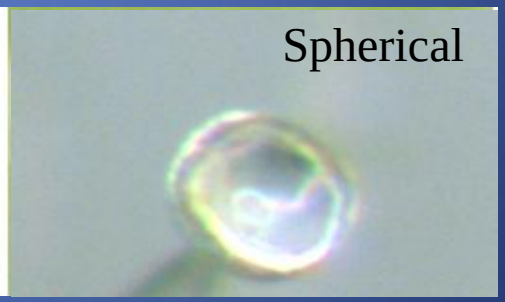


8059



LA1777

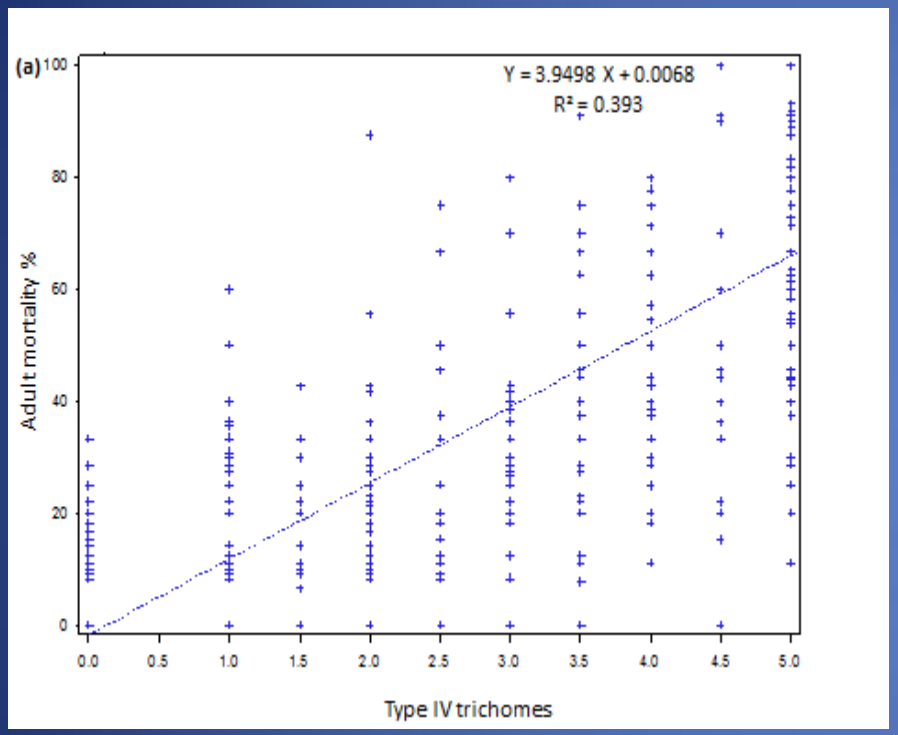


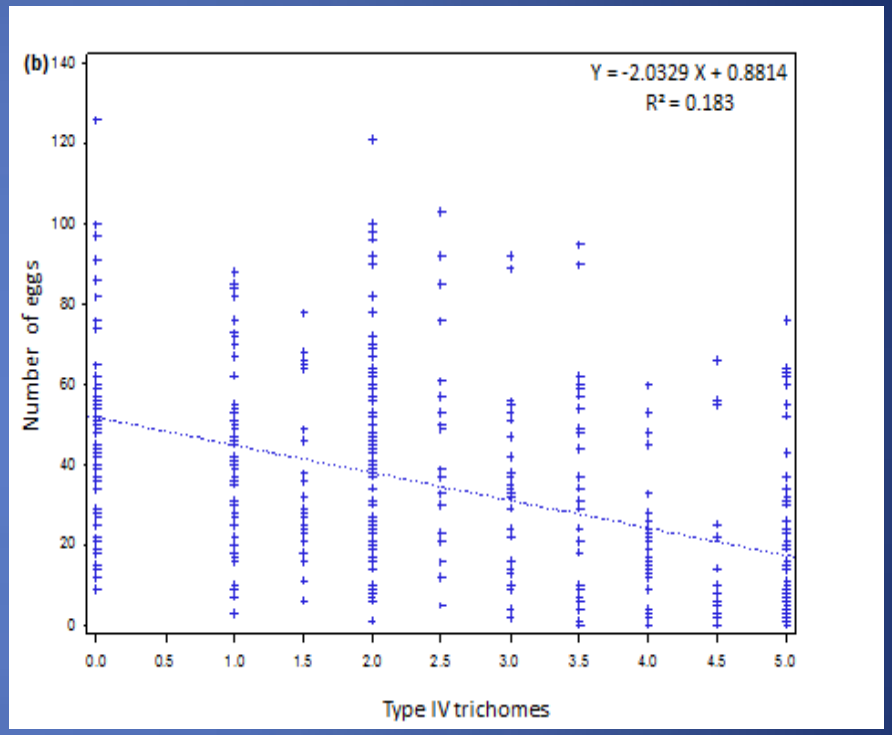


8059

LA1777

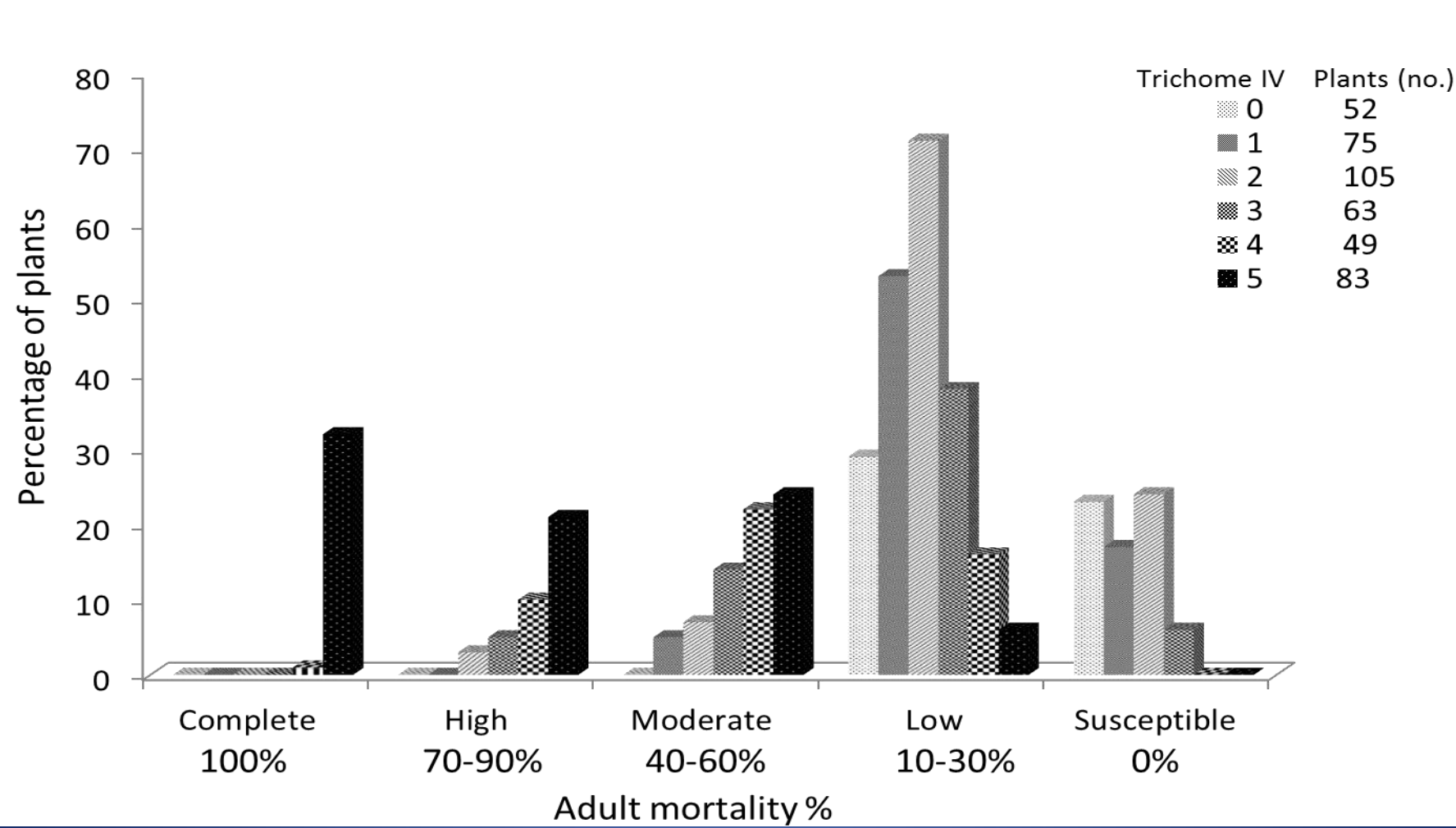
Relationship between type IV trichomes and SPWF resistance





Regression (R^2) of adult mortality and egg number with type IV trichome density on the 3th leaf node of $F_3BC_2F_2$ plants derived from susceptible tomato x LA1777- Spring 2011

Relationship between type IV trichome and SPWF resistance

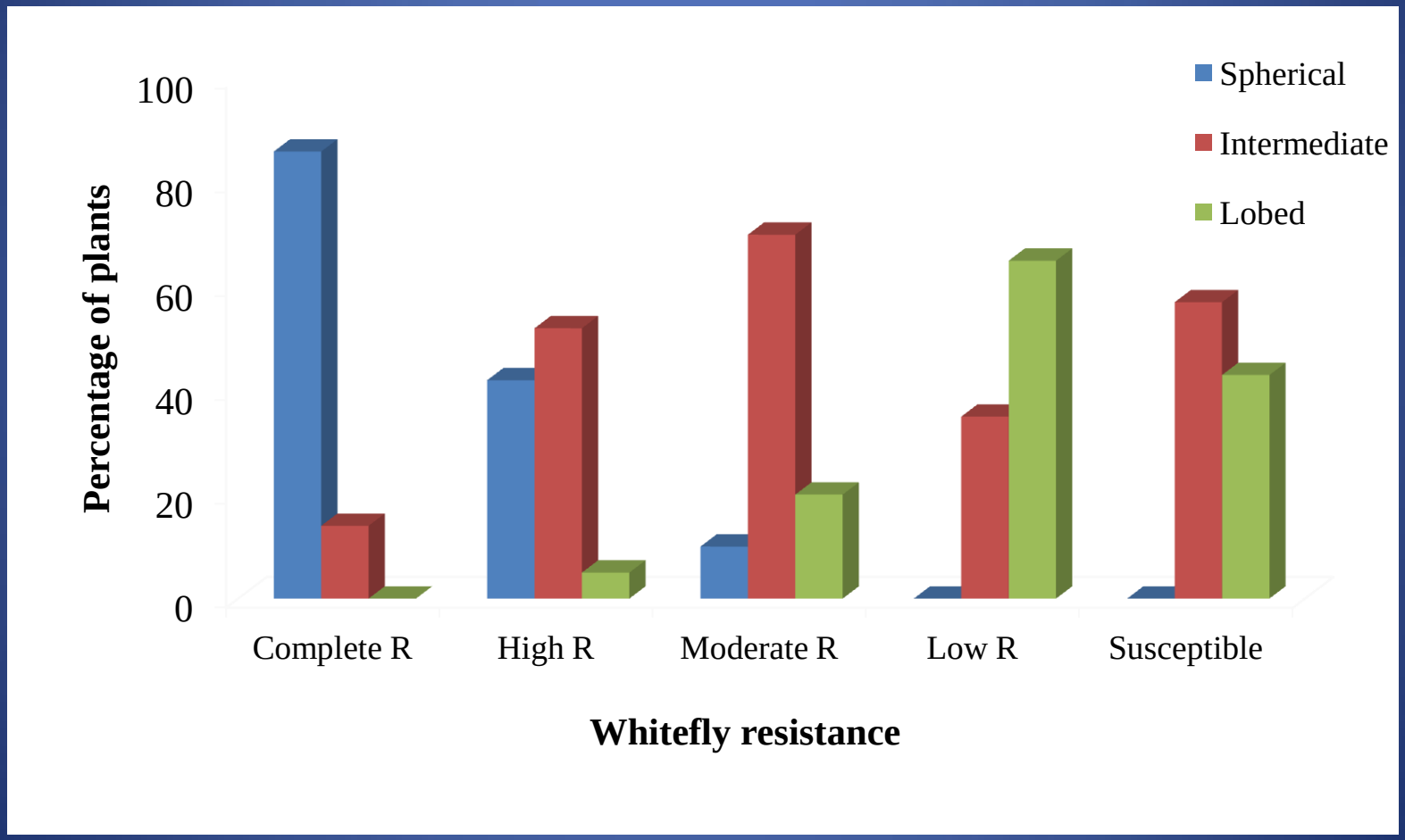


Correlation coefficients between trichome densities and SPWF resistance variables

	Choice bioassay	No-choice bioassay	
Trichome	Number of adult whitefly	Adult mortality	Number of eggs
Type IV	0.44^{**}	0.86^{**y}	0.89^{**}
Type VI lobed	0.03^{ns}	0.05^{ns}	0.00^{ns}
Type VI intermediate	0.19^{*}	0.011^{ns}	0.00^{ns}
Type VI spherical	0.34^{**}	0.77^{**}	0.79^{**}

[y]*,**, indicate significance at P ≤ 0.05 and P ≤ 0.01, respectively; NS = not significant.

Relationship between SPWF resistance and shape of type VI trichomes



Distribution of type IV and spherical shape of type VI trichomes in resistant plants

Line	Trichomes	
	Type IV	Spherical shape of type VI
Complete resistance lines		
WFB-341	5	5
WFB-204	5	5
WFB-437	5	2
WFB-540	5	2
WFB-441	5	1
WFB-146	5	3.5
WFB-47	5	0
High resistance lines		
WFB-340	5	3
WFB-196	5	5
WFB-432	4	3
WFB-135	5	5
WFB-542	4.5	3
WFB-448	1	4
WFB-76	4	3
WFB-169	5	0
WFB-283	4	0
WFB-281	5	0
WFB-319	5	0
WFB-423	4.5	0
WFB-93	5	0
WFB-544	5	0
WFB-48	3.5	0
WFB-446	5	0
WFB-530	4	0

4- Identificaton of chemical compounds associated with SPWF resistance from LA1777

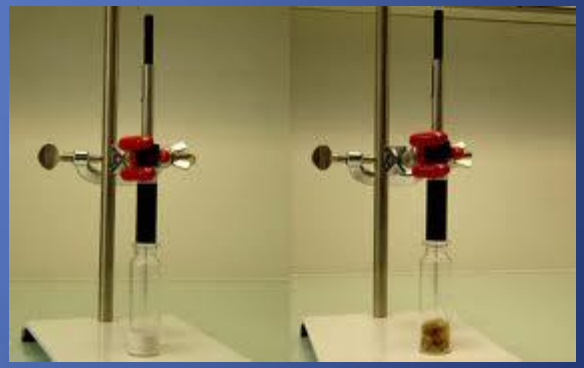
- Plant material:

Susceptible parent (Fla. 8059), resistant parent LA1777 and 50 tomato lines of $\mathbf{F_3BC_2F_2}$ classified from complete resistance to susceptible by no-choice test .

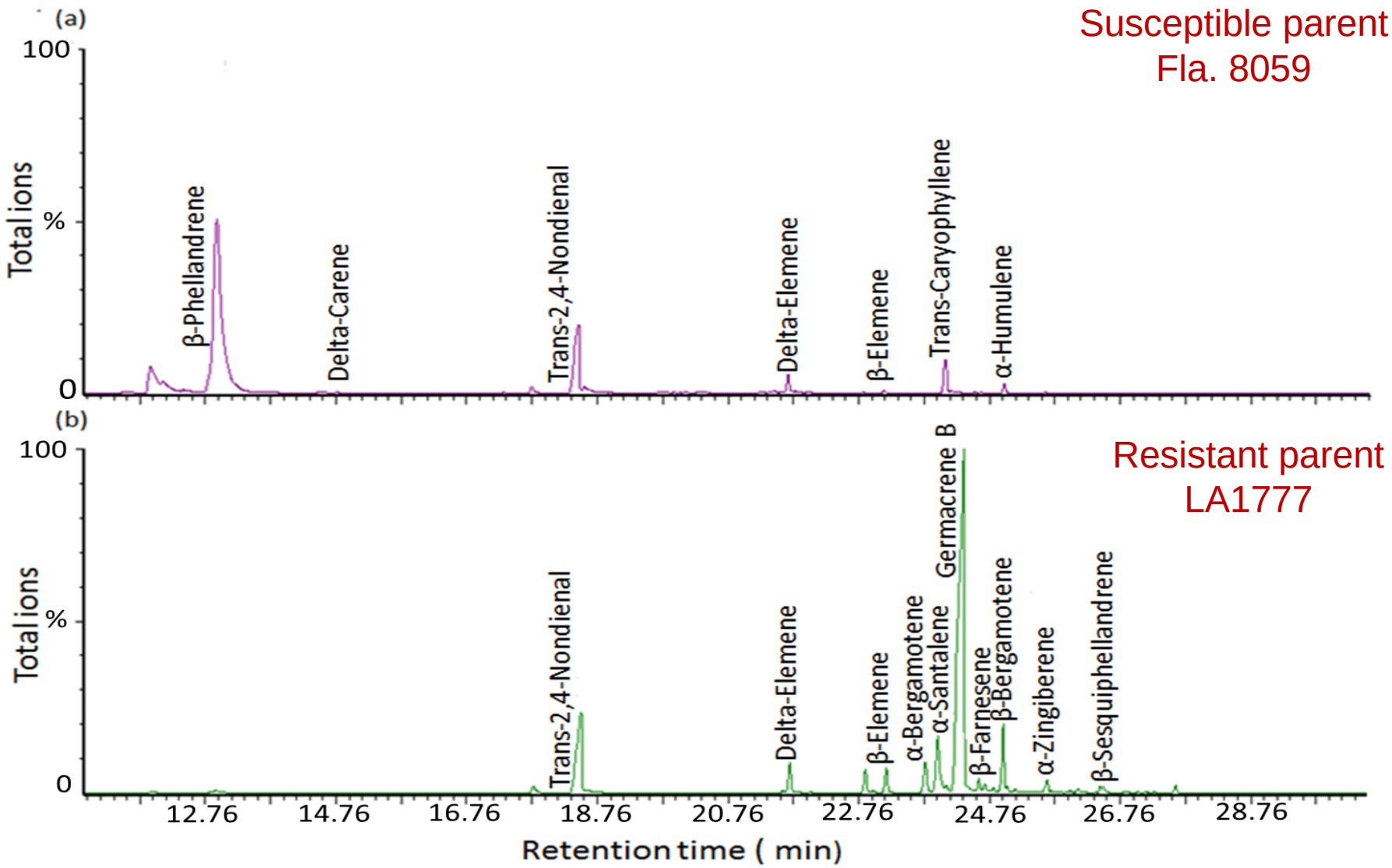
- Leaf extraction:

  Methylene chloride

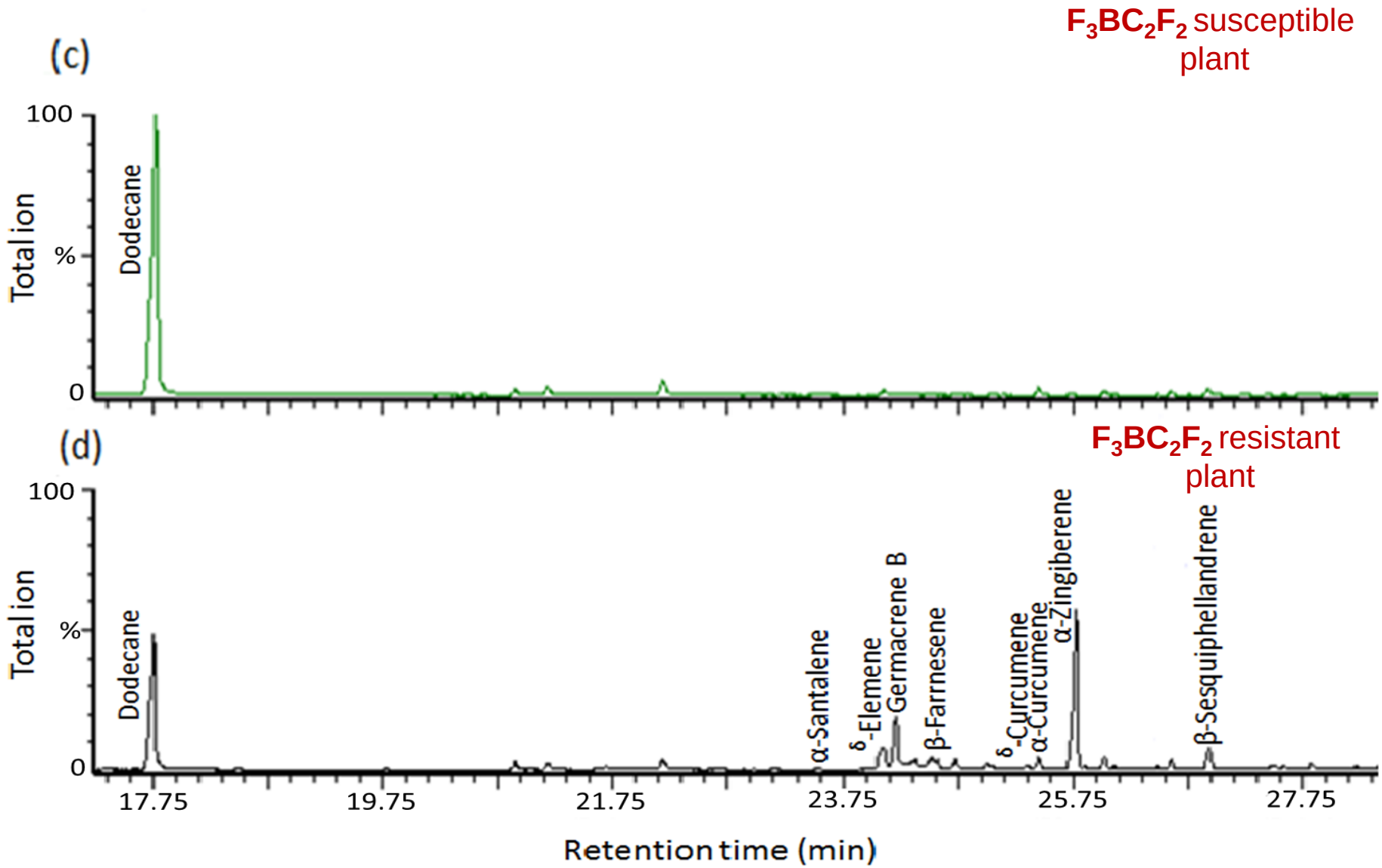
  Solid-phase micro extraction (SPME)



GC-MS analysis



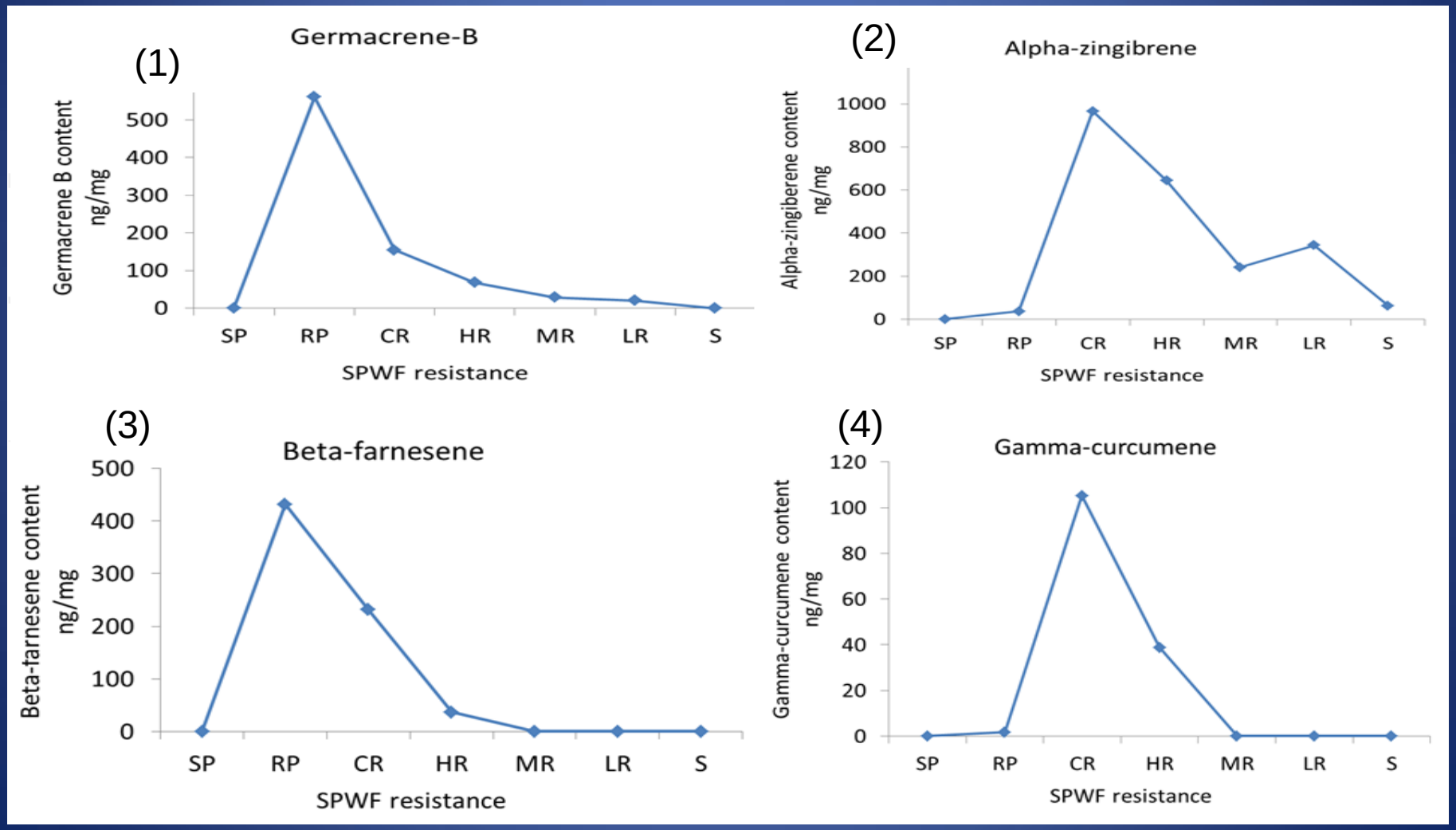
GC-MS analysis



Stepwise multiple regression analysis of putative compounds associated with whitefly resistance

Model		No-choice bioassay		Choice bioassay
		Adult mortality %	Eggs	Number of adult whitefly
Single compound analysis - Model R^2 (single compound).				
Compound 1	(Germacrene-B)	0.628 **	0.666 **	0.385* [y]
Compound 2	(Alpha-zingibrene)	0.188 *	0.214*	0.130*
Compound 3	(Beta-farnesene)	0.158 *	0.174 *	0.100 [ns]
Compound 4	(Gamma-curcumene)	0.134 *	0.123 [ns]	0.050 [ns]
Compound 5	(Delta-elemen)	0.124 [ns]	0.106 [ns]	0.083 [ns]
Compound 6	(Alpha-santalene)	0.121 [ns]	0.135 *	0.082 [ns]
Compound 7	(Sesquiphellandrene)	0.113 [ns]	0.126 [ns]	0.076 [ns]
Compound 8	(Alpha-bergamotene)	0.106 [ns]	0.119 [ns]	0.073 [ns]
Compound 9	(Beta-elemen)	0.106 [ns]	0.119 [ns]	0.072 [ns]
Compound 10	(Alpha-curcumene)	0.056 [ns]	0.056 [ns]	0.008 [ns]
Compound 11	(Trans-caryophelene)	0.045 [ns]	0.049 [ns]	0.027 [ns]
Compound 12	(Alpha-humelene)	0.003 [ns]	0.001 [ns]	0.001 [ns]
Important significant compounds model - Model R^2 (compound × compound).				
Model (2)				
1 + 4		0.753**	0.780**	0.431**
11 +1		0.678**	0.721**	0.415**
Model (3)				
11 + 1 + 4		0.771**	0.788**	---
8 + 1 + 7		0.761**	0.813**	0.451**
1 + 4 + 10		0.765**	0.792**	0.468**
Model (4)				
5 + 1 + 4 + 7		0.805**	0.832**	---
8 + 11 + 1 + 7		0.784**	0.838**	---
5 + 9 +1 + 4		0.803**	0.835**	---
5 + 11 + 1 + 4		0.802**	---	---
5 + 1 + 10 + 7		---	---	0.490**
Model (5)				
5 + 9 + 11 + 1 + 4		0.827**	0.846**	---
8 + 11 +1 + 4 + 7		0.827**	0.861**	---
Model (6)				
8 + 11 +1 + 4 + 10 + 7		---	0.866**	---
8 + 11 +1 + 4 + 2 + 7		0.842**	0.866**	---
Model (7)				
5 + 9 + 11 + 1 + 12 + 4		0.857**	---	---
Model (8)				
5 + 6 + 12 + 1 + 3 + 12 + 4 + 10		---	---	0.541**
Model (9)				
5 + 9 + 8 + 6 + 11 + 1 + 12 + 4 + 2		0.874**	---	---

Chemicals associated with SPWF resistance



Conclusions

- Molecular markers: missing one or more of the resistance loci. Hence, we will test 500 molecular markers covering the entire genome to find the missing locus/loci.

- Methodology selection study; The choice test allowed for the selection of smaller groups that could then be assayed in a no-choice assay. In this way large populations can be effectively assayed.

Conclusions

- Trichomes study; SPWF resistance was related to the density of type IV glandular trichomes and the spherical shape of type VI trichomes .

- Chemical study; the most important compounds for resistance were the sesquiterpenes gemacrene B, alpha-zingiberene and beta-farnesene, both singly and in synergistic combinations with each other and/or other sesquiterpene compounds.

Acknowledgements

Dr. Jay W. Scott
Dr. Samuel Hutton
Dr. Hugh Smith
Dr. Jose I. Reyes
Dr. Faraj Hijaz
Dr. Shawky Mostafa
Dr. Abdelwahab Etman
Dr. Tarek Shalaby

Jose Diaz
Tim Davis
Cathy Provenzano
Rosa March
Rudy Jones
Dolly Cummings
Ragy Ibrahim