Whitefly Resistance in Tomato

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Question

What is the oldest insect in the world?
The oldest definitive insect fossil, *Rhyniognatha hirsti*, is estimated to be 407 to 396 million years old (comes from near Aberdeen, Scotland)
Outline

I. General introduction: Whitefly damage, host resistance, and challenges

II. Identification of whitefly resistance

   *Solanum pimpinellifolium, S. galapagense, and S. lycopersicum var. cerasiforme*

III. WorldVeg strategy for whitefly resistance

IV. Identify mechanisms of whitefly resistance

V. WorldVeg breeding programs for whitefly resistance derived from *S. galapagense, and S. pimpinellifolium*
Economic importance

Tomato is the second most important vegetable crop.

Total world production 163.7 million ton with a net value $59.8 billion (FAOSTAT Database, 2017).
Of these, 60% come from Asia (mainly China and India)
Major insect pests of tomato

✓ Feed almost exclusively on foliage
  - Spider mites, dipterous leafminers

✓ Feed on both foliage and fruit
  - Lepidopterans *Helicoverpa zea,*
    *Spodoptera exigua,* and *Tuta absoluta*

✓ Feed on plant sap
  - Whiteflies
  - Aphids
  - Thrips
Whitefly damage

- Direct damage through feeding
- Indirect damage: transmission of viruses
- Chemical control is difficult
Major resistance sources

- Cultivated tomato: Susceptible
- Wild relatives:

  > 50 years ago!!!

  *Solanum pennellii* and *S. habrochaites*

  Recently

  *S. pimpinellifolium* and *S. galapagense*
Challenges of tomato breeding for insect resistance

- **Linkage drag**: Linkage of insect resistance genes with other genes from wild species conditioning poor horticultural traits
- **Polygenic Inheritance**
- **Limited information on markers linked to quantitative trait loci (QTL)** for insect resistance
- **Influence of environment** on insect resistance
- **Insect bioassays (choice and no-choice):**
  - Costly
  - Difficult with large plant populations
  - Labor intensive
Insect bioassays

Choice bioassay: Record numbers of adult, egg, nymph and pupa (3 weeks)

No-choice bioassay: Record numbers of dead and living adult whiteflies and eggs (1 week)
Linkage drag in *S. pennellii*

Cornell tomato lines developed from LA 716
High resistance but almost no fruits!!!

Lin et al. 2014
Breeding strategies

- Develop valid and efficient screening methods
- Identify novel sources of resistance
- Identify mechanisms of insect resistance
- Pyramiding insect-resistant genes
- Combine insect resistance and virus resistance
- Mapping insect resistance genes (phenotypic and metabolomics QTLs)
Identification of whitefly resistance in closely related wild relatives

In total 260 accessions:
11 accessions of *S. cheesmaniae*
18 accessions of *S. galapagense*
231 accessions of *S. pimpinellifolium*

- Trichome analysis
- No-choice bioassay: Record numbers of dead and living adult whiteflies and eggs (1 week)
- Choice bioassay: Record numbers of adult, egg, nymph and pupa (3 weeks)
Means of sweetpotato whitefly resistance parameters and type-trichome density in most resistant accessions compared to susceptible check CL5915 tomato line evaluated in choice bioassays

<table>
<thead>
<tr>
<th>Taxa and AVRDC accessions code</th>
<th>Other codes</th>
<th>Whitefly resistance parameters</th>
<th>Trichomes density/mm$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Eggs (no.)</td>
<td>Nymph (no.)</td>
</tr>
<tr>
<td>SOLANUM GALAPAGENSES</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VI063177</td>
<td>LA0530</td>
<td>7.3 f</td>
<td>0.0 d</td>
</tr>
<tr>
<td>VI037239</td>
<td>LA436</td>
<td>7.5 f</td>
<td>0.0 d</td>
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<tr>
<td>VI063174</td>
<td>LA0438</td>
<td>7.2 f</td>
<td>1.2 d</td>
</tr>
<tr>
<td>VI057400</td>
<td>LA483</td>
<td>13.8 ef</td>
<td>2.5 d</td>
</tr>
<tr>
<td>VI037340</td>
<td>LA1408</td>
<td>14.3 ef</td>
<td>3.2 d</td>
</tr>
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<td>VI045262</td>
<td>LA1141</td>
<td>18.2 ef</td>
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<td>SOLANUM CHEESMANIENSES</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>VI037240</td>
<td>LA483</td>
<td>35.7 def</td>
<td>6.7 d</td>
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<tr>
<td>SOLANUM PIMPINELLIFOLIUM</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>VI030462</td>
<td>PI390519</td>
<td>40.2 cdef</td>
<td>22.7 cd</td>
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<tr>
<td>SOLANUM LYCOPERICUM</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>CL5915 (CH45)</td>
<td></td>
<td>154.8 ab</td>
<td>133.0 a</td>
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</table>

$^a$Type-trichome densities were evaluated in a one mm$^2$ area during choice and no-choice bioassays

$^\gamma$Whitefly adults were counted 3 (3 DWF) and 19 (19 DWF) days after whitefly infestation.

Rakha et al. 2015.
Sweetpotato whitefly resistance parameter means and type-trichome densities in most resistant accessions compared to susceptible check CL5915 tomato line evaluated in no-choice bioassays

<table>
<thead>
<tr>
<th>Taxa and AVRDC accessions code</th>
<th>Other codes</th>
<th>Whitefly resistance parameters</th>
<th>Trichomes density/mm²</th>
<th>Type I</th>
<th>Type IV</th>
<th>Type V</th>
<th>Type VI</th>
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<tbody>
<tr>
<td>Solanum galapagense</td>
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<tr>
<td>VI037340</td>
<td>LA 1408</td>
<td>100.00 a</td>
<td>21.48 bcde</td>
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<td>11.3 bc</td>
<td>0.0 e</td>
<td>1.5 abcd</td>
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<td>97.50 a</td>
<td>15.75 cde</td>
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<td>92.50 a</td>
<td>26.38 bcde</td>
<td>1.0 a</td>
<td>11.8 bc</td>
<td>0.0 e</td>
<td>1.0 bc</td>
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<tr>
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<td>VI037245</td>
<td>LA 1036</td>
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<td>79.53 bc</td>
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<td>1.5 ef</td>
<td>14.8 bc</td>
<td>1.5 abcd</td>
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<td>66.90 bcd</td>
<td>32.17 bcde</td>
<td>0.0 c</td>
<td>18.5 ab</td>
<td>0.0 e</td>
<td>0.5 bc</td>
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</tr>
<tr>
<td>VI030462</td>
<td>PI 390519</td>
<td>93.39 a</td>
<td>4.66 e</td>
<td>1.0 a</td>
<td>19.0 ab</td>
<td>0.0 e</td>
<td>1.3 abcd</td>
</tr>
<tr>
<td>Solanum lycopersicum</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL5915 (CH45)</td>
<td></td>
<td>6.53 h</td>
<td>86.08 ab</td>
<td>0.5 b</td>
<td>0.0 f</td>
<td>18.4 bc</td>
<td>1.5 abcd</td>
</tr>
</tbody>
</table>

Type-trichome densities were evaluated in a one mm² area during choice and no-choice bioassays as described in Materials and Methods.

Means followed by different letters within columns are different by Duncan’s Multiple Range Test (P=0.05).

Most of these accessions were also resistant to spider mite (Rakha et al., 2016), and *Tuta absoluta* (Plant Breeding, submitted Feb. 2017)
Whitefly resistance in *S. lycopersicum* var. *cerasiforme*

>430 *cerasiforme* accessions were characterized for trichome types and density

We identified one accession with high level of resistance to whiteflies

No-choice bioassay

This accession was also resistant to spider mite and tomato fruitworm!!!
Identify mechanisms of whitefly resistance

The role of trichomes in insect resistance

**Physical defense:** glandular trichomes might act as a physical barrier, interfering with insect landing, feeding and oviposition

**Biochemical defense:** glandular trichomes produces some compounds that play a role as repellents/toxic to insect (acyl sugar, methyl ketones, sesquiterpenes and ……?)
Metabolite analyses

Collaboration with:
Plant Research International-
Wageningen UR
Drs. Ric de Vos, Roland Mumm

Targeted chemicals

**Acyl sugars:** S3 (Triacylsucrose), S4 (Tetracylsucrose), and S5 (Pentaacylsucrose) compounds

**Methylketones:** 2-tridecanone, 2-dodecanone, and 2-undecanone.

**Sesquiterpenes:** 7-epizingiberene, α-zingiberene, R-curcumene, γ-curcumene, α-santalene, β-bergamotene, germacrene-B, germacrene-D, β-caryophyllene, α-humulene, β-farnesene, δ-elemene, sesquithujene, β-farnesene and sesquiphellandrene.

**Monoterpenes:** carene, p-cymene, β-ocimene, α-terpinene, γ-terpinene, α-terpinolene β-myrcene, α-pinene and α-phellandrene.
107 apolar compounds

GCMS analysis

GCMS results, 17122015 to AVRDC.M1 (PCA-X)
Colored according to model terms

- beta-pinene (or sabinene??)
- alpha-pinene
- beta-caryophyllene
- delta-elemene
- alpha-humulene (or Selina-4(15),6-diene?)
- gamma-terpinene
- terpinolene
- alpha-phellandrene
- beta-phellandrene (or limonene???)
- beta-myrcene

R²[1] = 0.467 R²[2] = 0.199
PCA based on 107 GCMS compounds

S. pimpinellifolium
Resistant
Acyl sugar S4:27 (5,5,5,12)
Tomato populations derived from *S. pimpinellifolium* for whitefly resistance

*S. Lycopersicum* (CLN3682C) × *S. pimpinellifolium*

Female parent: Multiple disease resistance genes (Ty-3, Ty-2, Bwr-12, I2, Mi-1)

Two highly resistant plants (Rakha et al. 2015)

CLN3682C × F₂

F₁ → sib mated

F₂BC₁ → F₁BC₁ → F₃

7 R F₂ plants selected and sib mated

Four plants from controls, parents, F₁, and 172 plant F₂ derived from *S. pimpinellifolium* (VI030462) were phenotyped for whitefly resistance based on no-choice bioassays
Whitefly resistance derived from *S. pimpinellifolium*

Objectives:
- Map whitefly resistance QTL(s) in F2 and F2BC1 populations
- Develop tightly linked markers with whitefly resistance
- Combine whitefly resistance with tomato yellow leaf curl virus resistance genes (*Ty*-2, *Ty*-3)
Phenotypic data (No-choice assay)

Traits were continuously distributed indicating polygenic control.
Type IV trichome data

![Bar graph showing the distribution of Type IV trichome numbers across different intervals.](image)
Tomato populations derived from *S. galapagense*

*S. Lycopersicum* (CLN3682C) x *S. galapagense*
*S. Lycopersicum* (CLN3696A) x *S. galapagense*

Female parent:
Multiple disease resistance genes (Ty-3, Ty-2, Bwr-12, I2, Mi-1)

\[ \text{F}_1 \]

Si b-mated

\[ \text{F}_1 \text{BC}_1 \]
\[ \text{F}_1 \text{BC}_2 \]

\[ \text{F}_2 \text{BC}_1 \]

Si b-mated

\[ \text{F}_3 \]

F1BC1 plants were also backcrossed to the susceptible lines to produce F1BC2.

5 R F2 plants were also backcrossed to the susceptible lines to produce F2BC1.

5 R F2 plants selected and self-pollinated to produce F3.
Big Hairy Tomato!!!

S. galapagense

F1

Fresh-market tomato (F1BC1)

Processing Tomato (F1BC1)

AVRDC, September 2015
Conclusion

- High levels of whitefly resistance were detected in *S. galapagense*, *S. cheesmaniae*, *S. pimpinellifolium*, and *S. lycopersicum* var. *cerasiforme*
- Most whitefly-resistant accessions (*S. galapagense*, *S. cheesmaniae*, *S. pimpinellifolium*) were also found resistant to spider mite, tomato fruitworm and *Tuta absoluta*
- Identified *S. lycopersicum* var. *cerasiforme* accession was also provided broad spectrum insect resistance
- Large variation amongst susceptible and resistant accessions for both acyl sugars (126 compounds) and terpenes (107 compounds)
- *S. pimpinellifolium* (VI030462) separates from all others in both acyl sugar and terpene composition, suggesting the possible presence of different resistance mechanisms in this accession.
- Multiple QTLs associated with whitefly resistance were identified in *S. pimpinellifolium* indicating polygenic control
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