Using Resistance to Combat Root-Knot Knot Disease in tomato

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Florida Nematology Giants Retiring

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Resistance to Combat Tomato Root-Knot Disease

• Florida: little interest in using root-knot nematode resistant tomato cultivars

• California: majority of processing tomato are resistant cultivars

• Why resistance?

• Viable alternative?
How was tomato resistant to root-knot nematode (RKN) developed:

• 1940’s – RKN resistance from wild sp. *Solanum peruvianum* - *Mi-1* gene

• Resistance to 3 spp. *M. incognita*, *M. javanica*, and *M. arenaria*

• Several *Mi*-genes: *Mi-1* to *Mi-9* - only *Mi-1* incorporated in tomato cv’s
## Crops with nematode resistant genes - root-knot nematode

*Almost all resistance against “endoparasitic” nematodes*

<table>
<thead>
<tr>
<th>Host plant</th>
<th>Gene or source</th>
<th><em>Meloidogyne</em> spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carrot</td>
<td><em>Mj</em> - 1</td>
<td>Mi, Mj</td>
</tr>
<tr>
<td>Clover</td>
<td>TRKR</td>
<td><em>M. trifoliophila</em></td>
</tr>
<tr>
<td>Coffee</td>
<td><em>Mex</em> - 1</td>
<td><em>M. exigua</em></td>
</tr>
<tr>
<td>Common bean</td>
<td><em>Me 1, Me2, Me 3</em></td>
<td>Mi, Mi, Mj</td>
</tr>
<tr>
<td>Cotton</td>
<td><em>Rkn 1, RKN2</em></td>
<td>Mi</td>
</tr>
<tr>
<td>Cowpea</td>
<td>RK, Rk2, rk3</td>
<td>Ma, Mi, Mi, Mj</td>
</tr>
<tr>
<td>Grape</td>
<td><em>N, Mur1</em></td>
<td>Ma, Mi</td>
</tr>
<tr>
<td>Lucerne</td>
<td><em>Mj</em> - 1</td>
<td>Mi</td>
</tr>
<tr>
<td>Lima bean</td>
<td><em>Mjr -1, Mjg - 1</em></td>
<td>Mi, Mj</td>
</tr>
<tr>
<td>Groundnut (peanut)</td>
<td><em>Arachis</em> spp. hybrids</td>
<td>Ma, Mj</td>
</tr>
<tr>
<td>Pepper</td>
<td><em>Me1, 3, 4, 7; Mech1,2</em></td>
<td>Ma, Mi, Mj, <em>M. chitwoodi</em></td>
</tr>
<tr>
<td>Potato</td>
<td><em>Rmc1, MfaXIIsp1</em></td>
<td><em>M. chitwoodi</em>, <em>M. fallax</em>, Mi</td>
</tr>
<tr>
<td>Prunus (peach)</td>
<td><em>Ma</em></td>
<td>Mj, Mi, Ma, Mf</td>
</tr>
<tr>
<td>Soybean</td>
<td>2 QTLs</td>
<td>Mj</td>
</tr>
<tr>
<td>Sugarbeet</td>
<td><em>Beta vulgaris</em> spp.</td>
<td>Ma, <em>M. chitwoodi</em>, <em>M. fallax</em></td>
</tr>
<tr>
<td>Sweet potato</td>
<td><em>Mi, Mj, Ma</em></td>
<td>Mi</td>
</tr>
<tr>
<td>Tobacco</td>
<td><em>Rk</em></td>
<td>Mi</td>
</tr>
<tr>
<td><strong>Tomato</strong></td>
<td><em>Mi1 – Mi9</em></td>
<td>Ma, Mi, Mj</td>
</tr>
<tr>
<td>Wheat</td>
<td><em>Triticum tauschii</em></td>
<td>Mi, Mj, <em>M. chitwoodi</em></td>
</tr>
</tbody>
</table>
Root-knot nematodes (Meloidogyne spp.)

• #1 nematode in Florida + the world, many crops, many species in FL, Meloidogyne arenaria, Meloidogyne incognita, Meloidogyne javanica, Meloidogyne enterolobii, Meloidogyne floridensis, Meloidogyne haplanaria, Meloidogyne hapla
How does the *Mi-1* gene work:

*Mi-1* gene prevents formation of specialized cells required by the nematode for feeding – w/o “giant cells” nematode is unable to feed.
How does the *Mi-1* gene work:

Do RKN juveniles enter resistant tomato?

![Bar chart showing juvenile infectivity in different tomato cv's](image)

*Regmi and Desaeger, 2018*
Essence of Resistance? Effect on Reproduction

RKN reproduction on Tomato Tasti Lee lines with and w/o Mi gene (Dr. S. Hutton)

Regmi and Desaeger, 2018
There are limitations of the Mi-gene

Not effective against *Meloidogyne hapla*, *M. enterolobii* or *M. floridensis*

Brito et al., 2007

Known occurrences of resistance breaking populations of *M. incognita* and *M. javanica*

**California** (Kaloshian et al., 1996)  
**Cyprus** (Philis and Vakis, 1977)

**France** (Castagnone-Sereno et al., 1994; Jarquin-Barberena et al., 1991)  
**Greece** (Tzortzakakis et al., 2005)

**Israel** (Iberkleid et al., 2014)  
**Italy** (Molinari and Miacola, 1997)

**Morocco** (Eddaoudi et al., 1997)  
**Spain** (Ornat et al., 2001)

Temperature effects: temperatures above 28 °C are reported to cause gene to become nonfunctional

Discrepancy in reports about the loss of resistance:

≥ 32°C (Dropkins, 1969; Veremis and Roberts, 1996; Williamson, 1998)

< 32°C (Abdul-Baki et al., 1996; Ammati et al., 1986; Verdejo-Lucas et al., 2013)
Important questions for *Mi* tomatoes in Florida

1. Will high soil temperature under plastic mulch cause the resistant gene to become nonfunctional?
2. What degree of root galling will occur on resistant tomato?
3. Do both resistant and susceptible cultivars respond to soil fumigation?
4. What is the yield potential of a resistant versus a susceptible cultivar without and with soil fumigation?
5. What is the likelihood that fields in Florida transplanted with RKN resistant cultivars result in a resistant breaking race of *Meloidogyne* sp?
6. What RKN species are present in grower tomato production fields and why is this important for growers that want to use a RKN resistant cv?
## DWD - Field evaluations of rkn resistant tomato cultivars, Citra, FL

### Example of 1 of 5 field trials

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Vigor Rating (0-10)</th>
<th>XL (Kg/ha)</th>
<th>L (Kg/ha)</th>
<th>M (Kg/ha)</th>
<th>Marketable yield (Kg/ha)</th>
<th>Galling index (0-100%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BHN 602</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nontreated</td>
<td>4.8 c</td>
<td>4,001 bc</td>
<td>4,433 c</td>
<td>6,018 b</td>
<td>14,452 c (60)</td>
<td>58 a</td>
</tr>
<tr>
<td>1,3-D + chloropicrin 300 lbs</td>
<td>9.7 a</td>
<td>8,506 a</td>
<td>12,974 a</td>
<td>14,632 a</td>
<td>36,112 a (0)</td>
<td>1 b</td>
</tr>
<tr>
<td><strong>Amelia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nontreated</td>
<td>4.2 c</td>
<td>3,027 c</td>
<td>3,892 c</td>
<td>6,199 b</td>
<td>13,118 c (64)</td>
<td>1 b</td>
</tr>
<tr>
<td>1,3-D + chloropicrin 300 lbs</td>
<td>8.6 ab</td>
<td>5,839 b</td>
<td>9,371 b</td>
<td>14,777 a</td>
<td>29,987 b (17)</td>
<td>0 b</td>
</tr>
<tr>
<td><strong>Red Bounty</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nontreated</td>
<td>4.8 c</td>
<td>4,505 bc</td>
<td>3,640 c</td>
<td>6,199 b</td>
<td>14,344 c (60)</td>
<td>0 b</td>
</tr>
<tr>
<td>1,3-D + chloropicrin 300 lbs</td>
<td>8.4 b</td>
<td>9,118 a</td>
<td>8,001 b</td>
<td>13,154 a</td>
<td>30,273 b (16)</td>
<td>0 b</td>
</tr>
</tbody>
</table>
Tomato Field Experiments: Fall 2017, Spring 2018, GCREC

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Chemicals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Florida 47 (Mi -)</td>
<td>Pic100 (200 lb/acre)</td>
</tr>
<tr>
<td>Sanibel (Mi +)</td>
<td>Pic100 (200 lb/acre) + Nematicide (Nimitz, 2017; Salibro (exp), 2018)</td>
</tr>
<tr>
<td>Skyway (Mi +)</td>
<td>Nematicide only (2018)</td>
</tr>
<tr>
<td>Tasti Lee (Mi +)</td>
<td>None</td>
</tr>
</tbody>
</table>

GCREC farm, root-knot nematode species = *Meloidogyne javanica*
Effect of fumigation on plant growth and development, fall 2017

Fumigated bed (left) versus non-fumigated bed (right) 55 DAT (cv. FL47)

Non-fumigated bed (left) versus fumigated bed (right) 75 DAT (cv. FL47)
Plant vigor for different cv’s and nematicides, fall 2017

<table>
<thead>
<tr>
<th>Plant vigor index</th>
<th>Pic100+Nimitz</th>
<th>Pic100</th>
<th>None</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sanibel 45 DAT</td>
<td>0.45</td>
<td>0.40</td>
<td>0.35</td>
</tr>
<tr>
<td>SkyWay 45 DAT</td>
<td>0.43</td>
<td>0.38</td>
<td>0.33</td>
</tr>
<tr>
<td>Tasti Lee 45 DAT</td>
<td>0.42</td>
<td>0.37</td>
<td>0.32</td>
</tr>
<tr>
<td>Florida 47 45 DAT</td>
<td>0.41</td>
<td>0.36</td>
<td>0.31</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Plant vigor index</th>
<th>Pic100+Nimitz</th>
<th>Pic100</th>
<th>None</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sanibel 60 DAT</td>
<td>0.50</td>
<td>0.45</td>
<td>0.40</td>
</tr>
<tr>
<td>SkyWay 60 DAT</td>
<td>0.48</td>
<td>0.43</td>
<td>0.38</td>
</tr>
<tr>
<td>Tasti Lee 60 DAT</td>
<td>0.47</td>
<td>0.42</td>
<td>0.37</td>
</tr>
<tr>
<td>Florida 47 60 DAT</td>
<td>0.46</td>
<td>0.41</td>
<td>0.36</td>
</tr>
</tbody>
</table>

**p-values**
- Chem < 0.0001
- Var 0.1836
- Chem*Var 0.1303

**Chem p-value**
- Var 0.0357
- Chem*Var 0.4613

Note: * indicates significant difference.
RKN gall severity for different cv’s and nematicides, fall 2017

Gall percent

55 DAT  85 DAT  130 DAT

Soil Temp  Rainfall

Degree Fahrenheit

Inches

Soil Temp

Rainfall

1-Sep-17  1-Oct-17  1-Nov-17  1-Dec-17  1-Jan-18


None  Pic100  Pic100+Nimitiz
Yield for different tomato cultivars/nematicides, fall 2017

Yield (kg/plot)

<table>
<thead>
<tr>
<th></th>
<th>Pic100+Nimitz</th>
<th>Pic100</th>
<th>None</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sanibel</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skyway</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tasti Lee</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Florida 47</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p-value*

<table>
<thead>
<tr>
<th></th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trt</td>
<td>0.01</td>
</tr>
<tr>
<td>Var</td>
<td>0.12</td>
</tr>
<tr>
<td>Trt*Var</td>
<td>0.3</td>
</tr>
</tbody>
</table>
Spring 2018 - Tomato plant vigor for different cv’s / nematicides

Vigor Index (0-1)

50 DAT  64 DAT  78 DAT

None  Pic100  Salbro  Pic100+Salbro  None  Pic100  Salbro  Pic100+Salbro  None  Pic100  Salbro  Pic100+Salbro

Florida47  Sanibel  Skyway  TastiLee
Root-knot gall severity for different cultivars / nematicides, spring 2018

![Graph showing gall severity for different cultivars and nematicides over time. The x-axis represents time from 12-Feb-18 to 12-Jun-18, and the y-axis represents gall percent. The graph includes data for cultivars like Florida47, Sanibel, Skyway, and TastiLee, and nematicides like Pic100, Salibro, and Pic100+Salibro. The graph also includes data for soil temperature and rainfall.]
Yield for different tomato cultivars/nematicides, spring 2018

<table>
<thead>
<tr>
<th></th>
<th>Pic100+Salibro</th>
<th>Pic100</th>
<th>Salibro</th>
<th>None</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield kg/plot</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sanibel</td>
<td>70</td>
<td>60</td>
<td>55</td>
<td>50</td>
</tr>
<tr>
<td>Skyway</td>
<td>65</td>
<td>60</td>
<td>55</td>
<td>50</td>
</tr>
<tr>
<td>Tasti Lee</td>
<td>60</td>
<td>55</td>
<td>50</td>
<td>45</td>
</tr>
<tr>
<td>Florida 47</td>
<td>55</td>
<td>50</td>
<td>45</td>
<td>40</td>
</tr>
</tbody>
</table>

- **Var** 0.02
- **Chem** 0.02
- **Var*Chem** 0.95
Future for Root-Knot Resistant Tomato in Florida

• *Mi* works well in Florida ... no heat sensitivity issues
• New tomato cv’s with root-knot resistance
• Potential of *Mi* in IPM
  • Mi-1.2 gene also can confer resistance against potato aphid *Macrosiphum euphorbiae* and the whitefly *Bemisia tabaci*
• Research Needs - Host Plant Resistance
  • novel gene source identification and development
  • genetic studies on tolerance traits, nematode parasitism, virulence factors
  • Novel gene transfer techniques ... CRISPR project – Dr. Lee
  • Other potential areas such as induced resistance through elicitors
‘Elicitors’ can Induce Resistance in Plants against Pathogen Infection

• Elicitors are compounds > activate chemical defense in plants.
  • salicylic acid, methyl salicylate, benzothiadiazole, benzoic acid, chitosan, ...
  • use in crop protection and pest management is still in the very early stages

• Nicotinamide adenine dinucleotide (NAD) is a chemical elicitor of plant innate immunity and regulates plant defense responses to multiple biotic stresses
Tomato seedlings exposed to NAD for 24hrs by soil drench before nematode inoculation showed reduced nematode penetration 48hpi with RKN

NAD treatment on host plants reduces *M. hapla* penetration

<table>
<thead>
<tr>
<th>Tomato cultivar</th>
<th>Control</th>
<th>NAD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rutgers</strong></td>
<td><img src="#" alt="Control" /></td>
<td><img src="#" alt="NAD" /></td>
</tr>
<tr>
<td><strong>VFN</strong></td>
<td><img src="#" alt="Control" /></td>
<td><img src="#" alt="NAD" /></td>
</tr>
</tbody>
</table>

2018, N. Abdelsamad, H. Regmi, J. Desaeger, and P. DiGennaro
Thank you GCREC Nematology and sponsors
**Mi gene**

Introduced from *Solanum peruvianum* into *S. esculentum* by embryo rescue of interspecific cross (Smith, 1944)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Source</th>
<th>Properties</th>
<th>Genetics</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Mi</em></td>
<td><em>Solanum peruvianum</em> PI128657</td>
<td>Resistant to: <em>Ma; Mi, Mj</em>; resistance lost at &gt;28 °C</td>
<td>Mapped to short arm of chromosome 6; <strong>Cloned</strong></td>
</tr>
<tr>
<td><em>Mi</em>–2</td>
<td>PI270435-2R2</td>
<td>Resistant to <em>Mi</em> at 32 °C Resistant to: <em>Ma; Mi, Mj, Mh</em></td>
<td>Not linked to <em>Mi</em> or <em>Mi</em>-3, linked to <em>Mi</em>-8</td>
</tr>
<tr>
<td><em>Mi</em>–3</td>
<td>PI126443-1MH</td>
<td>Resistant to <em>Mi</em>-virulent <em>Mi</em> 557R</td>
<td>Mapped to short arm of chromosome 12; linked to <em>Mi</em>-5</td>
</tr>
<tr>
<td><em>Mi</em>–4</td>
<td>LA1708-1</td>
<td>Resistant to <em>Mi</em> and <em>Mj</em> at 32 °C</td>
<td></td>
</tr>
<tr>
<td><em>Mi</em>–5</td>
<td>PI126443-1MH</td>
<td>Resistant to <em>Mi</em> and <em>Mj</em> at 32 °C</td>
<td>linked to <em>Mi</em>-3, linked on chromosome 12</td>
</tr>
<tr>
<td><em>Mi</em>–6</td>
<td>PI1270435-3MH</td>
<td>Resistant to <em>Mi</em> at 32 °C</td>
<td>linked to <em>Mi</em>-7</td>
</tr>
<tr>
<td><em>Mi</em>–7</td>
<td>PI1270435-3MH</td>
<td>Resistant to <em>Mi</em>-virulent <em>Mi</em> 557R at 25°C</td>
<td>linked to <em>Mi</em>-6</td>
</tr>
<tr>
<td><em>Mi</em>–8</td>
<td>PI270435-2R2</td>
<td>Resistant to <em>Mi</em>-virulent <em>Mi</em> 557R at 25°C</td>
<td>linked to <em>Mi</em>-2</td>
</tr>
<tr>
<td><em>Mi</em>–9</td>
<td>LA2157</td>
<td>Resistant to <em>Ma; Mi, Mj</em> at 32°C</td>
<td>Mapped to short arm of chromosome 6</td>
</tr>
</tbody>
</table>

(Adapted from: Williamson, 1998; *Ammiraju et al.*, 2001)
Root-knot Nematode Life Cycle

*Meloidogyne* spp.

- Egg Mass
- Egg
- J1 in egg
- J2 Hatches
- Infective J2
- J2 Infects root
- Parasitic J2
- Adult Male
  - Adult male leaves root to seek female
  - 1st molt in egg
  - 2nd Molt
  - 3rd Molt
  - 4th Molt

- Adult Female
  - Adult Female
  - J3
  - J4

Drawings by C.S. Papp
Nicotinamide Adenine Dinucleotide (NAD)

- Pyridine nucleotide
- Primary metabolite
- Produced from Aspartate
- Ca²⁺ signaling
- ROS production

(Pétriacq et al., 2013)