Getting to the Root of ‘Flordaguard’ Rootstock Resistance:
Rootstock Alternatives and Current Efforts

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UF Stonefruit Field Day
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PSREU, Citra
Peach trees are composite genetic systems

- Genetically distinct scion
  - Unique fruit quality
  - Low-chill adaptation

- Genetically distinct rootstock
  - Locally-adapted
  - Nematode-resistant
Root-knot nematodes (RKN), *Meloidogyne* spp., are parasitic to many agricultural crops

- Four most common RKN species:
  1. *M. incognita* (MI) – the Southern RKN
  2. *M. javanica* (MJ) – the Javanese RKN
  3. *M. arenaria* (MA) – the peanut RKN
  4. *M. hapla* (MH) – the Northern RKN

Light micrographs of the anterior end of second-stage juveniles

Three major species present in most areas with tropical and Mediterranean climates

http://nematode.net/NN3_frontpage.cgi?navbar_selection=sp
ciestable&subnav_selection=Meloidogyne_javanica
Problem: the peach root-knot nematode infects known resistant peach rootstocks

- **Meloidogyne floridensis (MF)**
  common name: peach root-knot nematode

- Occurs only in Florida - first detected by Dr. Ralph Sharpe in 1960s in Gainesville, FL

- Initially identified as *M. incognita* Race 3

- Characterised as a new species based on morphology and unique esterase isozyme pattern (Handoo et al., 2004; Carneiro et al., 2000)

- Wide host range and overcomes nematode resistance of ‘Nemaguard’, ‘Okinawa’, ‘Guardian’ and ‘Nemared’ rootstocks
Comparison of esterase dehydrogenase profiles of *Meloidogyne floridensis* n. sp. (Mf) with that of *M. incognita* (Mi) and *M. javanica* (Mj).

Adult female (J4) and egg mass


I. Invasion
J2 juveniles penetrate the root and migrate intercellulary to reach the vascular cylinder

II. Induction
Induces feeding cells, becomes sedentary

III. Nutrient Acquisition
Vascularization of feeding site, nematode matures to adult

Endophytic phase in the root

Exophytic phase in the soil

J1 in eggs
J2
J3
J4
Male
Female
Eggs

Damage Caused by RKN in Peach

Tree stunting and reduced vigor
Damage Caused by RKN in Peach

- Early defoliation and reduced foliage resulting in the production of unpalatable “stress fruits”
Damage Caused by RKN in Peach

Galled roots

Nematode Management

No Quick Solutions...

- Can’t eliminate nematodes
- No post-plant nematicides available for many crop-nematode combinations
- No single practice will control nematodes, so two or more control methods must be used
- RKN-resistant cultivars – effective against nematode genotypes
• Three commonly used rootstock Nemaguard, Guardian, Nemared, are susceptible to *Meloidogyne floridensis*

• Nemaguard and Nemared require more winter chill for proper fruiting

• *Flordaguard has improved root-knot nematode resistance and low-chill adaptation*  
  – Used as standard rootstock for low-chill peach production in root-knot nematode infested non-alkaline soils

http://hos.ufl.edu/extension/stonefruit/stone-fruit-varieties
How do we manage the disease while maintaining production and staying competitive?
Current Efforts

I. Molecular characterization of resistance in Flordaguard
   ➢ Understand the genetic nature of resistance to *MF* in ‘Flordaguard’ peach
   ➢ Identify SSR markers associated with resistance against *MF* in various segregating populations

II. Evaluation of horticultural performance of peach rootstocks
   ➢ Identify potential rootstocks with resistance to *MF*
# Resistance spectrum of main *Prunus* RKN-resistant sources

(Claverie et al., 2004; Van Ghelder et al., 2010; Cao et al., 2011)

<table>
<thead>
<tr>
<th>Rootstock</th>
<th>MA</th>
<th>MI</th>
<th>MJ</th>
<th>MF</th>
<th>RKN resistance genotype</th>
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</thead>
<tbody>
<tr>
<td><strong>Almond (P. dulcis)</strong></td>
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<tr>
<td>‘Alnem1’</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>(R_{Mja} / R_{Mja})</td>
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<td><strong>Peach (P. persica)</strong></td>
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<tr>
<td>‘Shalil’</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>(R_{Mia} / r_{Mia})</td>
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<tr>
<td>‘Nemaguard’, ‘Nemared’</td>
<td>R</td>
<td>R</td>
<td>R/S*</td>
<td>S</td>
<td>(R_{MiaNem} / R_{MiaNem})</td>
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<td>((G \times N)_{15} = ‘Felinem’)</td>
<td>R</td>
<td>R</td>
<td>R/S*</td>
<td>S</td>
<td>(R_{MiaNem} / r_{MiaNem})</td>
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<td><strong>Myrobalan plum (P. cerasifera)</strong></td>
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<td>R</td>
<td>R</td>
<td>R</td>
<td>(Ma / ma)</td>
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<td><strong>Japanese plum (P. salicina)</strong></td>
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<td>Accession J.222</td>
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<td>R</td>
<td>R</td>
<td>R</td>
<td>(R_{jap} / r_{jap})</td>
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<tr>
<td><strong>Wild peach (P. kansuensis)</strong></td>
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<tr>
<td>‘Honggengansutao’</td>
<td>--</td>
<td>I**</td>
<td>--</td>
<td>--</td>
<td>(P_{kMi}) tested only on MI; possibly an allele of (R_{Mia})</td>
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</table>

*R/S – variable behaviour in function of *M. javanica* isolates

**I** = Immune
Parental Genotypes for the Crosses

- **Flordaguard** (*P. persica*)
  - *rr*
  - and hybrids with *P. kansuensis* *Rr*

- **Wild peach** (*P. kansuensis*)
  - *rr*

- **‘Okinawa’** (*P. persica*)
  - *RR*
  - and hybrids with *P. kansuensis* *Rr*

- **‘UF Sharp’** (*P. persica*)
  - *RR*

300 cu

325 cu
**Generating the mapping populations for phenotyping**

1. **Manual pollinations** (late Jan – early March)

2. **Pollen collection** (late Jan – early Feb)

3. **Fruit harvesting & seed extraction** (April – May)

4. **Seed stratification and germination** (Jun – Aug)

5. **Growing plants in greenhouse**

6. **Screening for MF resistance** (Dec - Mar)
Screening of parental genotypes with SSR markers

The number of SSRs is highly variable among individuals.

Markers A, C, and D can differentiate the two parental genotypes.

Current Efforts...

II. Evaluation of horticultural performance of peach rootstocks

- Identify potential rootstocks with resistance to MF
Peach rootstock field evaluation for Resistance to *M. floridensis*

- **Replaced trees**
- FG = 21
- P-22 = 6
- Ok = 4
- MP-29 = 3
- B = 3

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**Peach Rootstocks**
- Bulgars: UFSun
- Flordaguard
- Barton
- Okinawa
- MP-29
- P-22

**Rutgers Tomato**
**Experiment Design**

- Location: Citra, Florida
- Plot size: 200 ft x 130 ft
- Design: RCBD with 5 subsamples per treatment
- No. of rows (replicates): 10
- Spacing: 4ft between trees, 20ft between rows
- Treatments: Flordaguard (R), Barton (R), Okinawa (S), and 2 new USDA rootstocks (R)

*Photo courtesy of Dr. Andrew Nyczepir, USDA-ARS Byron, Georgia*
Setting up the microplots

Auger was used to excavate holes

Trenches at plot borders for installation of sprinkler irrigation system

Microplots were laid out in rows and fumigated soil was backfilled into pots
Budding on ‘Barton’ and ‘MP-29’
Nematode inoculation for resistance evaluation

Total of 45,000 juvenile nematodes inoculated per plant
2013 Scion and rootstock trunk circumferences (2 inches above and below graft union) of grafted trees in field microplots at Citra, FL.
Scion and rootstock trunk circumferences (2 inches above and below graft union) of grafted trees in field microplots

<table>
<thead>
<tr>
<th>Variety</th>
<th>Scion</th>
<th>Rootstock</th>
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<tr>
<td>Okinawa</td>
<td>a</td>
<td>a</td>
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<td>P-22</td>
<td>a</td>
<td>a</td>
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<tr>
<td>Flordaguard</td>
<td>b</td>
<td>b</td>
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<tr>
<td>Barton</td>
<td>bc</td>
<td>c</td>
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<tr>
<td>MP-29</td>
<td>c</td>
<td>c</td>
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Abbreviations:
- nursery-budded
- field-budded
Relative growth rates of scion based on trunk circumference (2 inches above graft union) at the end of 2013 growing season in field microplots.
Pearson correlations between the scion trunk circumference and selected growth (n = 50) *\text{, } P=0.02; **\text{, } P=0.001.

<table>
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<th>Parameter</th>
<th>Correlation coefficient, r</th>
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<td>Scion trunk circumference</td>
<td></td>
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<tr>
<td>Pruning weight</td>
<td>0.83**</td>
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<tr>
<td>Tree height</td>
<td>-0.28**</td>
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<tr>
<td>Tree spread</td>
<td>0.88**</td>
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<tr>
<td>Scion trunk relative growth rate</td>
<td>-0.33*</td>
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More studies underway...

II. Testing of ‘Flordaguard’ for durability of resistance against four main root-knot nematodes – *M. arenaria*, *M. incognita*, *M. javanica*, and *M. floridensis*

III. Histological characterization of resistance in ‘Flordaguard’
**Histological characterization of resistance**

Terminal root fragments, 10 mm long

1, 2, 3, 5, 8, 12, 17, 23, 30, 40 d post-inoculation

Examine for nematode penetration and root galling

Chemical fixation with 2% glutaraldehyde overnight

Processing – dehydration through graded ethanol series and clearing 2 d

Infiltration and embedding with epoxy resin

Microscope examinations

Sectioning, 5 \( \mu m \)

Staining

Stained slides

Unstained slides for phenolic compound examination

Histological characterization of resistance
Nematode management requires a concerted effort...

- **UF Stonefruit breeding program**
  - Continued efforts to develop improved rootstocks for the peach industry

- **Nurserymen**
  - Maintain nematode-free nursery stock
  - Ascertain rootstocks’ trueness-of-type

- **Growers**
  - Monitor nematode numbers
  - Report any signs of nematode infestation
  - Send soil/root samples to nematode diagnostics lab to identify nematode species
<table>
<thead>
<tr>
<th>MARKERS</th>
<th>Clones</th>
<th>Comm’l nursery</th>
<th>Comm’l orchard 1</th>
<th>Comm’l orchard 2</th>
<th>Seedlings</th>
<th>UF Beauty</th>
<th>Okinawa</th>
<th>Nemaguard</th>
<th>P. kansuensis</th>
<th>Tardy Nonpareil</th>
<th>Citra orchard</th>
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Assessing trueness-of-type

OUTCROSS!

TRUE-TO-TYPE
(red color fades when exposed to warm temperatures)
Resistance-breaking nematodes?

True-to-type ‘Flordaguard’ peach rootstock, confirmed from genotyping analyses
Collecting root and soil samples for nematode diagnostics

Send samples to: UF Nematode Assay Lab
nematology.ifas.ufl.edu
Acknowledgment

Florida Department of Agriculture and Consumer Services (FDACS) Specialty Crop Block Grant
Lineage of ‘Flordaguard’ Rootstock

Shau Thai ___op____Chico 11---
(PI 65821)

-- x ___op____H-91___op_Fla. 12-9___op_Fla. 17-64---
outcross

Prunus davidiana---
(C-26712)

---Fla. 4-115___op_Fla. 14-11
(Flordaguard)

either
(N.J. 5106137 x Okinawa)op
or
(Okinawa x Rancho 23/32)op

op = open pollination
x = unnumbered selection
N.J. 5106137 = J. H. Hale x (Elberta x Rutgers Redleaf)