Identification Of Disease Resistant Genotypes For Use In Walnut Rootstock Development

USDA-NIFA-SCRI Project
Disease-Resistant Walnut Rootstocks

**Long-term goal:** Develop, evaluate, and deploy walnut rootstocks with resistance to the major soil-borne pathogens.

**Target Pathogens:**
- *Agrobacterium tumefaciens* (crown gall)
- *Phytophthora* spp. (Phytophthora root/crown rot)
- *Pratylenchus vulnus* (lesion nematode)
Objectives:

1. Generate/exploit a genetically diverse *Juglans* germplasm collection
2. Identify *Juglans* germplasm resistant to key soil-borne pathogens
3. Generate and clonally propagate hybrid and elite disease-resistant genotypes for validation in field trials
4. Develop genomic tools to facilitate rootstock breeding
5. Deliver disease resistant rootstocks to growers
Evaluation of wild *Juglans* species for disease resistance

Exploiting the USDA-ARS Walnut Germplasm Collection

*J. ailantifolia*

*J. californica*

*J. cathyensis*

*J. hindsii*

*J. major*

*J. hopeiensis*

*J. mandshurica*

*J. microcarpa*

*J. nigra*

*J. regia*

*J. sinensis*

*Malli Aradhya*

*Chuck Leslie*
Germplasm Generation / Propagation for
disease resistance testing

Chuck Leslie
Malli Aradhya
Wes Hackett
Generation of Interspecific Full-sib Hybrids from Selected “Mother Trees”

J. microcarpa X J. regia ‘Serr’ pollen

C. Leslie,
D. Kluepfel,
M. Aradhya
In vitro Production of Clonal Plants from Hybrid Seed for Replicated Pathogen Resistance Testing
Plant Propagation Goal:

- Total of 43,800 fully acclimated hybrid clonal plants for pathology resistance screening.
- >37,000 of these were 31.01 and 31.09 genotypes;
  >15,000 compliments of Duarte Nursery.

Produced to date:

- Total of 43,800 fully acclimated hybrid clonal plants for pathogen resistance screening trials.
- >37,000 of these were 31.01 and 31.09 genotypes.

>15,000 compliments of Duarte Nursery.
Objectives:

1. Generate/exploit a genetically diverse *Juglans* germplasm collection (OP and interspecific hybrids)
2. Identify *Juglans* germplasm resistant to key soil-borne pathogens
3. Generate and clonally propagate hybrid and elite disease-resistant genotypes for validation in field trials
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Walnut Germplasm Screen
Inoculation method

Stab technique using *A. tumefaciens* infested blade
Rating System

1 = no disease symptoms
2 = gall symptoms with stem girdling less than 25%
3 = gall symptoms and stem girdling between 25% and 50%
4 = galls symptoms with stem girdling >50%
Walnut Germplasm Screen for Crown Gall resistance (hybrids and open pollinated genotypes)
Pathogen Resistance Screening

Phytophthora crown and root rots—Browne Lab
Assessing resistance to *Phytophthora*

2-3 month exposure to
*P. cinnamomi, P. citricola*

Resistance assessed according to:
• Survival duration; (ratings, 0 to 5)
• Crown length rotted (%) (measured)
• Root length rotted (%) (visual)
Assessing resistance to *Phytophthora*
Assessing Resistance to Nematodes

Goal: Select for resistance/tolerance to root-lesion and root-knot nematode (Westphal, McKenry)

Activities:

- Field screen of clones; inoculated with RKN and RLN.
- Selected candidates planted into a replicated nursery for continued evaluation.
- New potential candidates with high levels of resistance in seedling derivatives.
Results: Evaluating MS1 – hybrid clones

Outcome/ Deliverables

- Two years of testing provides the most meaningful nematode data.
- Experimental genotypes show greater nematode resistance than levels observed in standard industry clones.

Future effort: Validate nematode resistance/tolerance of top candidates.
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Field evaluation of “Elite” clonal rootstock germplasm

5 Elite genotypes are in field trials.
5 new clones are ready to enter field trials

In vitro propagation under commercial conditions.
*(compliments of Sierra Gold)*

Shoot/root development

Hardening off

Field trials
### Field evaluation of “Elite” clonal rootstock germplasm

<table>
<thead>
<tr>
<th>Location</th>
<th>Advisor</th>
<th>Potential Site Problems</th>
<th>VX211, RX1, Vlach, Sdling PDX</th>
<th>Experimental Germplasm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lindcove REC Tulare Co.</td>
<td>Fichtner</td>
<td><em>Phytophthora</em>, lesion nematode</td>
<td>Yes</td>
<td>K3, JM4, JM8, 11-99</td>
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<tr>
<td>Lake Co.</td>
<td>Elkins</td>
<td><em>Armillaria</em> root rot, Nematodes, Crown Gall</td>
<td>Yes</td>
<td>K3, JM4, JM8, CC</td>
</tr>
<tr>
<td>Glenn Co.</td>
<td>Lightle</td>
<td><em>Phytophthora</em>, lesion nematode, Crown Gall</td>
<td>Yes</td>
<td>K3, JM4, JM8</td>
</tr>
<tr>
<td>Sutter Co. SGN</td>
<td>Hasey</td>
<td>Marginal Soil</td>
<td>Yes</td>
<td>K3, JM4, JM8, 11-99, 3S-17, CC</td>
</tr>
<tr>
<td>Solano Co. UCD Armstrong</td>
<td>Pope</td>
<td>Inoculated: CG <em>Phytophthora</em> spp</td>
<td>Yes (no sdlg PDX)</td>
<td>K3, JM4, JM8, 11-99, 3S-17</td>
</tr>
</tbody>
</table>

**Potential Site Problems**
- VX211, RX1, Vlach, Sdling PDX
- *Phytophthora*, lesion nematode
- *Armillaria* root rot, Nematodes, Crown Gall
- Marginal Soil
- Inoculated: CG *Phytophthora* spp
Objectives:

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Genetic Linkage Map of DJUG 31.01 (Juglans microcarpa)
Conclusions/Results

Crossed the “best” *Juglans* species with disease resistance with *J. regia* and invitro propagated hybrid progeny from these crosses (“Paradox”-like seedlings)

Disease resistant OP and some interspecific hybrids (*J. microcarpa x J. regia*) have been identified and will be used to map the genetic loci which mediate disease resistance.

Elite putative disease resistant hybrids have been clonally propagated and are being examined in field tests at multiple locations in the CA central valley

Developing Genomic information for future use in breeding
Current Conventional Breeding and Selection

- Effective but Time consuming
- Resource Intensive
- Complex Disease Testing

Genomics & Genetics

- ID genes associated with disease resistance. i.e. Genetic Marker discovery
- Genetic and Physical maps

Evaluate progeny from a Conventional Cross

- Juvenile selection

Walnut Rootstock Improvement

Pathogen resistance
Adaptability to varied soil conditions
Productivity/Longevity
Sustainability
Exploiting walnut wild relatives to identify disease resistant genotypes for use in commercial rootstock development.

USDA-NIFA-SCRI Project
**Goal:**
- Rootstock clones resistant to *Phytophthora* spp., markers for resistance to *Phytophthora*

**Activities:**
- Screening (2 *Phytophthora* species with: 290 clones, 38 genetic backgrounds; 13,800 plants tested since 2010, 2986 plants in 2016)
- Orchard trials, diagnostics

**Products:**
- Clones of *Juglans microcarpa x J. regia* and *J. cathayensis x reg* putatively low in susceptibility to *P. cinnamomi* and *P. citricola*.
- Orchard-validated resistance to *P. cinnamomi* in ‘RX1’, orchard trials established w/ ‘STJM4’, ‘29JM8’, & ‘3s17’