Concepts of Apple Rootstock Breeding and Selection: A Journey Through the Development of New Apple Rootstocks

G. Fazio, H. Aldwinckle, T. Robinson
Introduction

- Breeding work for the Geneva® rootstocks was initiated by Drs. Cummins and Aldwinckle in 1967.
- The USDA/Cornell program is actively breeding and selecting new rootstocks (about 2,500 in the pipeline) – Dr. Aldwinckle and Dr. Robinson represent Cornell University in the program.
- The program, has always focused on developing yield efficient, disease resistant rootstocks (fire blight, etc).
- It is now focusing on characterization of other important traits such as replant disease resistance, drought tolerance, cold tolerance, etc.
Apple Harvest Doud family farm (1916, Miami Co. Indiana)
Auvil Fruit Farm (Vantage, WA 2005 – next to Columbia River)
Benefits from the implementation of dwarfing rootstocks

Less sprays

Less ladder accidents

Increased productivity
Improving Rootstocks for Superior Tree Performance

- Fruit Color and Quality
- Fruit Size
- Disease Resistance

- Plant Architecture – Dwarfing
  - Molecular mapping and selection tools
  - Genomics
- Yield and productivity (Nutrition)
- Precocity
- Abiotic Stress Resistance (Cold)
- Disease Resistance
  - Fire blight ($40M 2000 epidemic, MI)
  - Replant disease complex
- TRANSGENIC ROOTSTOCKS for a plethora of traits
Active Apple Rootstock Breeding Programs 1970s and 80s

Sweden
Norway
Russia
Poland
Czech
Germany
Japan
Quebec
Vineland
Michigan
Geneva
Malling
Active Apple Rootstock Breeding Programs 2005

- Russia
- Japan
- S. Korea
- China
- New Zealand
- Geneva
# New and Experimental Apple Rootstocks in the U.S.

<table>
<thead>
<tr>
<th>Polish</th>
<th>Czhec</th>
<th>Malling</th>
<th>Russia</th>
<th>Vineland</th>
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# Geneva Rootstock Selection Traits

<table>
<thead>
<tr>
<th>TRAIT</th>
<th>EVALUATION YEARS</th>
<th>LOCATION</th>
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<tr>
<td><strong>Fire Blight resistance</strong></td>
<td>1 or 7</td>
<td>Greenhouse/Field</td>
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<tr>
<td>Phytophthora resistance</td>
<td>1</td>
<td>Greenhouse</td>
</tr>
<tr>
<td><strong>Replant Disease Complex</strong></td>
<td>1 or 7</td>
<td>Greenhouse/field</td>
</tr>
<tr>
<td>Wholly apple aphid res.</td>
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<td>Greenhouse</td>
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<tr>
<td>Juvenility - Spines</td>
<td>3-4</td>
<td>Field/Stoolbed</td>
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<tr>
<td>Stoolbed rooting</td>
<td>3-4</td>
<td>Field/Stoolbed</td>
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<tr>
<td>Growth habit - Brittleness</td>
<td>3-4</td>
<td>Field/Stoolbed</td>
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<tr>
<td>Dwarfing</td>
<td>8-12</td>
<td>Orchard</td>
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<tr>
<td>Precocity</td>
<td>8</td>
<td>Orchard</td>
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<tr>
<td>Suckering</td>
<td>8</td>
<td>Orchard</td>
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<tr>
<td>Yield – Biennial bearing</td>
<td>12</td>
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<tr>
<td><strong>Cold hardiness</strong></td>
<td>15</td>
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<tr>
<td><strong>Drought tolerance</strong></td>
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<tr>
<td><strong>Graft union compatibility</strong></td>
<td>5</td>
<td>Orchard</td>
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</table>
Insects and diseases of apple rootstocks

- Fire blight (*Erwinia amylovora*)
- Crown rot, root rot (*Phytophthora spp.*)
- Woolly Apple Aphid (*Eriosoma lanigerum*)
- Southern Blight (*Sclerotium rolsfsii*)
- White root rot (*Rosellinia necatrix*)
- Texas root rot (*Phymatotrichum omnivora*)
Apple Rootstock Breeding: Resources and Activities

Lab & Greenhouse
- Seedling inoculations
- Molecular markers
- Tissue culture
- Plant pathology
- ETC.

Orchard Production
- Individual yield and growth
- Disease incidence
- Scion compatibility
- ETC.

Nursery
- Liner production
- Tree Production
- Stoolbed evaluation
- Transplant Evaluation
- ETC.

Apple Rootstock Breeding is a very resource intensive endeavor.
Apple Rootstock Breeding and Selection Protocols

**Objective 1.1**

<table>
<thead>
<tr>
<th>Stage 1</th>
<th>Stage 2</th>
<th>Stage 3</th>
<th>Stage 4</th>
<th>Stage 5</th>
<th>Stage 6</th>
<th>Stage 7</th>
<th>Stage 8</th>
<th>Stage 9</th>
<th>Stage 10</th>
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<tr>
<td>Years 1-2</td>
<td>Years 3-4</td>
<td>Years 5-6</td>
<td>Years 7-12</td>
<td>Years 10-15</td>
<td>Years 16-18</td>
<td>Years 19-21</td>
<td>Years 22-24</td>
<td>Years 25-27</td>
<td>Years 27-30</td>
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</table>

1. Select elite parents
2. Generate F1 populations
3. Stratify and plant populations
4. MAS for dwarfing, precocity, disease resistance, etc.
5. Disease screening
6. Plant selected stools
7. Stool selection
8. Propagation and grafting
9. First test orchard
10. First test evaluation and selection
11. Elite stoolbed establishment
12. Stress tolerance tests - drought, cold hardiness, graft union strength
13. Elite liner and tree production
14. Elite stoolbed selection and distribution to nurseries
15. Intermediate replicated orchard
16. Intermediate orchard evaluation
17. Commercial stoolbed evaluation
18. NC-140 and cooperator trials (national and international)
19. Commercial production ramp up Patent and UPOV protection
20. Commercial sale

**Critical Juncture:** Molecular information about allelic constitution at disease resistance loci and about other important traits can be used to bypass first test orchard.

**Repeated inoculations with Fireblight, Wooly Apple Aphid, Phytophthora, Powdery Mildew and comparison to known standards**

**Highly replicated first test orchard at multiple sites**

**Contingent on goodness of MAS**

**Feedback from Horticultural Traits evaluations, disease resistance evaluations, stress tolerance evaluations will be combined with molecular data and used in subsequent cycles of selection. Elite material from these evaluations are used as parents in subsequent cycles.**

**Stress Tolerance Tests will have to be highly replicated at each location - therefore only a few selections at a time will be tested. Grower cooperators in cold or drought prone areas in the US will be selected for cold hardiness and drought stress. For graft union compatibility and strength a set of rootstocks will be grafted with multiple scions and graft unions tested after 3 years.**

**Feedback from Horticultural Traits evaluations, disease resistance evaluations, stress tolerance evaluations will be combined with molecular data and used in subsequent cycles of selection. Elite material from these evaluations are used as parents in subsequent cycles.**

**Commercial stoolbed selection and distribution to nurseries**

**Commercial production ramp up** Patent and UPOV protection

**Commercial sale**
Criteria for Parent Selection – Phenotype and Molecular Markers

- Dwarfing
- Precocity
- Disease Resistance
  - Fire Blight
  - Phytophthora
  - Powdery Mildew
  - Apple Scab
- Yield and Field Performance
- “New” Gene Pools

AD13 SCAR scab marker (Boudichevskaia et al. 2006)

EM M01 SCAR powdery mildew marker (Evans et al. 2003)
New Gene Pools at the Plant Genetic Resources Unit (PGRU) Geneva, New York

*Malus* - Apple - 3995 accessions 2430 clones (grafted) and 1565 seedlots from wild

2808 wild *Malus* seedlings from 310 populations from Kazakhstan, Russia, China & Turkey
Malus sieversii from Kazakhstan 1989 - 1996

Site 9 w/ depletion by grazing animals

Excellent apple-scab resistance from some sites

Site 5

Site 6
Gene Pool Identification – Combining SSR, SCAR Markers

[Diagram showing genetic relationships between different markers and gene pools, with coefficients indicated.]
Crossing Parents – Stage 1
Crossing Parents – Stage 1
Seed Harvest – Stage 1 – 2,000-10,000 seeds per cross
Disease Screens – Stage 1 – 3,000 to 10,000 seedlings/year
Disease Screens – Stage 1 – 3,000 to 10,000 seedlings/year
Fire blight - *Erwinia amylovora*

- Major disease for apple rootstocks in North America
- Bacterial disease with strain differentiation
- Resistance sources available
- Rootstock infection routes:
  - suckers
  - injuries
  - systemic movement of bacteria from scion
Fire Blight Screening – Stage 1
500 to 2,000 seedlings
Integration of Marker Assisted Selection – Stage 2

- High throughput PCR markers – SCARs, SSRs
- Target traits:
  - Dwarfing
  - Powdery mildew resistance
  - Scab resistance
  - Wooly apple aphid resistance
- Use published and “in house” markers
Propagation and Evaluation of Layering Stool-Bed Properties
Harvest of Rootstock Liners – Evaluation of Rooting
Rootstock Liners in Tree Nursery for Budding/Grafting
First Test Orchard – Stage 3

- 3-10 replicates per rootstock genotype
- 50-100 different genotype selections every year
- All grafted with same scion
- Evaluated for 8-12 years
Early field selection of precocious genotypes – Stage 4
Expansion of Layering Beds to Increase Replications – Stage 4
Evaluation of Layering Stool Beds – Stage 5
Rootstock Liner Evaluation – Stage 5
Second Test for Resistance to Biotic Stresses – Stage 5

- Fire Blight Inoculations with Multiple Strains
- Water Logging test with Phytophthora Inoculation
- Inoculation with Wooly Apple Aphid (*Eriosoma lanigerum*)
Replicated Orchard Trials in Multiple Locations – Stage 6

- Precocity
- Yield
- Fruit Size
- Dwarfing
- Tree Survival
- Disease Incidence
- Tree Architecture
- Burr Knots
Descriptive Statistics

Variable: CUM-YEFF

Anderson-Darling Normality Test
A-Squared: 17.106
P-Value: 0.000

Mean: 2.59040
StDev: 1.19212
Variance: 1.42114
Skewness: 1.75248
Kurtosis: 3.70960
N: 317

Minimum: 0.24800
1st Quartile: 1.87200
Median: 2.20500
3rd Quartile: 2.91550
Maximum: 8.18500

95% Confidence Interval for Mu
2.45867 2.72214

95% Confidence Interval for Sigma
1.10598 1.29292

95% Confidence Interval for Median
2.13806 2.34268
Replicated Orchard Trials in Multiple Locations – Stage 6

Descriptive Statistics

### Variable: Suckers

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<th>Statistics</th>
<th>Value</th>
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<td>StDev</td>
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<td>Minimum</td>
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<tr>
<td>1st Quartile</td>
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<tr>
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<td>3rd Quartile</td>
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Anderson-Darling Normality Test

- A-Squared: 27.087
- P-Value: 0.000

Confidence Intervals

- 95% Confidence Interval for Mu: 95% CI
- 95% Confidence Interval for Sigma: 95% CI
- 95% Confidence Interval for Median: 95% CI
Timing of fire blight screens

Number of genotypes observed

Number of replications per genotype

Transgene mediated resistance

Rootstock blight orchard trials

Strain specific direct inoculation screens

Time

Number of plants

1

2
Screening for Resistance to Fire Blight (*E. amylovora*)

- **Greenhouse inoculations on liners**
- **Field inoculations on finished trees**
Commercial Stool Bed Trials – Stage 7

- On site trials of elite rootstocks at commercial nursery locations
- Evaluate liner productivity and quality under commercial conditions
- Generates nursery stock for major orchard trials (NC-140, large grower trials)
Trials with NC-140 Cooperators –
Stage 8
Drought Tolerance Tests

Work of Dr. PARRA
Graft Union Strength Tests

Pictures Courtesy of Mike Parker (NC State University)
On Farm and Nursery Trials in Several U.S. Locations – Stage 9

- Large scale trials planted in WA, PA, MI, NY
- Trials include 20-45 different genotypes
Nursery Tree Measurements on 10-15 Trees per Rootstocks

- Tree Height
- Total Number of Branches
- Branch Height
- Branch Angle: 0=Flat, 90=Upright
- Branch Length
Branch Angles of Brookfield Gala Trees on Several Dwarfing Rootstocks for 7 Whirls

Branch Angle (0=Flat 90=Upright) vs Whirl (3 branches)

- 2034
- 2406
- 3041
- 3041
- 4210
- 4213
- 4214
- 5935
- B9
- 4814
- G11
- G16
- G16
- G?
- M9
Stages of Micro-Propagation
Prior to Release – Stage 10
Commercial Release and Continued Testing – Stage 10

- Program has released 6 new rootstock genotypes to date.
- G.16 and G.30 G.202, G.41, G.935, G.11 are commercially available in U.S.
- Release decision for six more elite rootstock genotypes expected in 2008.
Large Scale Production of Rootstock Liners
Production of High Quality Nursery Trees and Adoption By Growers

Nursery trees on Geneva 202 rootstocks and planted in high density orchard.
QTL Mapping of Apple Rootstock Yield & Disease Resistance Traits

- Dr. Wan Yizhen
- Construct a molecular map of Apple Rootstock using microsatellites, SNP, SCAR.
- Map and develop markers for plant architecture and disease resistance traits.
- Develop basic knowledge on Chinese apomictic species for seed propagated rootstocks.
- Transgenic approaches for improving rootstock performance.
Research Work on Apple Rootstocks Requires Many Collaborators and Institutions

- **Cornell University:**
  - T. Robinson (Orchard Systems)
  - I. Merwin (Horticulture - Replant)
  - H. Aldwinckle (Plant Pathology)
  - L. Cheng (Physiology)
  - S. Brown (Scion Breeding)
- **Michigan State University:**
  - R. Perry (Rootstocks)
  - S. VanNocker (Genomics)
- **Washington State University:**
  - B. Barrit (Scion Breeding)
  - D. Main (BioInformatics)
- **USDA ARS PGRU:**
  - A. Baldo (BioInformatics)
  - P. Forsline (Apple Collection)
- **USDA ARS AFRS Kearneysville:**
  - J. Norelli (Transgenics)
  - C. Bassett (Stress Physiology)
- **USDA ARS Wenatchee:**
  - M. Mazzola (Plant Pathology)
  - Y. Zhu (Genomics)
- **PENN State University:**
  - T. McNellis (Genomics)
  - J. Schupp (Horticulture)
- **Over 40 scientists as NC-140 collaborators**
- **Washington Tree Fruit Research Commission**
Genomic Revolution

- We know that M.7 rootstock is less precocious than M.9. Do we know why?
- We know that M.9 dwarfs more than M.26? Do we know why?
- Through Genomics much is being discovered about how rootstocks do all that they do.
  - NSF funded project that aims to discover what genes are turned on and off in the apple scion by different rootstocks. (Dr. McNellis, Penn State)
- Tree architecture modified by apple rootstocks…. 
- Wealth of new genetic material
The Geneva® Apple Rootstock Breeding Program

THANK YOU

Todd Holleran, Sarah Bauer, Yizhen Wan

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The Road Ahead (2003 NC-140 Mtgs. Door County, Wisconsin)