

Tree Fruit Rootstocks and Novel Breeding Technologies – the Hidden Treasure



The Geneva Apple Rootstock Breeding Team

Plant Genetic Resources Unit (PGRU) Geneva, New York

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



Wild *Malus* seedlings from 310 populations from Kazakhstan, Russia, China & Turkey



National Germplasm Repositories



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Apple Rootstock Breeding Program
Plant Genetic Resources Unit, Geneva, NY



The Geneva® Apple Rootstock Breeding Program



James Cummins Terence Robinson Herb Aldwinckle and Awais Khan



Charlie



Sarah Bauer



Todd Holleran



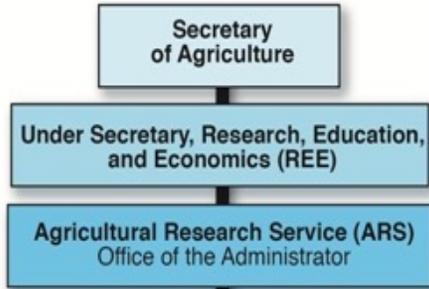
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ARS Organization

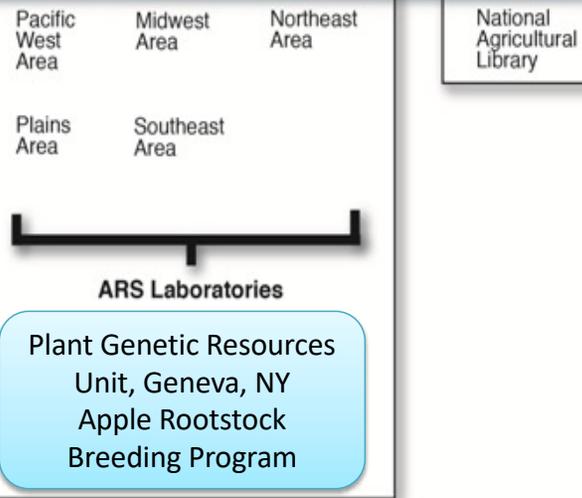


ARS has about 2,000 scientists in 700 research projects working at 90+ locations, including overseas labs. The National Agricultural Library and the National Arboretum are also part of ARS.

Central Program Planning, Coordination, & Support

Office of National Programs	Administrative & Financial Management	Legislative Affairs
Office of International Research Programs	Office of Chief Information Officer	Office of Outreach Diversity, and Equal Opportunity
Office of Technology Transfer	Information Staff	
Budget & Program Management Staff	Office of Scientific Quality Review	

Field Research Implementation & Information Delivery



ARS scientists

Are federal employees, working directly under the Department of Agriculture (USDA) on U.S. agricultural problems.



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Courtesy of Dr. Tim Rinehart

ARS MISSION

ARS delivers scientific solutions to national and global agricultural challenges.

ARS will deliver cutting-edge, scientific tools and innovative solutions for American farmers, producers, industry, and communities to support the nourishment and well-being of all people; sustain our nation's agroecosystems and natural resources; and ensure the economic competitiveness and excellence of our agriculture.

ARS VISION

Global leadership in agricultural discoveries through scientific excellence.

ARS CORE VALUES

Scientific excellence, creativity, innovation, integrity, leadership, collaboration, accountability, transparency, diversity, respect, inclusiveness, and public service.

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complementary federal research



USDA's principal in-house research agency. In consultation with industry, ARS **National Program Leaders (NPLs) assign research objectives and funding** to 700 projects that are distributed at ARS labs across the U.S. ARS scientists at those locations perform the research to meet objectives and fulfil the mission. Base funding is perpetual and timelines are typically 10 years or more.



National Institute of Food and Agriculture's (NIFA) advances agricultural research, education, and extension through extramural grant programs. **NIFA National Program Leaders distribute funding through competitive grant programs** to scientists, mostly university groups. Grant programs address specific agricultural problems or commodities authorized in the Farm Bill. Grant-based research funding typically lasts 3-5 years.

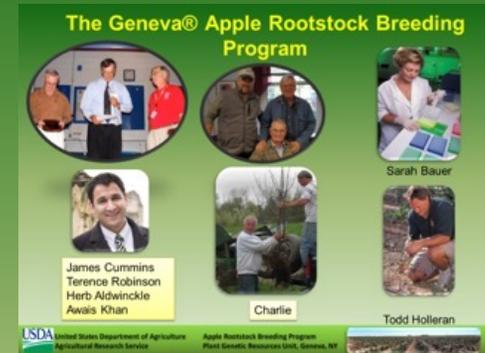


Apple Rootstock Breeding Partnership between USDA-ARS and Cornell University

Leadership,
Breeding and
Genetics, human
resources, funds



Horticulture,
Plant Pathology,
human resources,
land



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Commercial apple trees are a combination of two different genetic types: the **ROOTSTOCK** and the **SCION (aerial system) which bears fruit.**

1. The rootstock mother plants are layered with sawdust in a stoolbed to generate rooted **rootstock** shoots



2. Rooted **rootstock** shoots are harvested from the mother plant and planted in a nursery



3. A bud from a **scion** variety like Gala or Granny Smith is grafted on the **rootstock**



4. The **scion** bud grows into a shoot and then into a mature apple tree. The rootstock will influence the productivity, size and precocity of the apple tree.



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Courtesy of Richard Adams



Courtesy of Richard Adams



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Budding/Grafting in Apples

Chip bud

Whip/tongue

Saddle



Source: Royal Horticultural Society



Source: Adelaide Kitchen Gardeners



Source: Chris Vernon

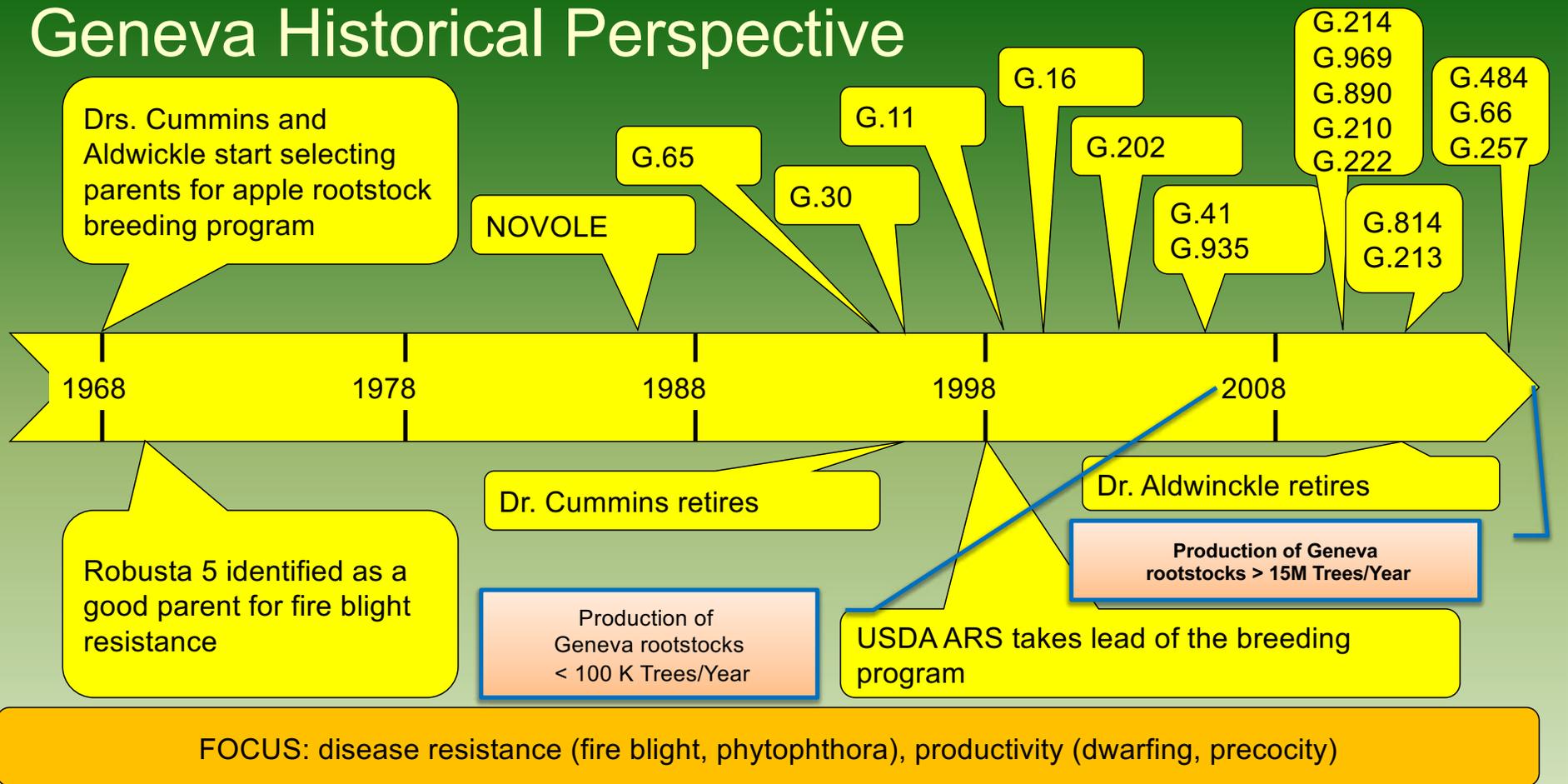


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Geneva Historical Perspective



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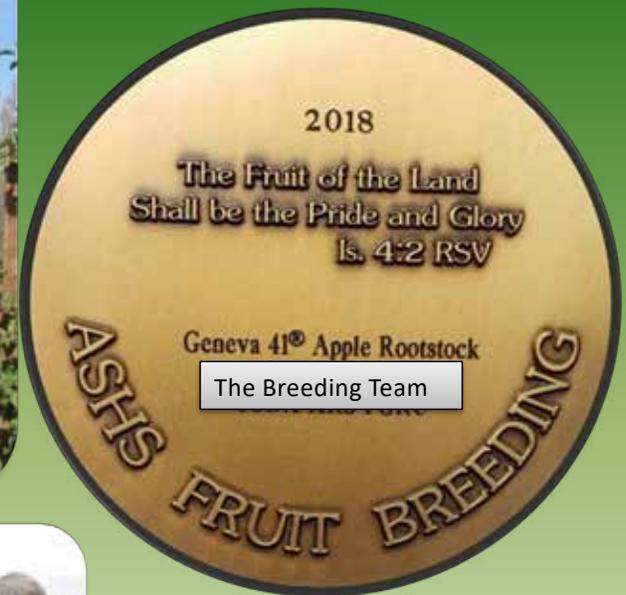


G.41

- M.9 vigor
- Very high yield efficient
- Highly productive
- Very precocious
- Resistant to replant disease
- Very cold hardy
- Does well in warmer climates (Mexico)
- Highly Resistant to Fire Blight and Crown Rot and Woolly Apple Aphid
- Requires tissue culture mother plants for stoolbed



G.41 Fuji CIV 2008



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G.210

- Vigor similar to M.7
- Precocious, productive
- Yield efficiency similar or better than M.9
- Resistant to apple replant disease.
- Resistance to woolly apple aphid, fire blight, and crown rot.
- Good rooting in stoolbed few spines.
- Mostly for Organic Production



G.890

- Vigor between M.7 and MM.106
- Replacements for G.30
- Free standing
- Precocious, productive
- Yield efficiency similar or better than M.9
- Resistance to woolly apple aphid, fire blight, and crown rot.
- Tolerance to apple replant disease.
- Good rooting in stoolbed few spines.
- Mostly for processing industry



G.202

- Size similar to M.26
- Precocious, productive
- Resistant to woolly apple aphid, fire blight, and crown rot
- Tolerant to apple replant disease
- Good choice for weak growing cultivars like Honeycrisp
- Moderate rooting in stoolbed



G.214

- Vigor similar to M.9 Paj.2
- Highly yield efficient
- Highly productive (most U.S. trials yields 100-125% of M.9 check)
- Good precocity
- Resistant to Fire Blight, Crown Rot and Woolly Apple Aphid
- Replant tolerant
- Very good stool bed propagation
- Strong Graft Union



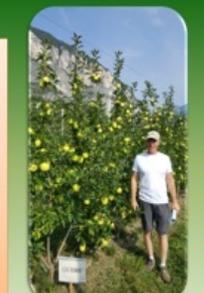
G.11

- Tree size similar to M.9 T337.
- Very high yield efficiency
- Large fruit size
- Partial tolerance to Replant Disease
- Resistant to Fire Blight but not immune.
- Resistant to Crown Rot
- Susceptible to Woolly Apple Aphid
- Good rooting in stoolbed
- Advances Maturation (France, Gala and Red Delicious)



G.969

- Vigor between M.26 and M.7
- Very efficient and productive
- Very cold tolerant
- Resistant to fire blight
- Resistant to Phytophthora
- Resistant to Woolly Apple Aphid
- Good Anchorage
- Excellent rootstock for weak scions like Honeycrisp



G.213

- Vigor similar to M.9 Paj.2
- Good yield efficiency
- High productivity 100-125% of M.9 check
- Very good precocity
- Resistant to Fire Blight, Crown Rot and Woolly Apple Aphid
- Replant tolerant
- Some spines in stool bed propagation
- Reduces chill requirement



G.213
VanAcker
Williamson, NY



G.213
Vacaria,
Brazil



G.814

- Size similar to Pajam 2
- Precocious, productive
- Promotes larger Fruit Size
- Upright branches
- Immune to fire blight, and resistant to crown rot
- Very tolerant to apple replant disease
- Susceptible to Woolly Apple Aphid
- Very susceptible to viruses (ASPV, ASGV, ACLV)
- Good rooting in stoolbed



G.202

- Size similar to M.26
- Precocious, productive
- Resistant to woolly apple aphid, fire blight, and crown rot
- Tolerant to apple replant disease
- Good choice for weak growing cultivars like Honeycrisp
- Moderate rooting in stoolbed



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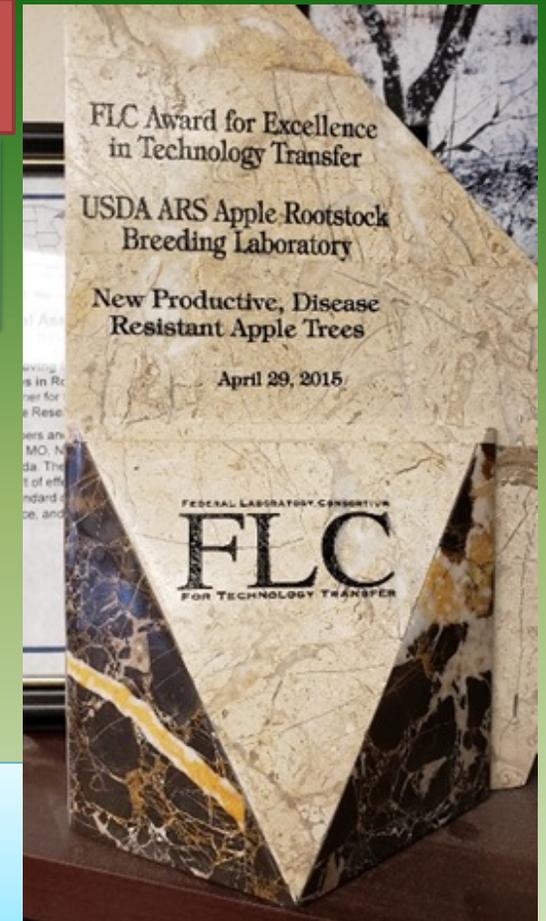
GOOD Tech Transfer is Essential for Success!!

Apple Rootstock Breeding Program (USDA ARS and Cornell Partnership) was Awardee of the Federal Labs Consortium 2015 Excellence in Technology Transfer – competing against other federal labs (NASA, U.S. Army, Naval Research Laboratory, etc.)



- Feedback on nursery performance.
- Support research.
- Word of mouth Extension
- May be able to do things that some other public institutions can't

Really?!?!? Apple Rootstocks?





Left: Honeycrisp tree on G.969 rootstock grafted with clean, virus free wood growing well. Above and right: Rootstock field trial at a Stemilt Growers orchard in Quincy, WA featuring Geneva rootstocks. These growers' trials are essential for field performance evaluation.



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Apple trees on Geneva rootstocks at Willow Drive Nursery (family owned) in Ephrata, WA.



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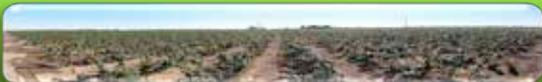


Geneva Rootstock Propagation Beds at Willow Drive Nursery in Ephrata, WA



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Geneva Apple Rootstock Propagation Beds in Full Production at Cameron Nursery in Eltopia, WA

Experimental Apple Rootstocks being tested at Cameron Nursery after being planted in June of 2021. Before propagation beds look like the ones above, they need to be tested for several years to monitor rooting and architecture properties.



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Apple trees raised in pots and apple rootstock propagation beds at Kit Johnston Nursery in Oregon



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Tissue culture (micropropagation) derived Geneva rootstocks at different stages all the way to grafted finished potted trees ready for transplant into the orchard. (North American Plants, OR)



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The next set of California and US (NC140) cider apple trials being propagated at Sierra Gold Nursery, Yuba City, CA. Dr. Micah Stevens showing off the experimental material.



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In addition to growing experimental rootstocks and trees, Sierra Gold produces millions of cherry, peach, almond and apple trees



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Organic Rootstock Field Trial at Lodi Fruit Farms (Replacement trees where trees on different rootstocks had died)



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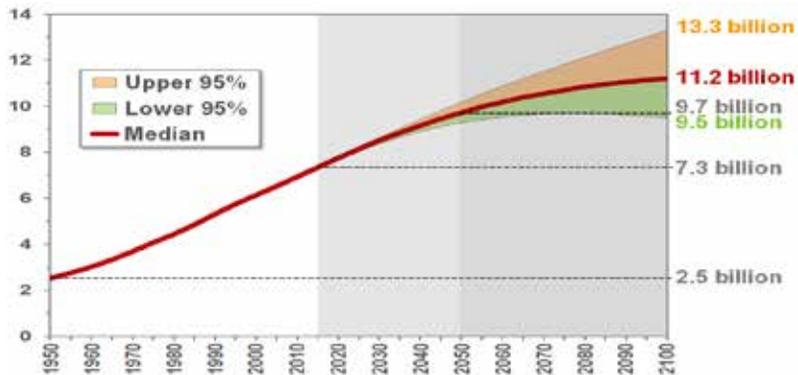
Geneva[®] rootstock field day in Brazil



Geneva[®] rootstock field day in Mexico



POBLACIÓN MUNDIAL-2050



PRODUCCIÓN ALIMENTOS



Dr. Ignasi Iglesias

SOSTENIBILIDAD AMBIENTAL



SOSTENIBILIDAD DE RENTAS



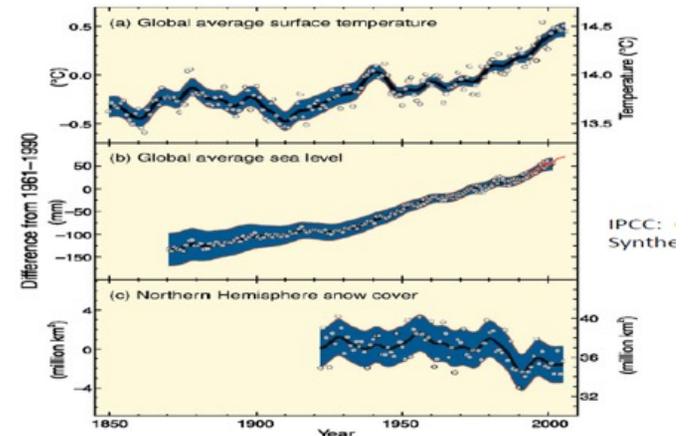
COSTES



USO EFICIENTE RECURSOS + INN. TEC.



CAMBIO CLIMÁTICO



COSTE MANO DE OBRA-2019



Dr. Ignasi Iglesias

País	Coste horario (€/h)	Coste diario (€/dia) 8h
Francia	12,2	97,6
Italia (Emilia Romagna)	10,7	85,6
Israel (Rosh Pina)*	10,2	81,6
España	8,6	68,8
Portugal	5,7	45,6
Grècia	3,7	29,6
Sèrbia	2,6	20,8
Polònia	2,5	20,0
Brasil	4,3	34,4
Chile	2,7	21,6 (fijo)
	6,5	52,0 (temporal+cereza)
USA (Califor.-Washing.)	15,0-24,0	120-192
Argentina	5,6	44,8 (temporal) / (30 al 2008)
Armènia	2,6	20,8
Georgia	2,4	19,2
Rússia meridional	2,6	20,8
Bulgària	1,6	12,8
Rumania	1,5	12,0
Ucraïna	1,3	10,4
Turquia	1,6-2,2	12,8-17,6
Marruecos	0,9-1,5	7,2-12,0
Tunez	0,8	6,4

(*): 8.000 m³/ha water = 3.500 €/ha

Iglesias, 2019



Dr. Ignasi Iglesias





**What is
more
productive?**

**Same Fuji
apple scion.**

**Different
rootstocks!**



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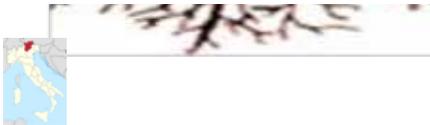
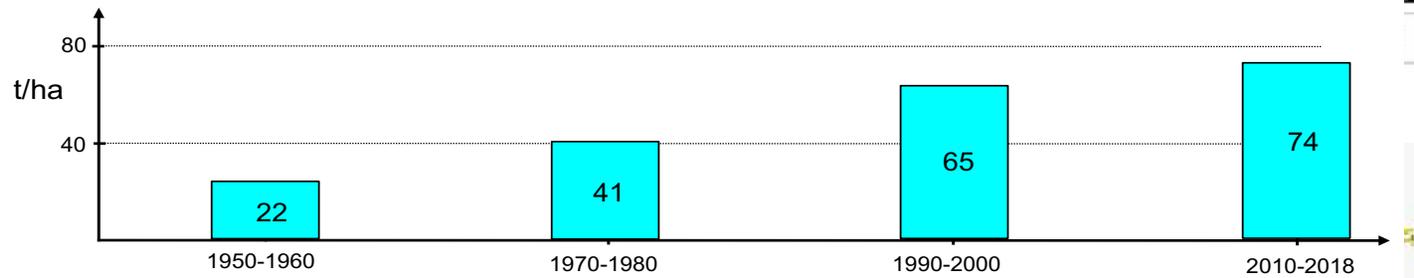
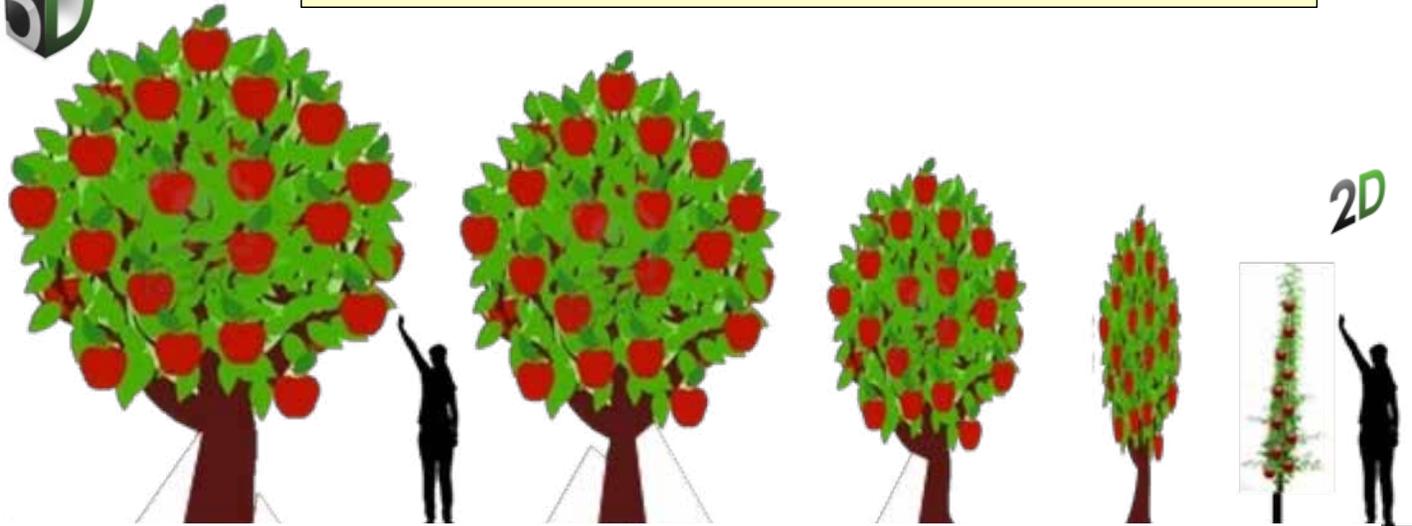
**Apple Rootstock Breeding Program
Plant Genetic Resources Unit, Geneva, NY**



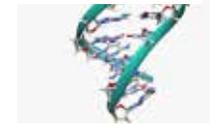
Why this transition from 3D to 2D has been it possible?



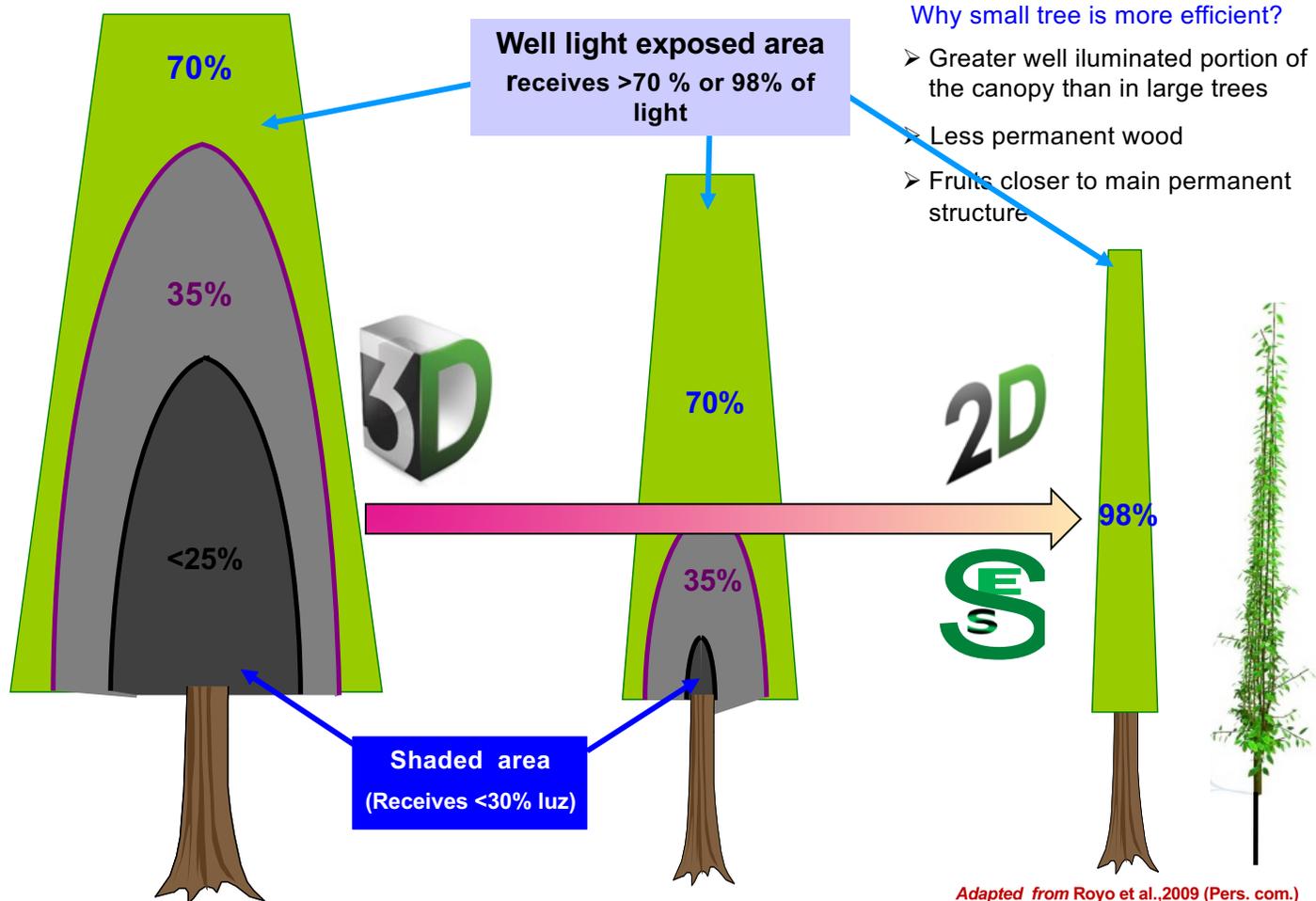
By changing the shape of canopy to do it more accesible



By genetics: breeding for new rootstocks



The effect of volume canopy on yield & light exposition



Benefits from the implementation of dwarfing rootstocks



Less ladder accidents, improved ergonomics for picking



Increased productivity and efficiency

Less sprays, easier mechanization

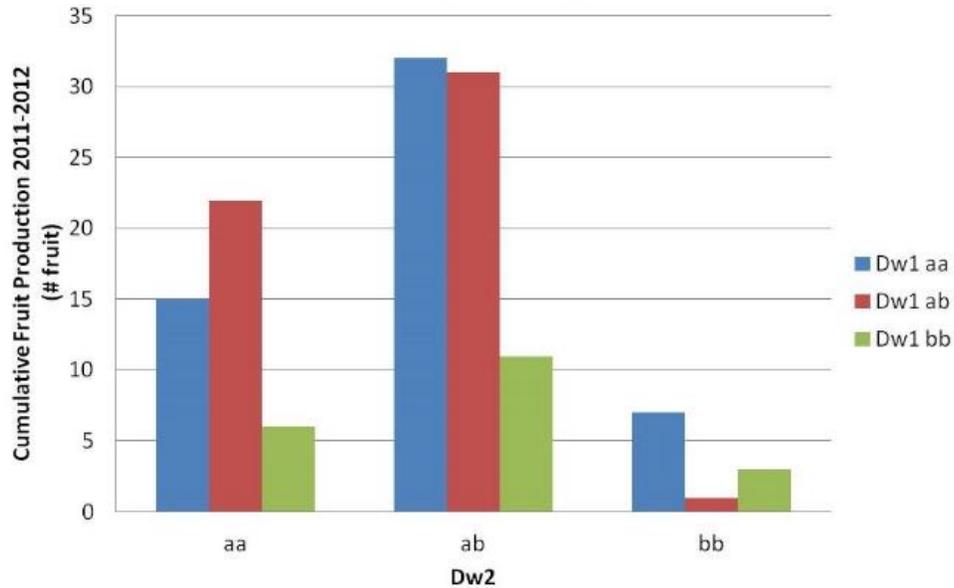


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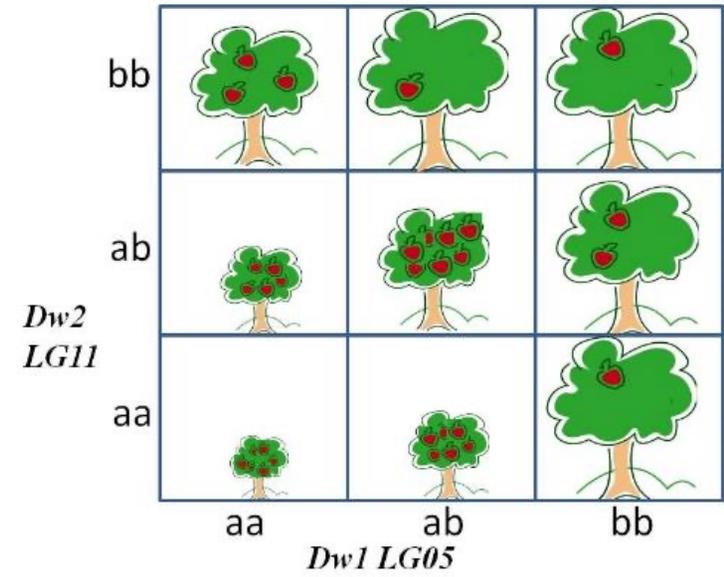
Cumulative Fruit Production
Least Square Means Dw1*Dw2
 Wilks lambda=.48548, F(20, 166.78)=2.0300, p=.00827



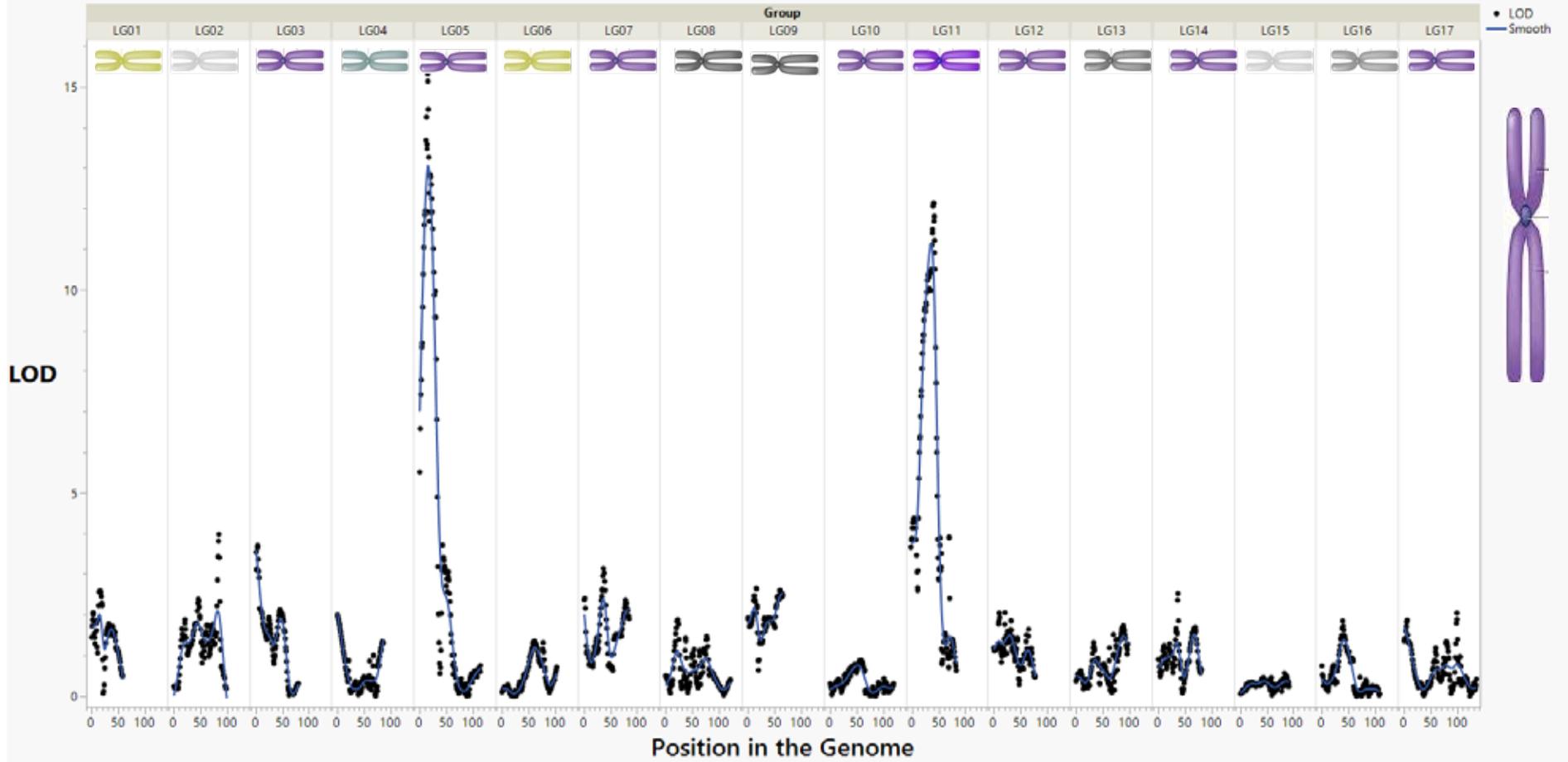
Possible to predict
 Tree Size
 on the basis of allelic combinations
 Dw1 aa ab bb with DW2 aa ab bb

J. Amer. Soc. Hort. Sci. 139(2):1-12, 2014
Dw2, a New Dwarfing Locus in Apple Rootstocks and Its Relationship to Induction of Early Bearing in Apple Scions
 Gennaro Fazio¹
 Plant Genetics Resources Unit, USDA-ARS, 630 W. North Street, Geneva, NY 14456
 Yizhen Wan
 Apple Research Center, College of Horticulture, Northwest A&F University, Yangling, Shaanxi 712100, China
 Dariusz Kviklys
 Lithuanian Institute of Horticulture, Babai, Lithuania
 Letija Romero
 Facultad de Ciencias Agrotecnológicas, Ciudad Universitaria s/n Campus 1 C.P. 31310 A.P. 24 Chihuahua, Mexico
 Richard Adams
 Willow Drive Nursery, 3539 RD 5 NW, Ephrata, WA 98823
 David Strickland
 Department of Molecular & Cellular Biology, Dartmouth University, Hanover, NH 03755
 Terence Robinson
 Department of Horticulture, NYSAES, Cornell University, Geneva, NY 14456

Highest fruit production in heterozygous plants



Mean Tree Diameter

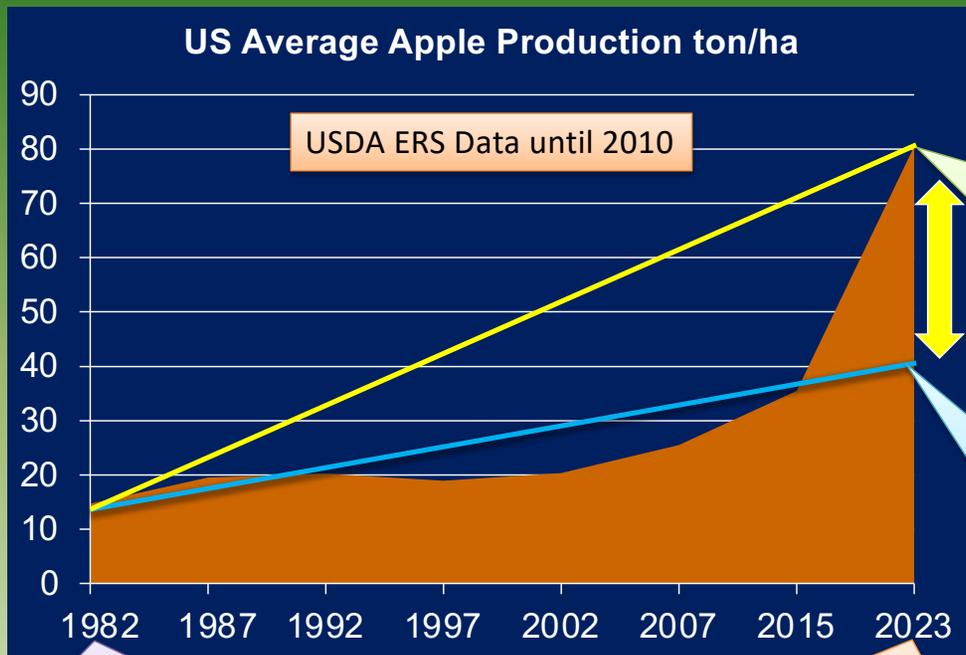


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Economic Impact of Dwarfing Precocious Apple Rootstocks on U.S. Apple Production



61% Increase due to the application of **dwarfing** and **early bearing** rootstocks

38% Increase due to better management practices and inputs (fertilizers, sprays, etc.)

40% of rootstocks sold were dwarfing and early bearing

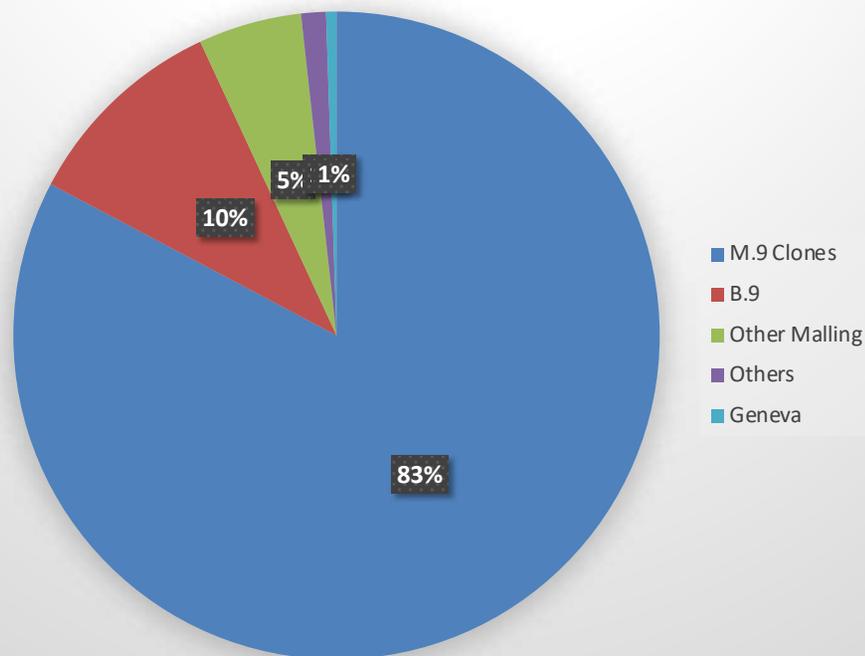
93% of rootstocks sold were dwarfing and early bearing

Farmgate value of U.S Apples is \$ 3.6 Billion. There are 2-3 major genes involved in **dwarfing** and **early bearing** in apple rootstocks. One can extrapolate that at least 50% (\$1.8B) of that farmgate value can be attributed to the application of genes involved in the implementation of dwarfing and early bearing

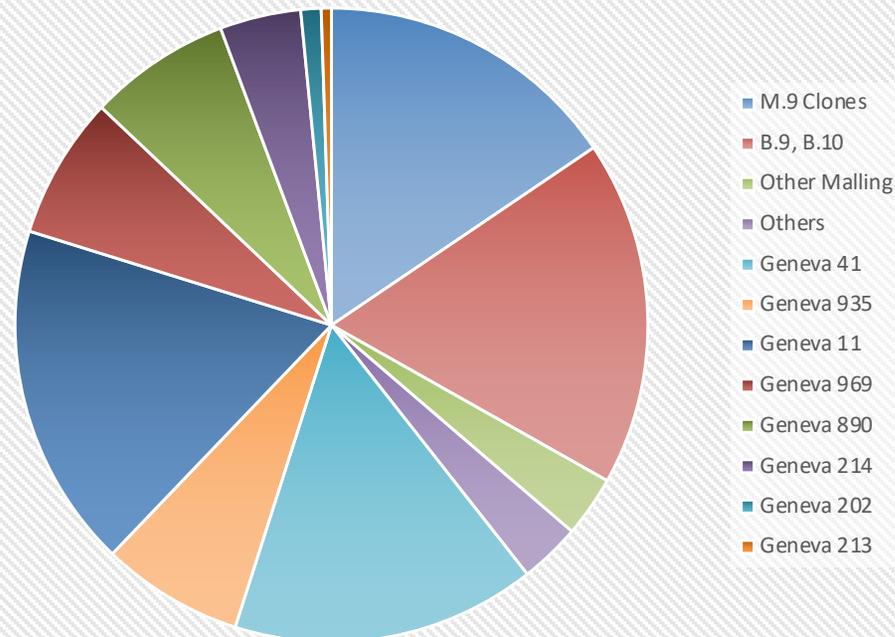


Genetic (Phenotypic) Diversity is the Key!

Estimate of U.S. Rootstock Sales Composition 2001 (%)



Estimate of U.S. Rootstock Sales Composition 2022 (%)

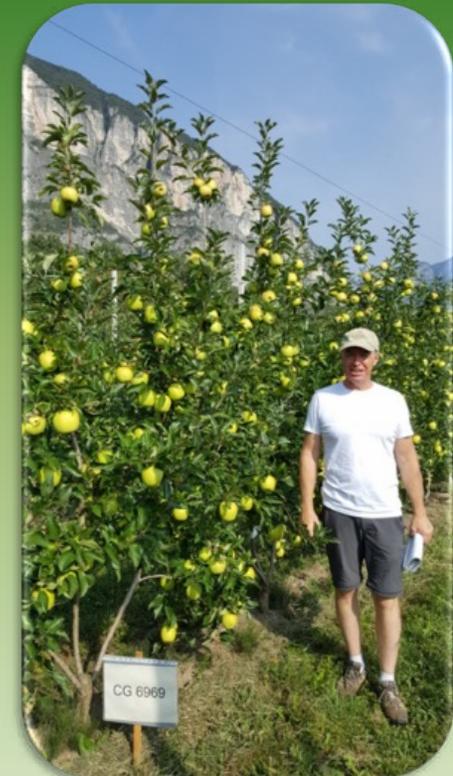


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Diverse rootstocks can support different training system dynamics: fruiting wall vs V trellis, single vs multi axes.



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How do we provide **Designer Solutions** for these interactions?



Decade Level Research needed for rootstocks!



Scientia Horticulturae
Volume 240, 27 February 2019, Pages 506-517



Effect of tree type and rootstock on the long-term performance of 'Gala', 'Fuji' and 'Honeycrisp' apple trees trained to Tall Spindle under New York State climatic conditions

Gemma Reig^{a,b}, Jaime Lordan^b, Mario Miranda Sazo^c, Stephen Anthony Hoying^d, Michael J. Fargione^d, Gabino Hernan Reginato^e, Daniel J. Donahue^f, Poliana Francescato^g, Gennaro Fazio^{h,i}, Terence Lee Robinson^b

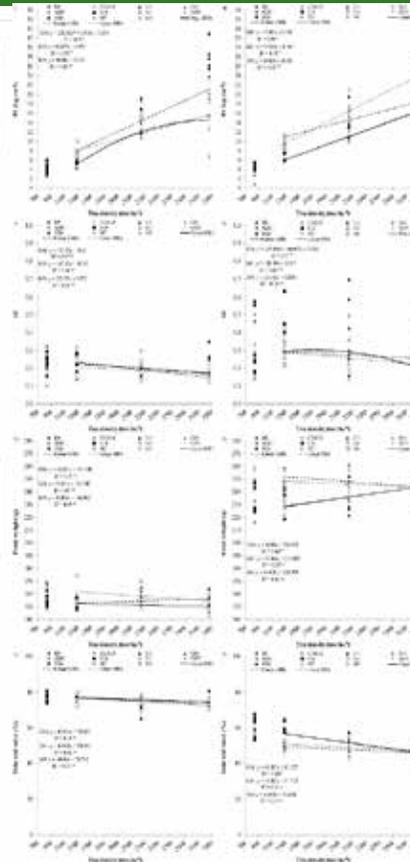


Scientia Horticulturae
Volume 244, 26 January 2019, Pages 277-293



Long-term performance of 'Gala', Fuji' and 'Honeycrisp' apple trees grafted on Geneva® rootstocks and trained to four production systems under New York State climatic conditions

Gemma Reig^{a,b}, Jaime Lordan^b, Mario Miranda Sazo^c, Stephen Hoying^d, Michael Fargione^d, Gabino Reginato^e, Daniel J. Donahue^f, Poliana Francescato^g, Gennaro Fazio^{h,i}, Terence Robinson^b



Long-term Performance of 'Delicious' Apple Trees Grafted on Geneva® Rootstocks and Trained to Four High-density Systems under New York State Climatic Conditions

in HortScience

Authors: Gemma Reig¹, Jaime Lordan², Stephen Hoying³, Michael Fargione⁴, Da...

View More

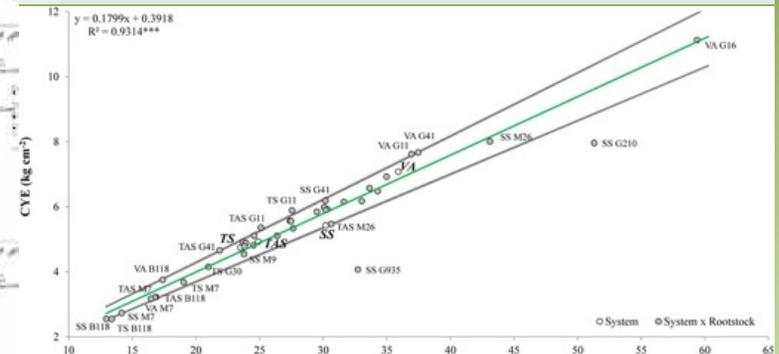
DOI: <https://doi.org/10.21273/HORTSCI14904-20>

Article Category: Research Article

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Online Publication Date: 21 Aug 2020

Volume/Issue: Volume 55: Issue 10



Regression relationship of cumulative crop load (CCL) with cumulative yield efficiency (CYE) for four orchard systems and 40 treatments. The linear regression is represented by the green line, whereas the 95% confidence interval is represented by the gray lines. Regressions were analyzed by a Student t-test (*P < 0.05; **P < 0.01; ***P < 0.001; ns, not significant). SS = Super Spindle; TAS = Triple Axis Spindle; TS = Tall Spindle; VA = Vertical Axis.



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LONG TERM PERFORMANCE

Average life of an apple orchard is 15 years; therefore, decade long research is needed to properly evaluate rootstock performance “Long Term” is key for Tech Transfer

HORTSCIENCE 55(10):1538–1550. 2020. <https://doi.org/10.21273/HORTSCI14904-20>

Long-term Performance of ‘Delicious’ Apple Trees Grafted on Geneva® Rootstocks and Trained to Four High-density Systems under New York State Climatic Conditions

Effect of tree type and rootstock on the long-term performance of ‘Gala’, ‘Fuji’ and ‘Honeycrisp’ apple trees trained to Tall Spindle under New York State climatic conditions



Long-term performance of ‘Gala’, Fuji’ and ‘Honeycrisp’ apple trees grafted on Geneva® rootstocks and trained to four production systems under New York State climatic conditions



I. Mineral nutrient profiles and relationships of ‘Honeycrisp’ grown on a genetically diverse set of rootstocks under Western New York climatic conditions

II. Horticultural performance of ‘Honeycrisp’ grown on a genetically diverse set of rootstocks under Western New York climatic conditions



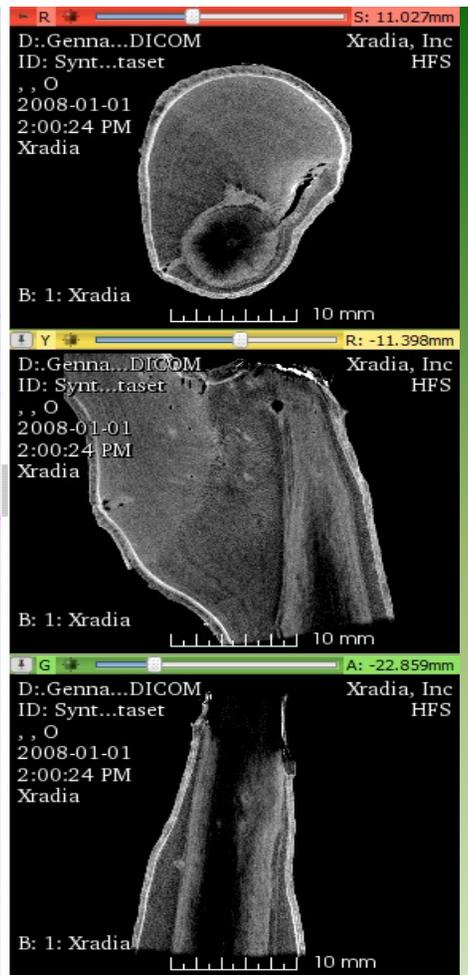
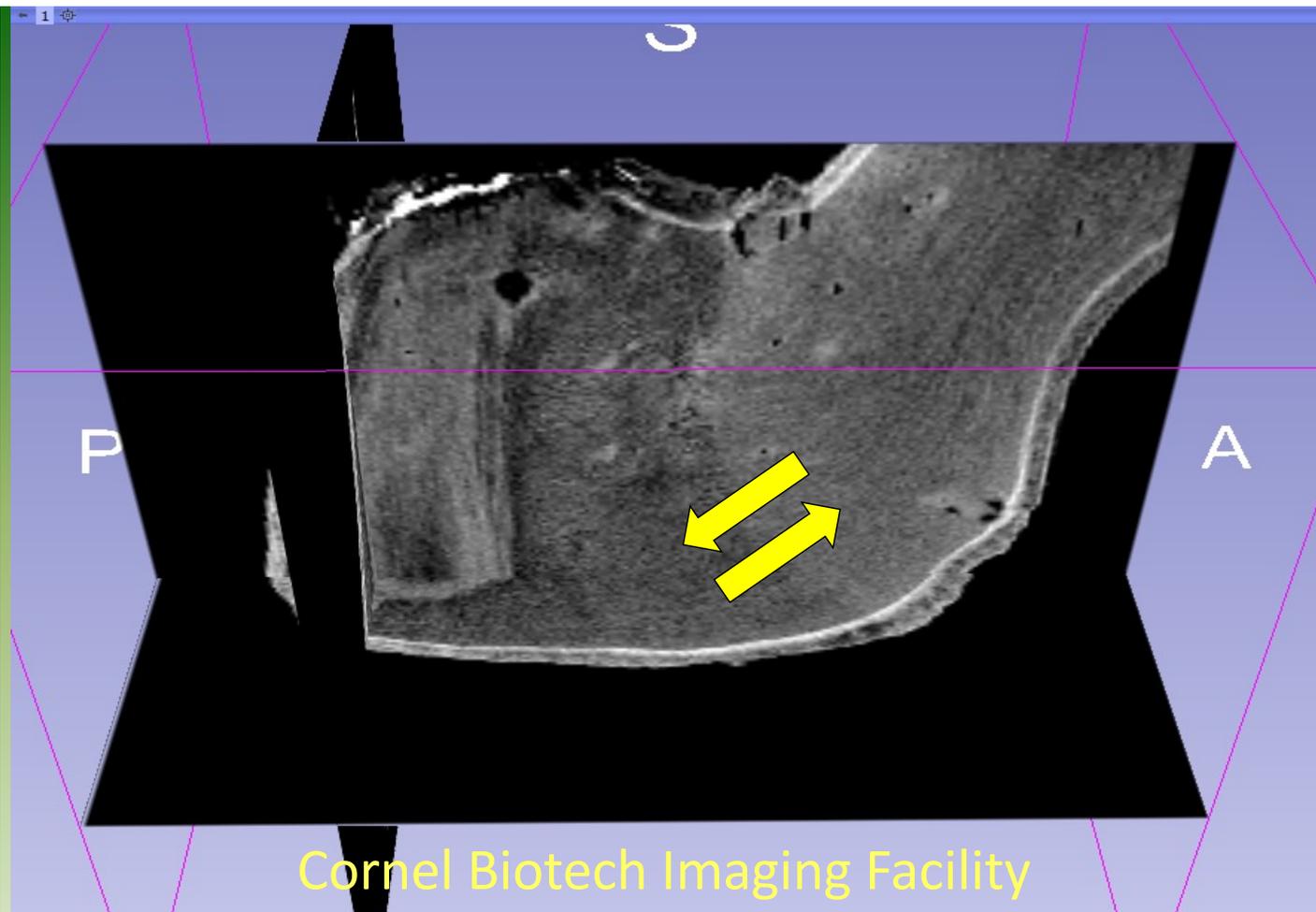
HORTSCIENCE · <https://doi.org/10.21273/HORTSCI15492-20>

Performance of Semi-dwarf Apple Rootstocks in Two-dimensional Training Systems

Nicola Dallabetta, Andrea Guerra, and Jonathan Pasqualini
FEM-IASMA, Technology Transfer Center, San Michele a/A, TN, Italy

Gennaro Fazio
U.S. Department of Agriculture Agricultural Research Service, Plant Genetics Resources Unit, Cornell AgriTech, Geneva, NY 14456; and Horticulture Section, School of Integrative Plant Sciences, Cornell AgriTech, Cornell University, Geneva, NY 14456

Additional index words. fruit size, fruit quality, yield efficiency, planar training system, mechanical pruning, crop value



Cornel Biotech Imaging Facility



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Examples of root(stock) induced traits on the scion

- Dwarfing and Early Bearing of Scion
- Branch angle modification and increased branching
- Increased flowering and bud break in low chill environments
- Hormone balances through the graft union
- Mineral nutrient concentration in the scion
- Increased whole tree tolerance to fire blight

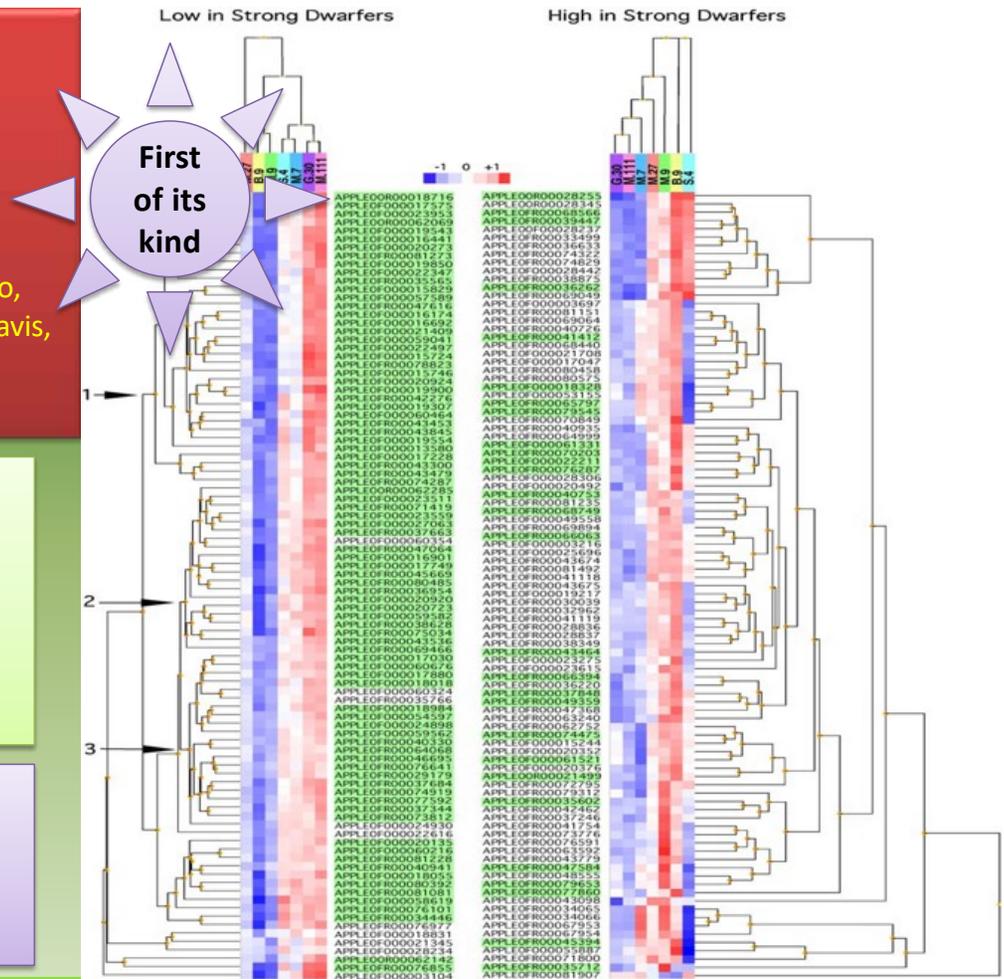


Rootstock-regulated gene expression patterns in apple tree scions

Philip J. Jensen, Izabela Makalowska, Naomi Altman, Gennaro Fazio, Craig Praul, Siela N. Maximova, Robert M. Crassweller, James W. Travis, Timothy W. McNellis
Tree Genetics & Genomes (2010) 6:57–72

• How do different root(stock) genotypes communicate with scion tissues such that they modify global and specific gene expression of the same scion in a unique way?

- Same Gala Scion
- Based on Nimblegen Apple EST Chip
- 26,020 unigene contigs
- 60 mer oligonucleotides
- 3-6x internal replication



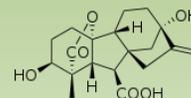
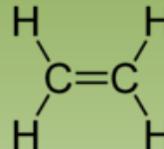
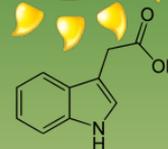
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How Do Rootstocks Affect Fruit Quality?

- Changes in tree architecture cause different exposure to the sun.
- Changes in water availability
- Changes in nutrient availability
- Changes in phytohormone status
- And more....



K

P

Fe

Cu

Ca

Zn

Na

Mo

Mn

Mg



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CRISIS CLIMÁTICA · Informe de la Organización Meteorológica Mundial

La concentración de gases de efecto invernadero alcanza un nuevo récord

Los niveles de CO₂ llegaron a las 407,8 partes por millón en 2018, una cifra nunca antes vista. Los expertos advierten de que seguirá aumentando "con efectos cada vez más graves"



El secretario general de la OMM, Petteri Taalas, presenta el informe sobre el calentamiento global. FABRICE COFFRINI AFP

La concentración de gases de efecto invernadero en la atmósfera ha alcanzado un nuevo récord histórico. Los científicos, además, advierten de que el incremento está

25. nov. 2019

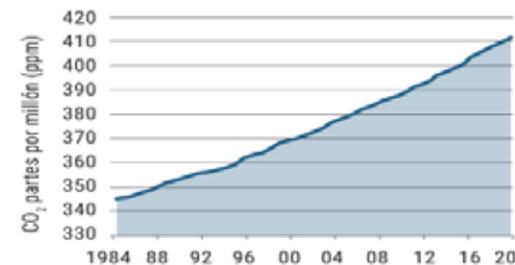
La cifra es la más elevada desde que existen mediciones y, según estiman los expertos, equivale a la concentración de CO₂ que se dio en la Tierra hace entre tres y cinco millones de años, un dato que se obtiene al analizar las burbujas de aire que quedan atrapadas en el hielo.

"En ese entonces, la temperatura era de 2 a 3 grados centígrados más cálida y el nivel del mar, entre 10 y 20 metros superior al actual", indica el secretario general de la OMM, Petteri Taalas.

Además, "hay una diferencia fundamental", advierte Emilio Cuevas, director del Observatorio Atmosférico de Izaña (AEMET), uno de los puntos desde los que se miden las concentraciones de gases. "Esas variaciones que había antes se producían de forma natural a lo largo de miles de millones de años, pero los cambios que estamos viendo ahora mismo se están produciendo en décadas".

AUMENTO DE DIÓXIDO DE CARBONO

Tendencia interanual (+1,9 ppm/año)



FUENTE: Aemet.

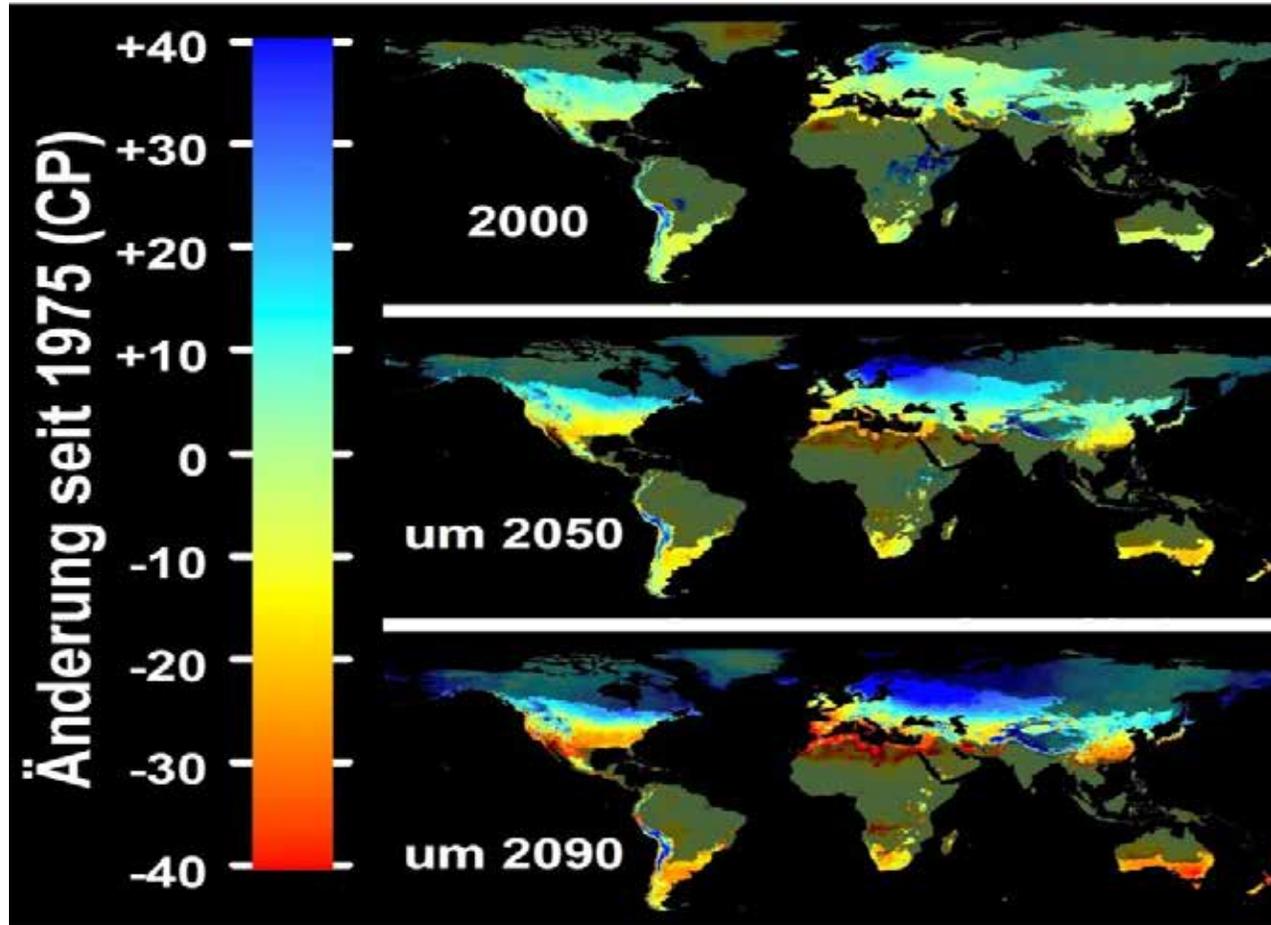


Dr. Ignasi Iglesias

Prediction of lack of winter chill worldwide



Dr. Ignasi Iglesias



Luedeling, Blanke et al. 2013:
Int J Biometeorology 57, 679f

Impact on chilling hours

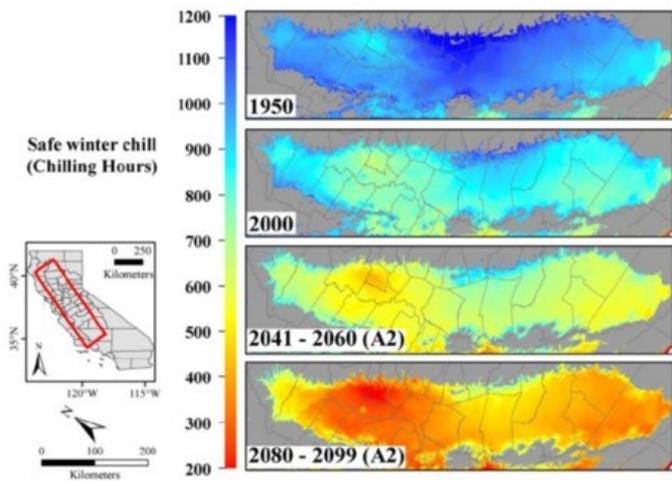
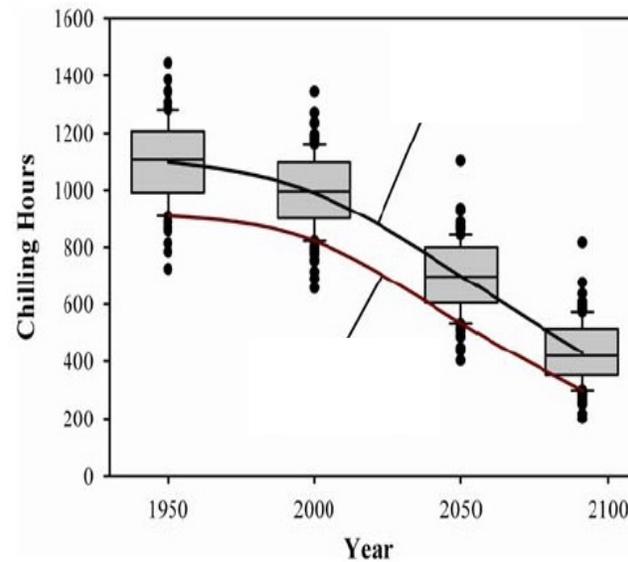


Figure 3. Safe winter chill in California's Central Valley in 1950, 2000, 2041-2060 and 2080-2099, calculated with the Chilling Hours Model. Future winter chill was quantified using the A2 IPCC greenhouse gas emissions scenario. doi:10.1371/journal.pone.0006166.g003



Dr. Ignasi Iglesias

OPEN ACCESS Freely available online

PLoS one

Climatic Changes Lead to Declining Winter Chill for Fruit and Nut Trees in California during 1950–2099

Eike Luedeling^{1,2*}, Minghua Zhang^{1*}, Evan H. Girvetz³

Some Geneva® apple rootstocks show increased productivity in low chill environments by causing more floral and vegetative buds to break. What is the signal that lowers the endodormancy requirement of high chill scion varieties?



ISHS Acta Horticulturae 1228: XI International Symposium on Integrating Canopy, Rootstock and Environmental Physiology in Orchard Systems

Chilling requirement and budburst uniformity of cultivar 'Maxi Gala' grafted on different rootstocks

Authors: T.A. Macedo, G.F. Sander, M.F. Michelon, J.F. Carminatti, A.R. Rufato, L. Rufato, T.L. Robinson
Keywords: *Malus domestica* B., chill hours, CG series, Marubakaido, interstem
DOI: [10.17680/ActaHortic.2018.1228.36](https://doi.org/10.17680/ActaHortic.2018.1228.36)



Scientia Horticulturae
Volume 256, 15 October 2019, 108651



Productivity and quality of 'Fuji Suprema' apple fruit in different rootstocks and growing conditions

Tiago Afonso de Macedo ^a, Pricila Santos da Silva ^b, Guilherme Fontanella Sander ^b, Juliana Fátima Welter ^b, Leo Rufato ^c, Andrea de Rossi ^d

Low Chilling Comparison between Gala grafted on G.213 and M.9 in Brazil (2014)



Work of Dr. Tiago Afonso de Macedo & Dr. Rufato

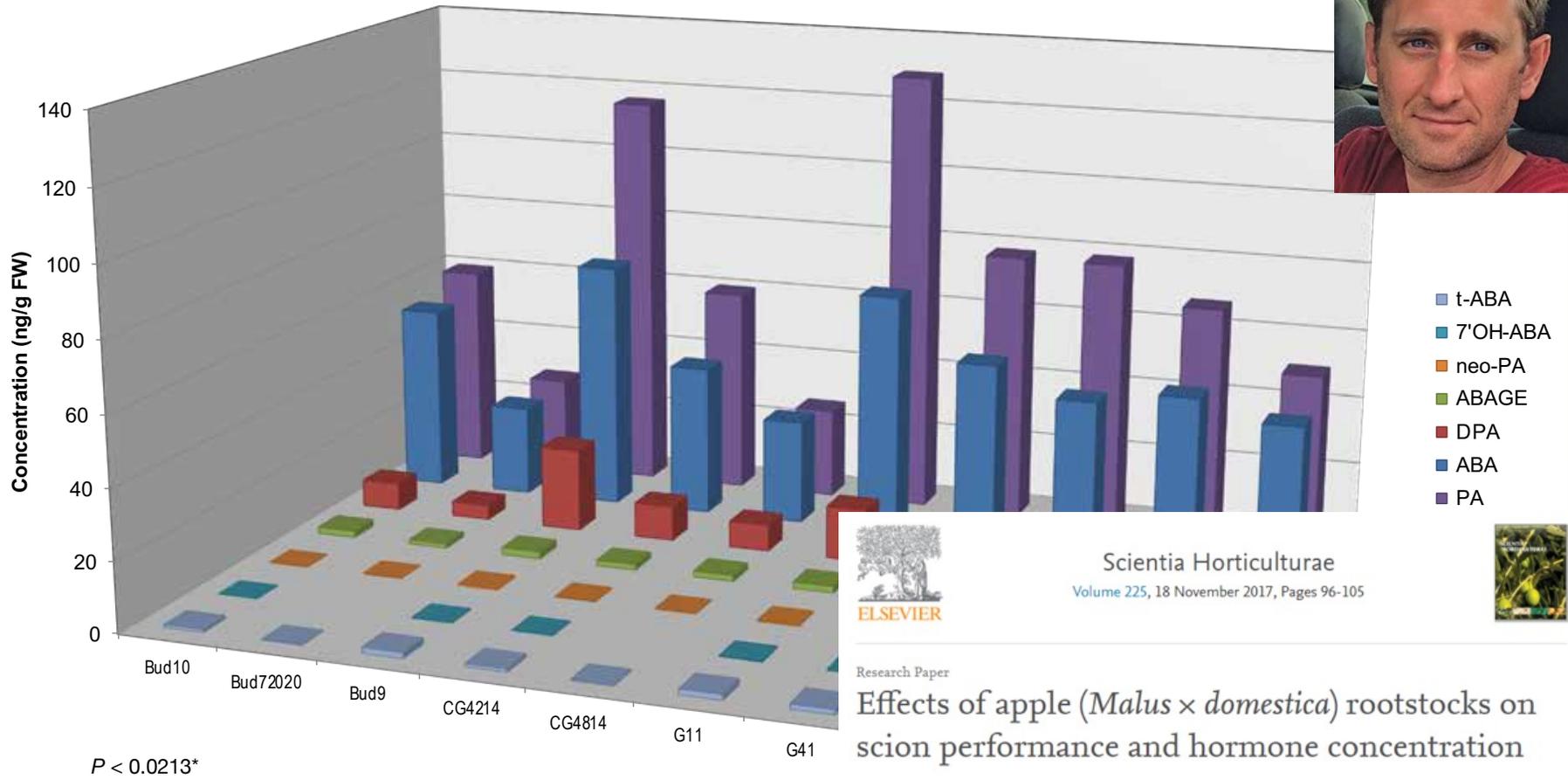


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ABA and ABA catabolites content in apple tree xylem tissue under different rootstocks



Scientia Horticulturae
Volume 225, 18 November 2017, Pages 96-105



Research Paper

Effects of apple (*Malus × domestica*) rootstocks on scion performance and hormone concentration

J. Lordan ^a, G. Fazio ^{a, b}, P. Francescato ^a, T. Robinson ^a



G. 41

**Courtesy Betsy Beers
WSU Wenatchee**



M.9



**United States Department of Agriculture
Agricultural Research Service**

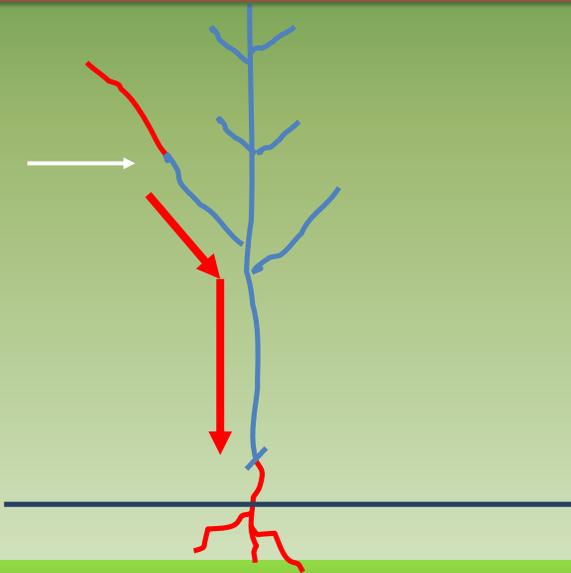
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The Rootstock Phase of Fire Blight is a Major Problem:

- Infection of susceptible rootstocks results in the death of the tree.
- Blight can enter the tree through blooms, mechanical wounds and insect wounds (burr knots, suckers)
- Fire Blight bacteria can travel from the top of the tree to the rootstock in less than 2 weeks.

Infection site
– Bloom or
Shoot

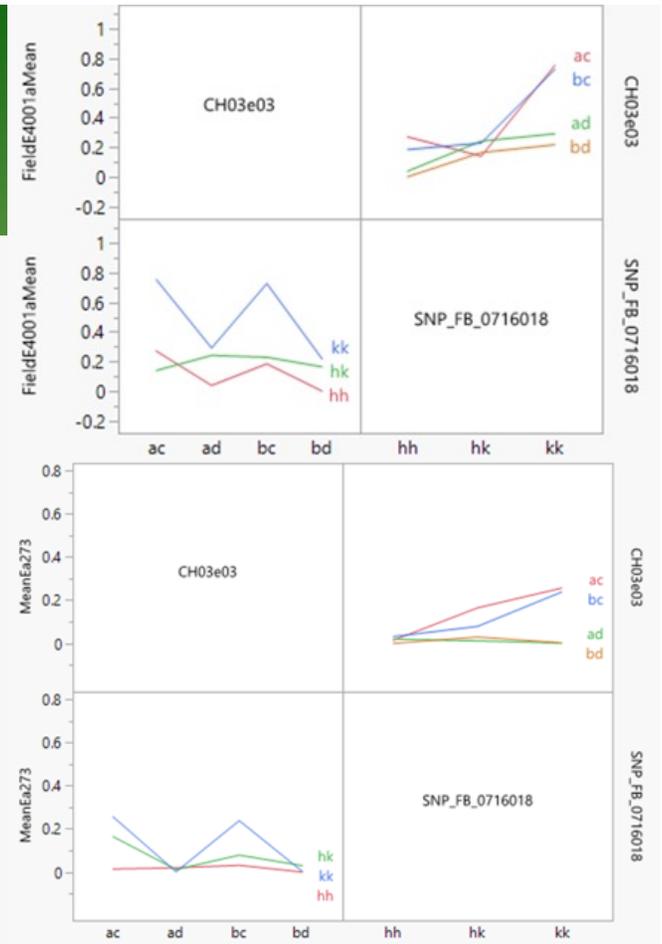
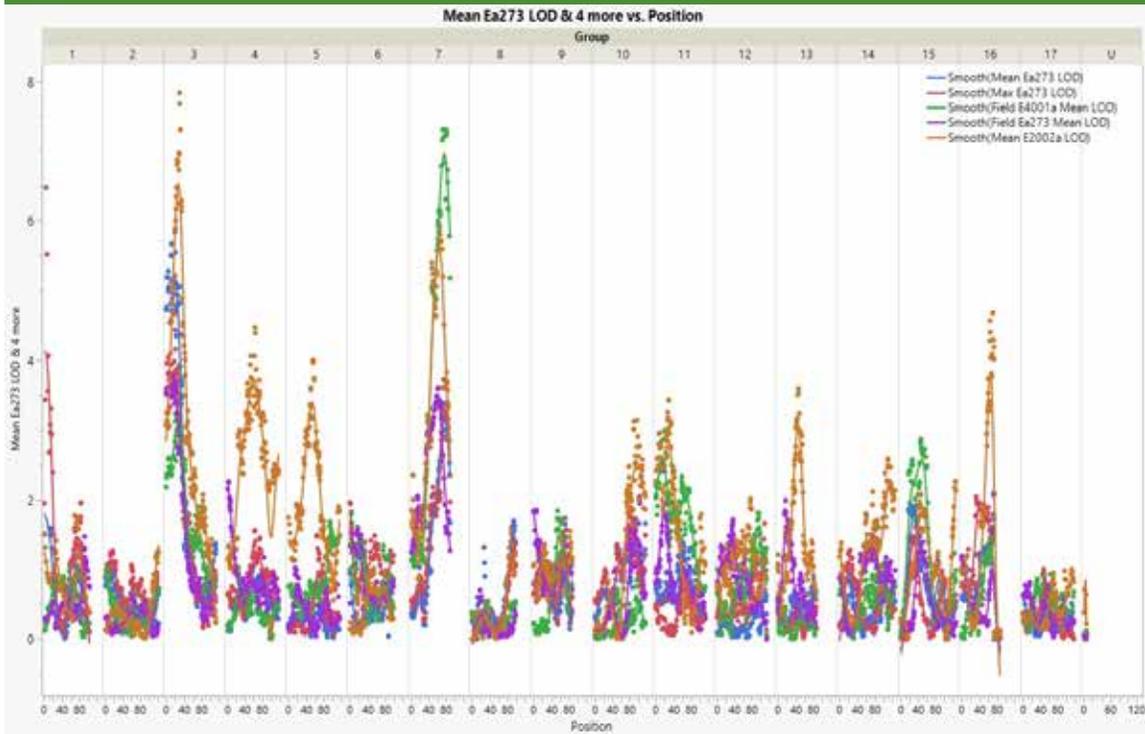


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QTLs for fire blight resistance on same population – different strains and years



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Targeted Metabolic Profiling Indicates Apple Rootstock Genotype-Specific Differences in Primary and Secondary Metabolite Production and Validate Quantitative Contribution From Vegetative Growth

Rachel Leisso^{1*}, Dave Rudell² and Mark Mazzola³

¹Montana State University Western Agriculture Research Center, Corvallis, MT, United States, ²Physiology and Pathology of Tree Fruits Research, Agricultural Research Service (ARS), United States Department of Agriculture (USDA), Wenatchee, WA, United States

Previous reports regarding rhizodeposits from apple roots are limited, and complicated by microbes, which readily colonize root systems and contribute to modify rhizodeposit metabolite composition. This study delineates methods for collection of apple rhizodeposits under axenic conditions, indicates rootstock genotype-specific differences

OPEN ACCESS

Edited by:
Philip Zedler

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journal homepage: www.elsevier.com/locate/soilbio



Metabolic composition of apple rootstock rhizodeposits differs in a genotype-specific manner and affects growth of subsequent plantings

Rachel Leisso¹, David Rudell, Mark Mazzola

USDA-ARS Tree Fruit Research Laboratory, 1104 N. Western Avenue, Wenatchee, WA 98801, United States



ARTICLE INFO

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ABSTRACT

Apple replant disease (ARD) negatively impacts apple tree health and reduces crop yield in new orchards established on sites previously grown to the same or related species. Use of tolerant rootstock genotypes can diminish the growth limiting effects of ARD, and while current research characterizes differential root gene expression by ARD tolerance among genotypes, the potential role of genotype-specific rhizodeposits contributing to ARD tolerance has not been intensively examined. A Q-TOF LC/MS metabolic profiling approach targeting phenolic compounds was used to characterize water-soluble phenolic rhizodeposit metabolites collected from water percolated through the rhizosphere of apple rootstocks planted in pasteurized quartz sand. Four rootstock genotypes (two with ARD field tolerance, G935 and

ARTICLE

Genotype-specific suppression of multiple defense pathways in apple root during infection by *Pythium ultimum*

Yanmin Zhu¹, Jonathan Shao², Zhe Zhou³ and Robert E. Davis²

Open Access



Abstract

The genotype-specific defense activation in the roots of perennial tree crops to soilborne necrotrophic pathogens remains largely unknown. A recent phenotyping study indicated that the apple rootstock genotypes B9 and G935 have contrasting resistance responses to infection by *Pythium ultimum*. In the current study, a comparative transcriptome analysis by Illumina Solexa HiSeq 3000 platform was carried out to identify the global transcriptional regulation networks between the susceptible B9 and the resistant G935 to *P. ultimum* infection. Thirty-six libraries were sequenced to cover three timepoints after pathogen inoculation, with three biological replicates for each sample. The transcriptomes in the roots of the susceptible genotype B9 were reflected by overrepresented differentially expressed genes (DEGs) with downregulated patterns and systematic suppression of cellular processes at 48 h post inoculation (hpi). In contrast, DEGs with annotated functions, such as kinase receptors, MAPK signaling, JA biosynthesis enzymes, transcription factors, and transporters, were readily induced at 24 hpi and continued up-regulation at 48 hpi in G935 roots. The earlier and stronger defense activation is likely associated with an effective inhibition of necrosis progression in G935 roots. Lack of effector-triggered immunity or existence of a susceptibility gene could contribute to the severely disturbed transcriptome and susceptibility in B9 roots. The identified DEGs constitute a valuable resource for hypothesis-driven studies to elucidate the resistance/tolerance mechanisms in apple roots and validating their potential association with resistance traits.

Transcriptomic analysis of molecular responses in *Malus domestica* 'M26' roots affected by apple replant disease

Stefan Weiß¹, Melanie Bartsch¹, Traud Winkelmann¹

Received: 13 August 2016 / Accepted: 23 March 2017 / Published online: 19 April 2017
© Springer Science+Business Media Dordrecht 2017

Abstract

Key message Gene expression studies in roots of apple replant disease affected plants suggested defense reactions towards biotic stress to occur which did not lead to adequate responses to the biotic stressors.
Abstract Apple replant disease (ARD) leads to growth inhibition and fruit yield reduction in replanted populations and results in economic losses for tree nurseries and fruit producers. The etiology is not well understood on a molecular level and causal agents show a great diversity indicating that no definitive cause, which applies to the majority of cases, has been found out yet. Hence, it is pivotal to gain a better understanding of the molecular and physiological reactions of the plant when affected by ARD and later to overcome the disease, for example by developing tolerant

upregulated whereas for several genes, metabolism lower expression was the verification of MACE1 data, candidate via RT-qPCR and a strong positive both datasets was observed. Comparing roots cultivated in ARD soil or y-triald guests that typical defense reactions to take place in ARD affected plants but responding to the biotic stressors attack to the observed growth depressions in a
Keywords Biotic stress response · Gm Growth depression · MACE1 · Phytoalexin Quantitative real-time PCR



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**Color,
Firmness,
Maturity,
Sugar, Size
are
affected
by Apple
Rootstocks**



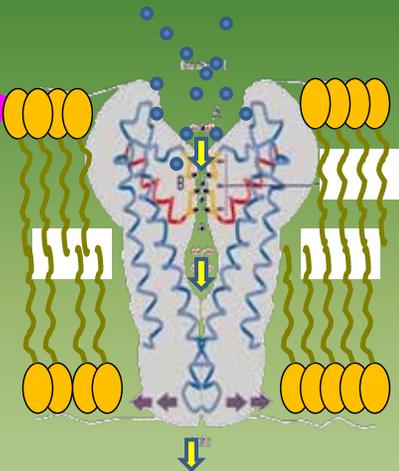
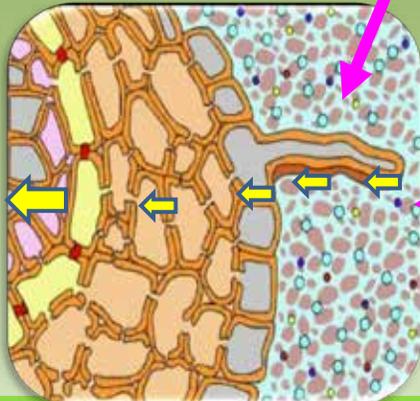
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Journey of nutrients from soil to scion

Different sinks competing for same resources



Different apple rootstocks genotypes may possess different absorbance and/or transport efficiencies.



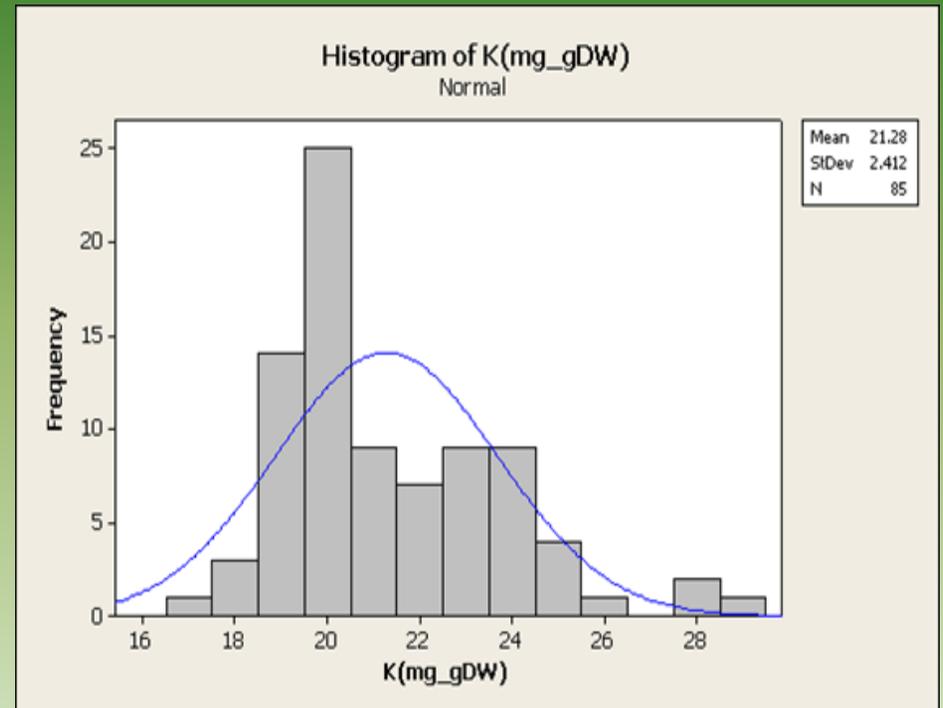
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What sparked our interest in root mediated ionomics (nutrient uptake)?

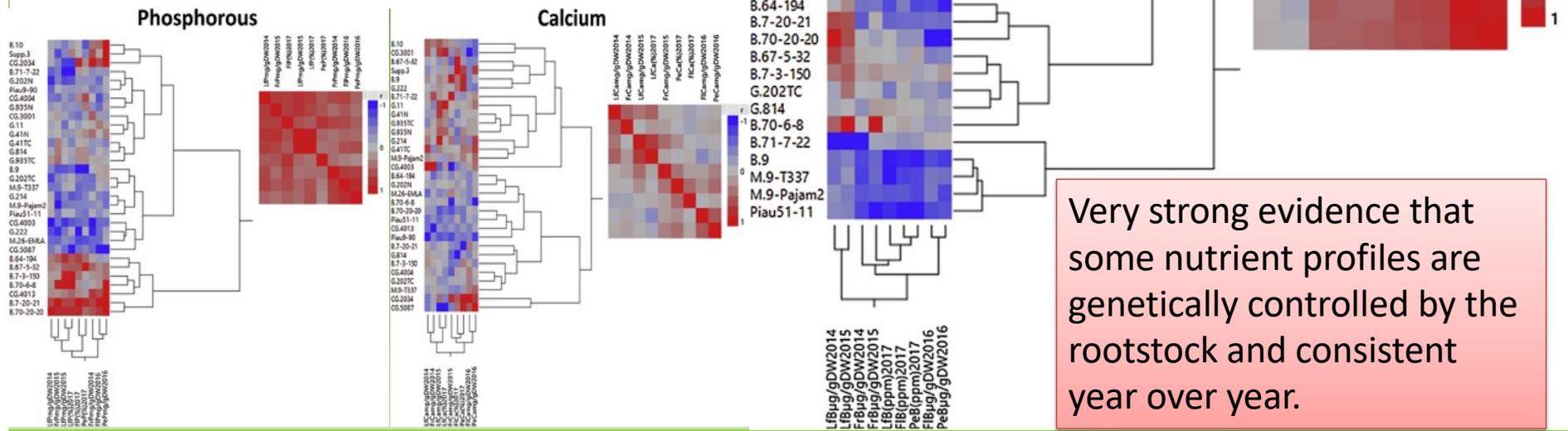
- Apple rootstocks response/interaction to a range of soil conditions (pH, water, soil borne diseases, soil type)
- Possibility to mitigate fruit disorders associated with nutrient deficiencies (calcium-bitterpit)
- Possibility to improve efficiency of fertilizer applications

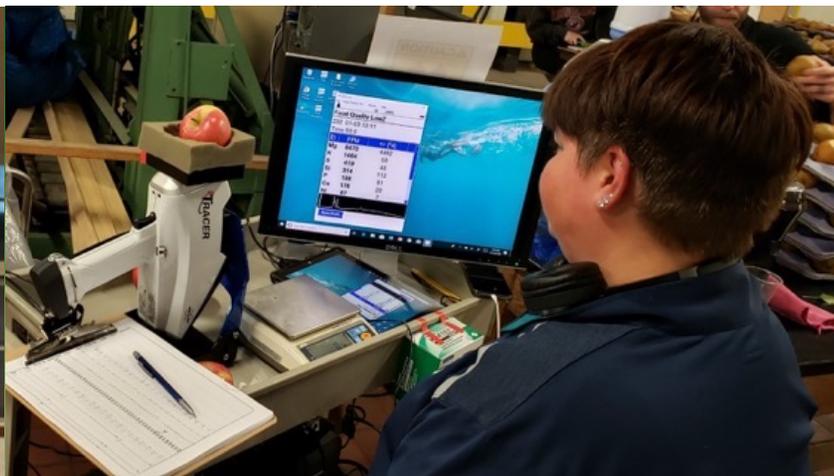




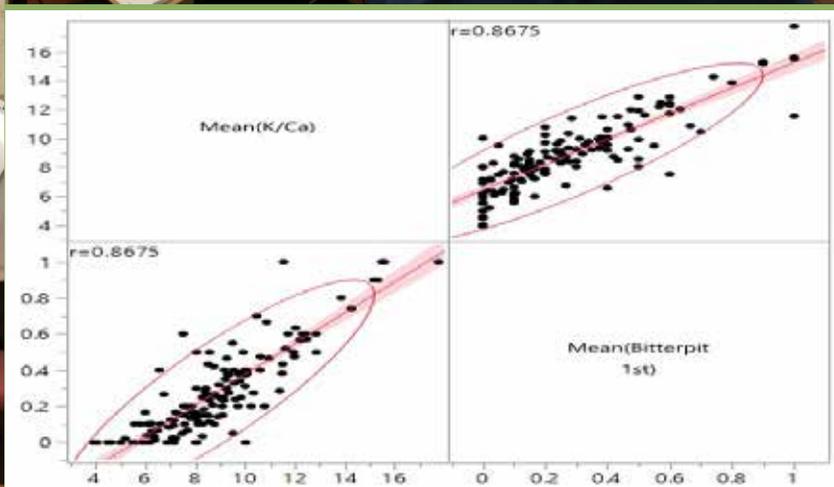
I. Mineral nutrient profiles and relationships of 'Honeycrisp' grown on a genetically diverse set of rootstocks under Western New York climatic conditions

Gennaro Fazio ^{a, b, ✉}, Jaume Lordan ^{b, c}, Michael A. Grusak ^d, Poliana Francescato ^b, Terence L. Robinson ^b





Bitter Pit of Honeycrisp apples is affected by many variables including some that are modulated by rootstocks: Nutrient Balance (IONOMICS) involving K, N, Mg, and Ca. Crop load, and some responses to climate.



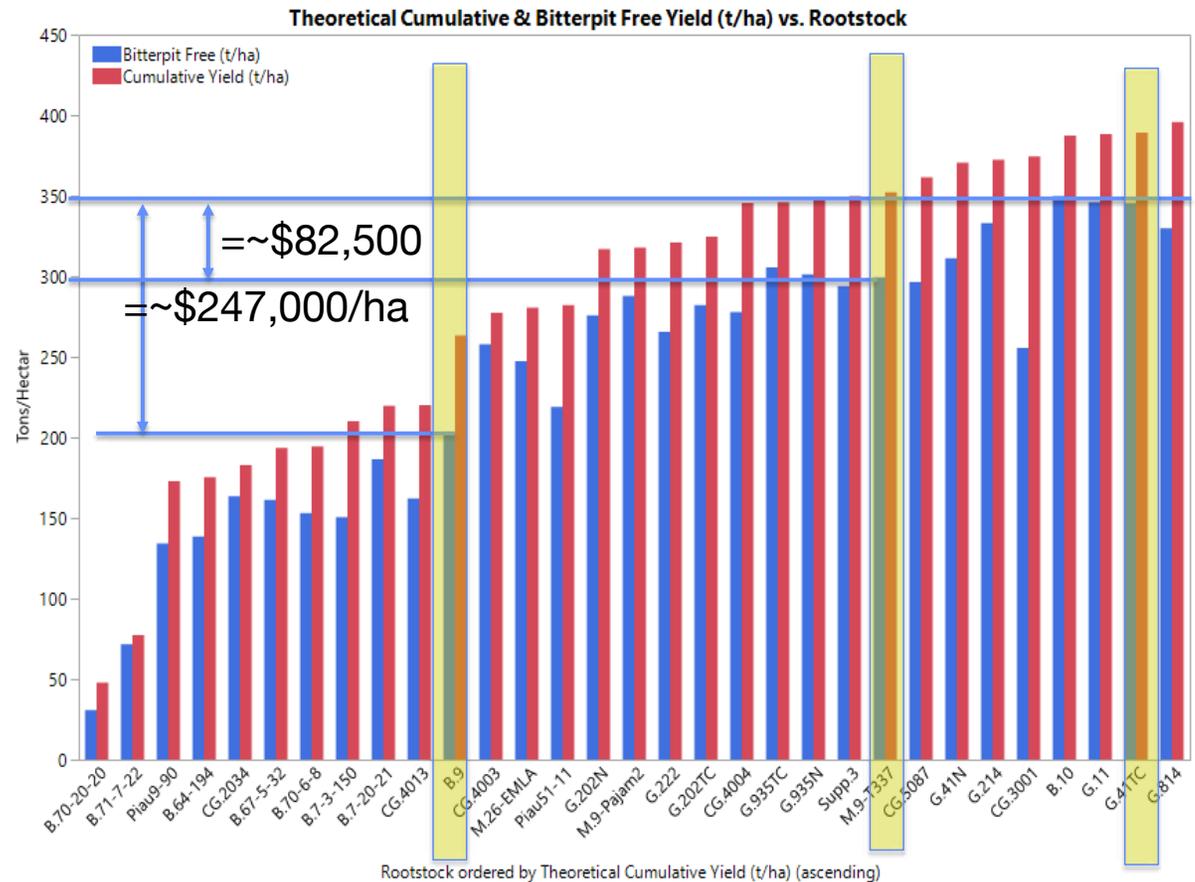
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Economic effects of rootstock influenced bitter pit and yield potential

- Rootstocks affect cumulative yield of the orchard
- Rootstocks affect the K/Ca ratio which in turn affects the percentage of bitter pit free apple yield
- The combination of the two can result in significant money \$\$\$ gained or lost.



Phenotypic Diversity and QTL Mapping of Absorption and Translocation of Nutrients by Apple Rootstocks

By GENNARO FAZIO¹, DARIUS KVIKLYS², MICHAEL A GRUSAK³
 and TERENCE ROBINSON⁴

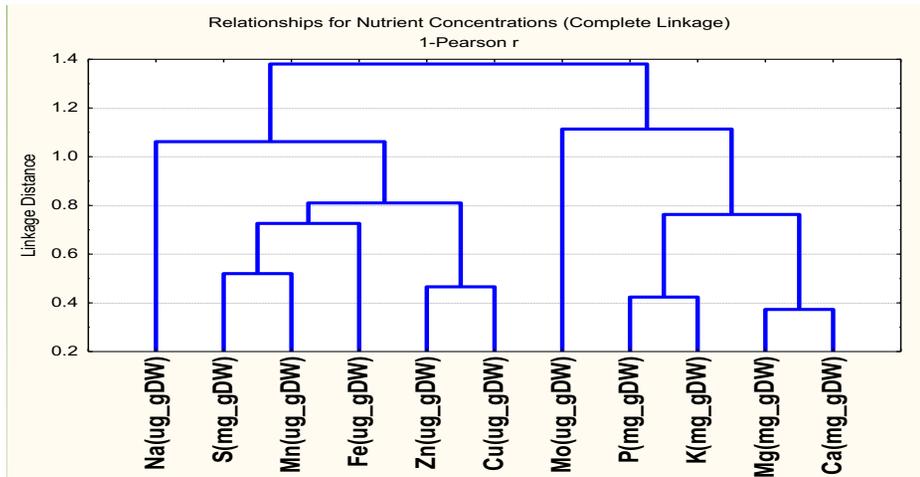
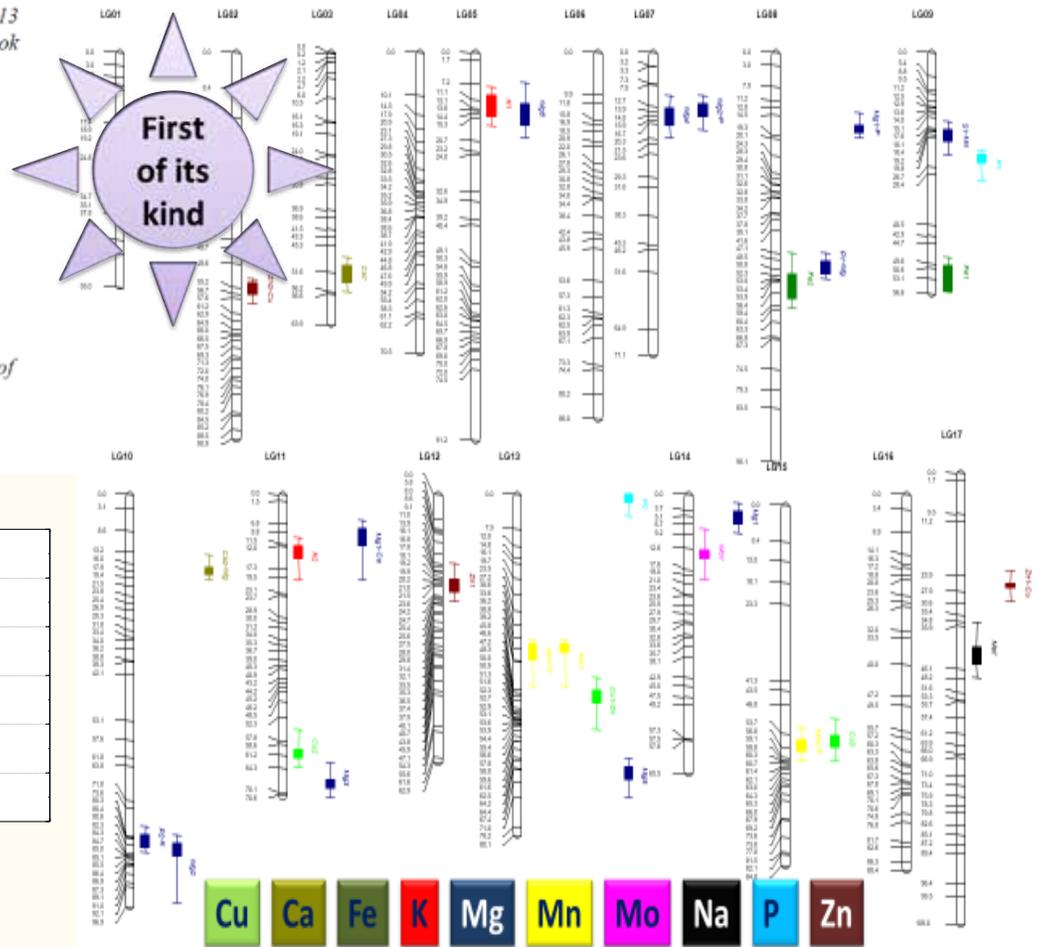
¹Plant Genetics Resources Unit, USDA-ARS, Geneva, NY

²Institute of Horticulture, Lithuanian Research Center for Agriculture and Forestry, Bабтай, Lithuania

³USDA-ARS Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, Texas

⁴Dept of Horticultural Sciences, NYSAES, Cornell University, Geneva, New York

Corresponding Author Email: gennaro.fazio@ars.usda.gov

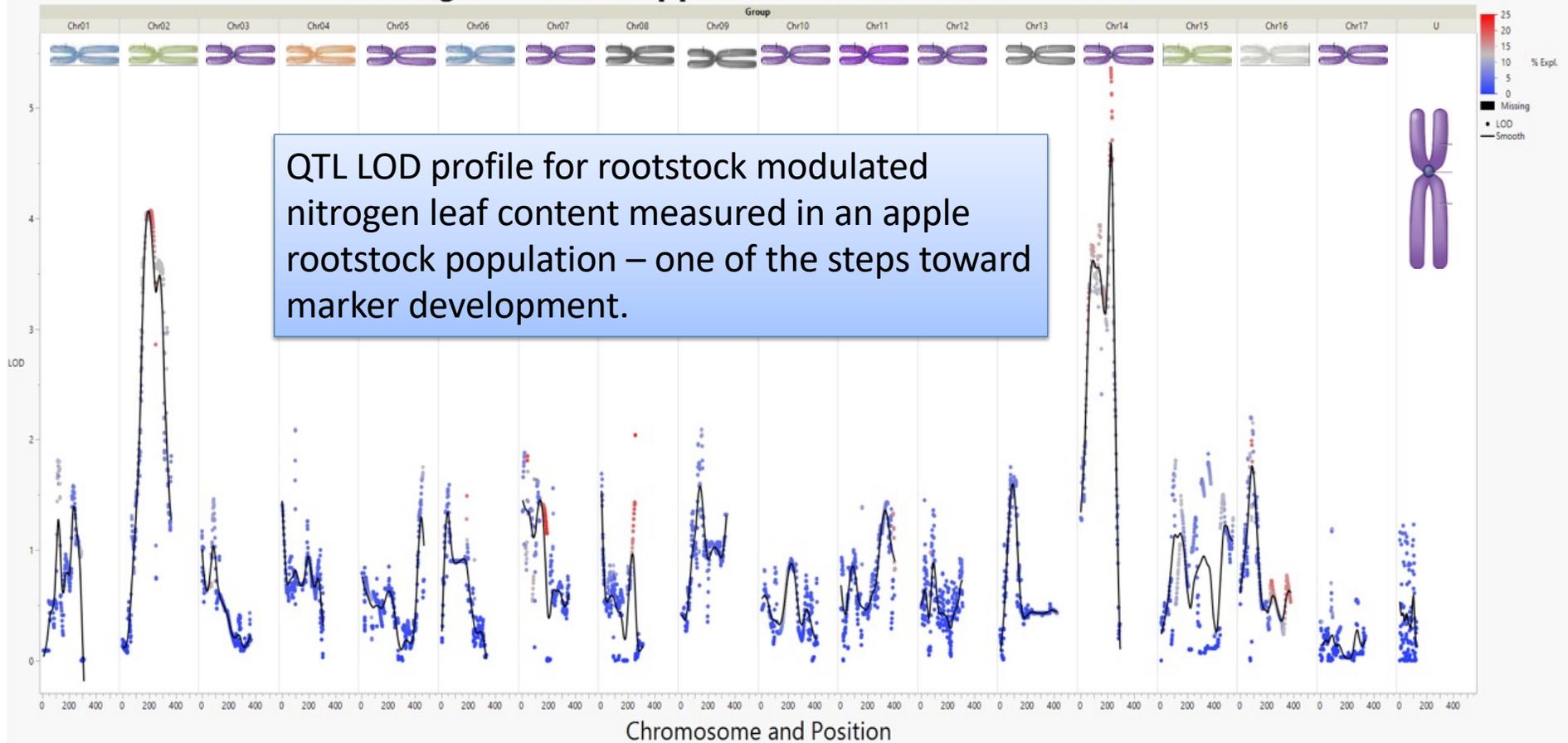


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Nitrogen QTLs in Apple Rootstock Chromosomes



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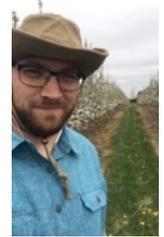


Geneva 213 and Geneva 214 at Cameron Nursery in Eltopia, WA



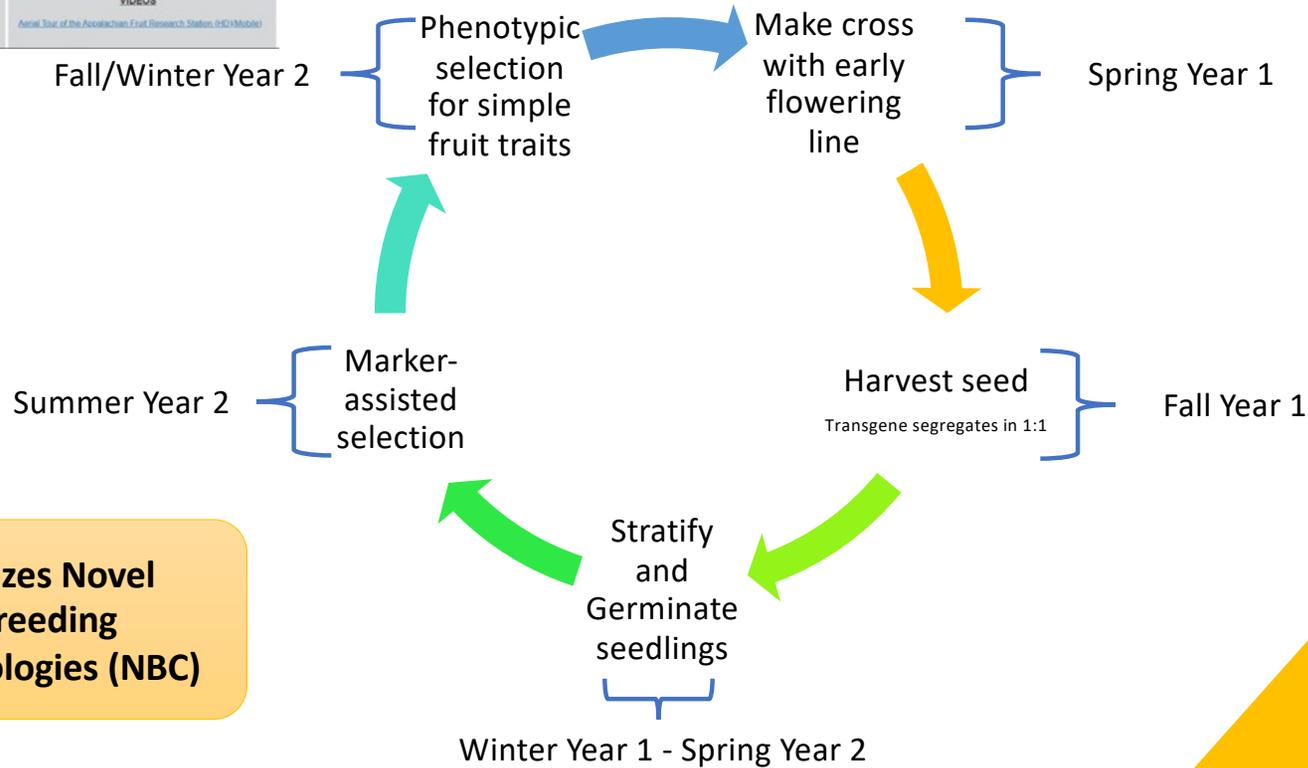


AFRS Rapid cycle breeding system timeline



Dr. Chris Gottschalk

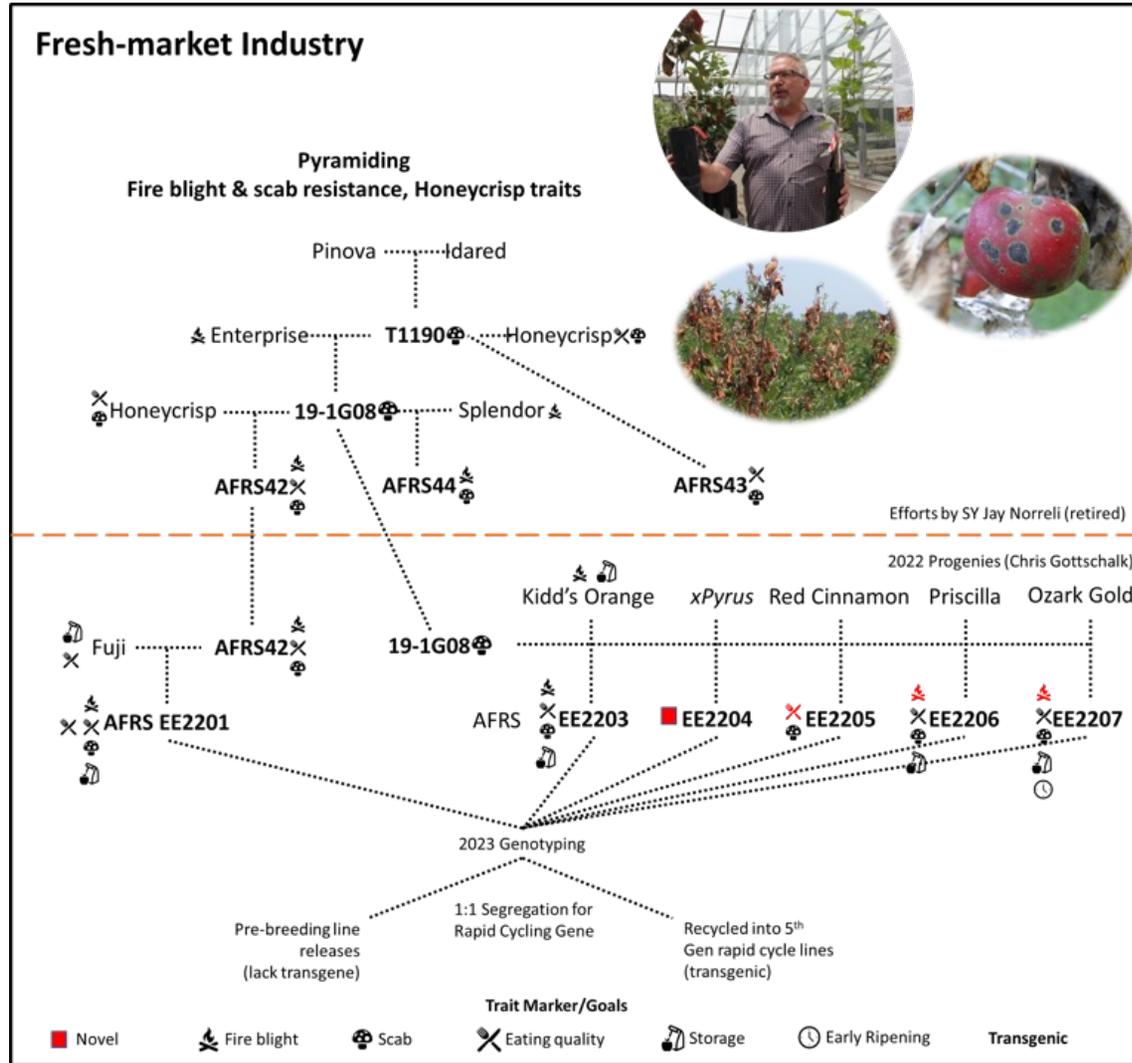
Utilizes Novel Breeding Technologies (NBC)





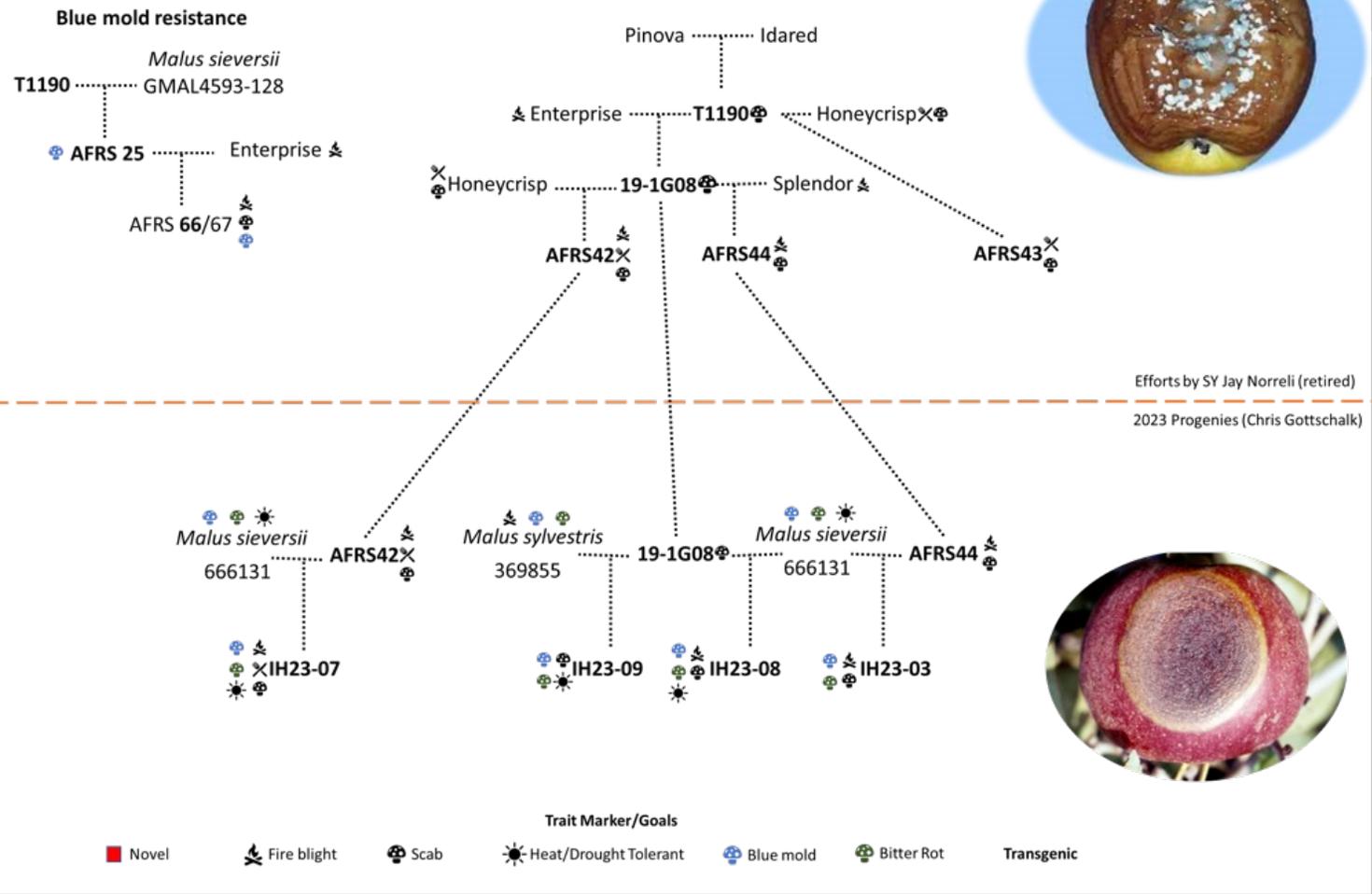
Dr. Chris Gottschalk

Current progress

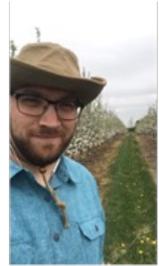


New directions for post-harvest rot resistance

Post-Harvest Rot Resistance Breeding

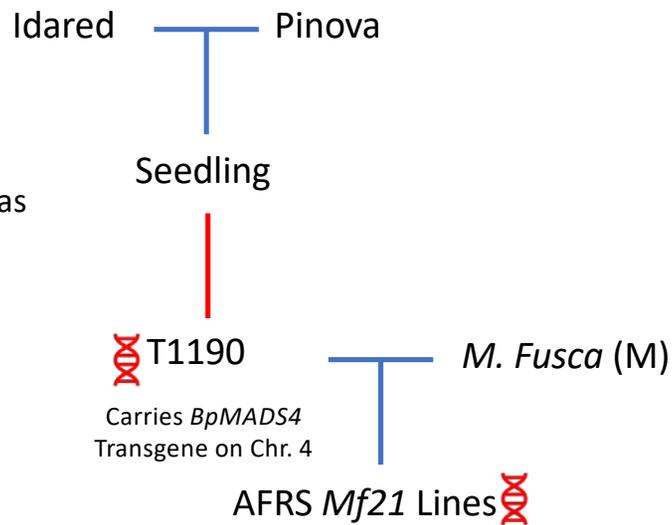


Introgression Breeding Using Rapid Cycle



Dr. Chris
Gottschalk

Allows us to target novel genetics such as *Mfu10* loci for fire blight resistance.



Use of a TFL RNAi Cassette Screen for Seedless Traits



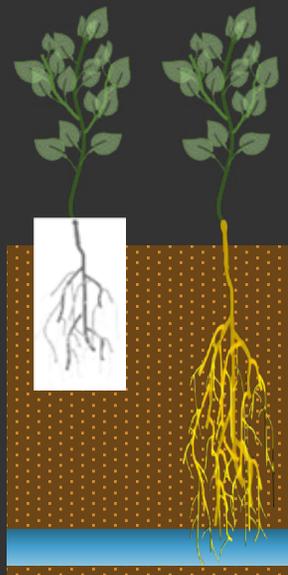
Dr. Chris Dardick



<u>Construct</u>	<u># of Lines/Plants</u>	<u>Flowering</u>	<u>Non-flowering</u>	<u>Seeds</u>
Seedless1	5/26	0%	100%	?
Seedless2	4/19	0%	100%	?
Seedless3	9/45	0%	100%	?
TFL RNAi	9/43	35%	65%	No
Seedless1 + TFL RNAi	6/34	76%	24%	No
Seedless2 + TFL RNAi	7/30	53%	47%	No
Seedless3 + TFL RNAi	7/62	50%	50%	No

*Charrier, A., Vergne, E., Dousset, N., Richer, A., Petiteau, A., & Chevreau, E. (2019). Efficient Targeted Mutagenesis in Apple and First Time Edition of Pear Using the CRISPR-Cas9 System. *Frontiers in plant science*, 10, 40.

Enhancing drought resilience in apple through *DRO1*?



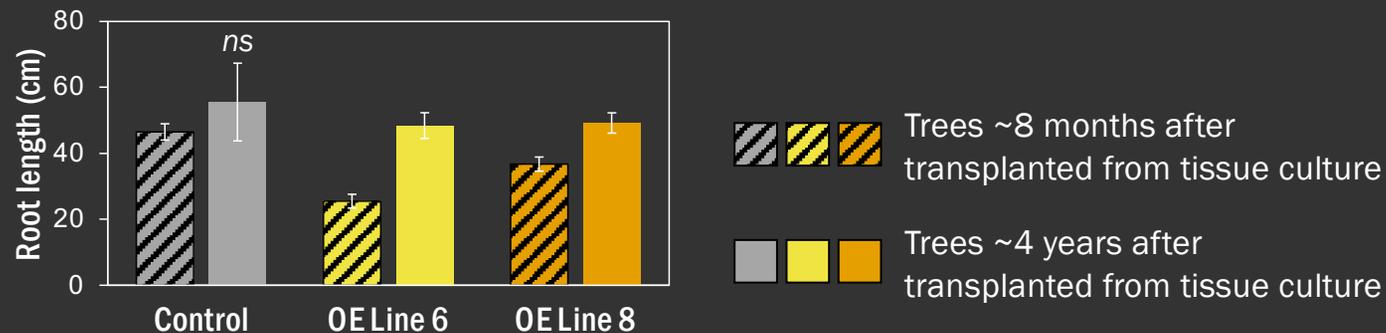
- Hypothesis:
Deeper rooting of 'M.26' apple due to *DEEPER ROOTING 1* (*DRO1*) overexpression allows water access in deeper soil domains and hence better drought tolerance (avoidance)



Dr. Lisa Tang

Root morphology of apple overexpressing *PpeDRO1*

- At 8 months after transplanted, roots of both *DRO1* OE lines were **shorter** than non-transgenic control
- For 4-yr-old trees, *no* significance difference in root length or root biomass between transgenic OE lines and control



- But drought tolerance in apple was altered by *DRO1* overexpression

Apple saplings overexpressing *PpeDRO1* under drought

- 3-month saplings after no water for 4 weeks:



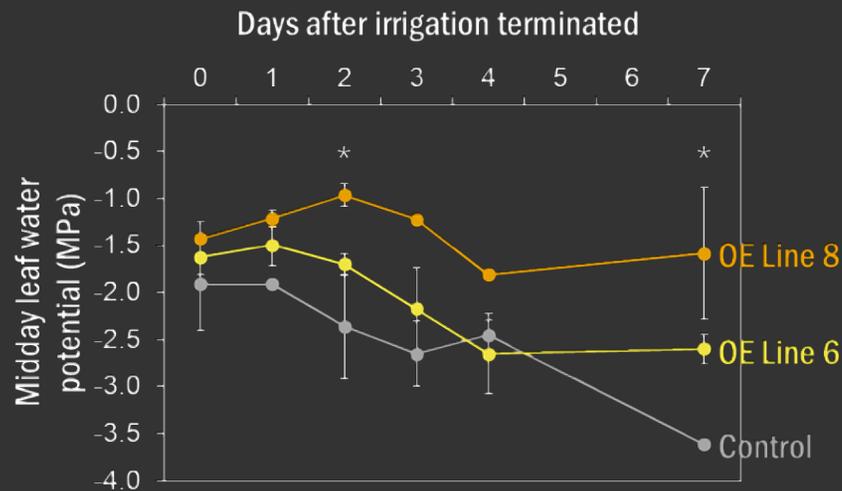
▲
Non-transgenic control ▶



▲
◀ *PpeDRO1* OE line

Young apple trees overexpressing *PpeDR01* under drought

- 4-year-old non-nearing trees, after no water for 7 days:
Both *DR01* OE Lines were **less stressed** than non-transgenic control



→ Investigation of the mechanism underlying increased drought tolerance by *DR01* OE is underway

Aeroponics Systems to study apple roots

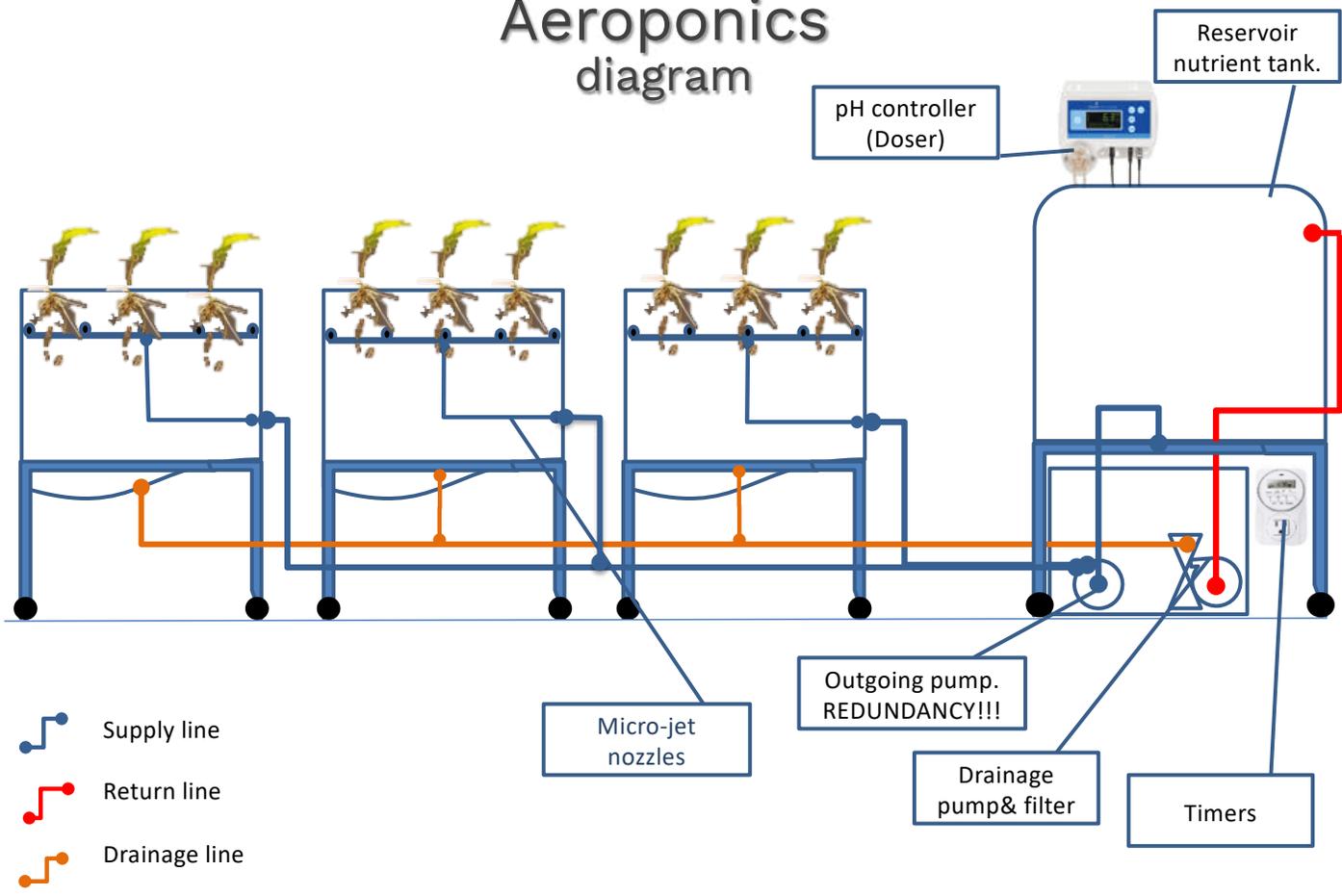


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Aeroponics diagram



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Apple root phenotypic diversity in aeroponics

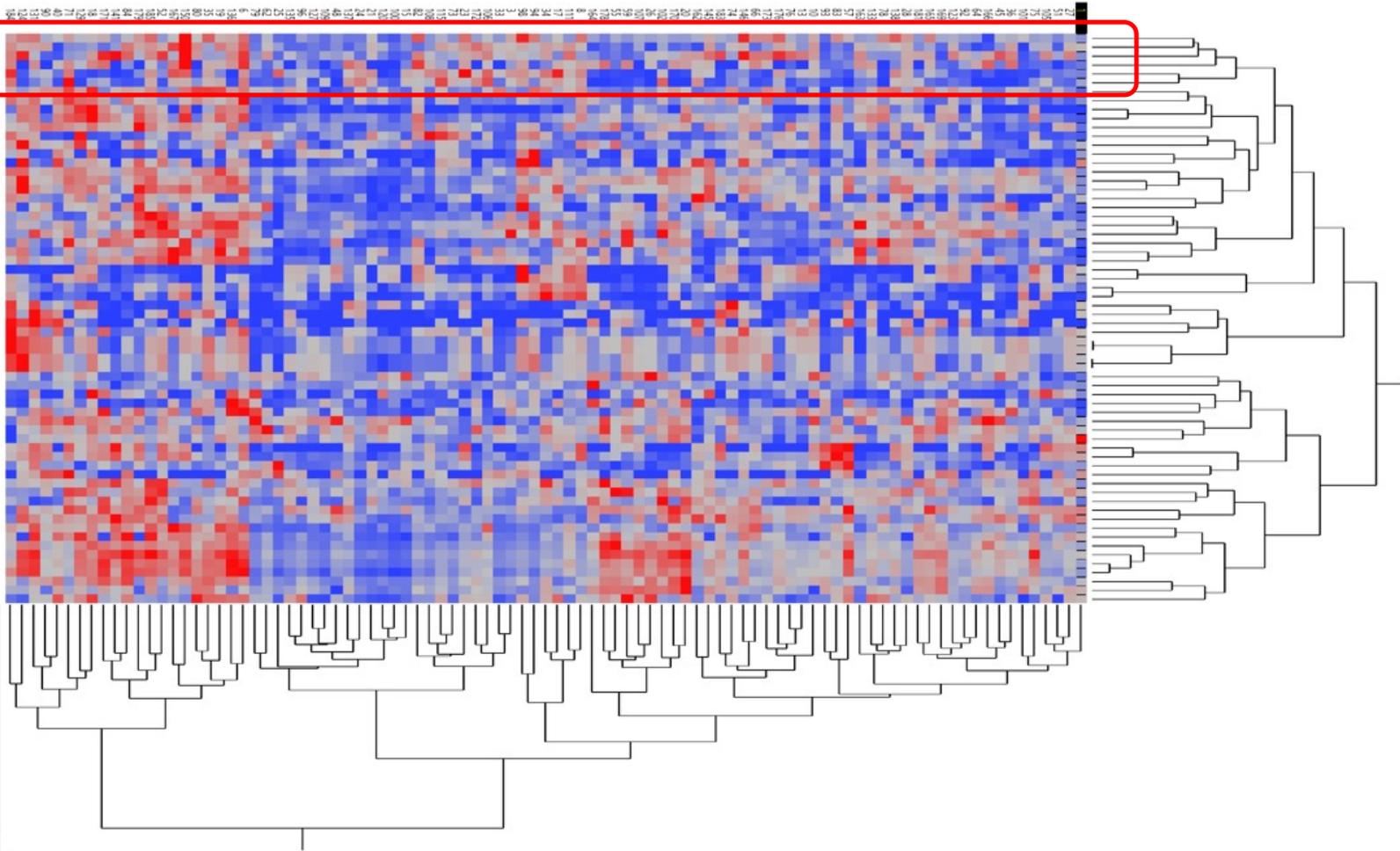


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PhloridzinS10
 procyanidin_11_23 2
 4_methyl_catechol 2
 caffeic 2
 benzoic 2
 reynoutrin 2
 quinic 2
 4-p_coumaroylquinic 2
 chlorogenicDimer 2
 chlorogenic 2
 STM3 2
 4-hydroxybenzoic 2
 p_coumaric 2
 STM13_2
 kaempferol_3_gluc 2
 vanillic 2
 flava_coumary 2
 STM14_2
 kaempferol_2_gluc 2
 ericofuran1 2
 flava1 2
 flava2 2
 TCtriterprenoid471 2
 pomonic 2
 annurcoic 2
 TC-triterprenoid_487 2
 flavaglycosideUnk3 2
 flavaglycosideUnk4 2
 phloretin_phloridzin 2
 STM_43_157_0362 2
 flavaglycosideUnk1 2
 rutin 2
 kaempfer2rutinosi 2
 quercetin 2
 isoquercitrin 2
 hyperinQu_3_OgalHyp 2
 quercitrin 2
 resveratrol 2
 gallic 2
 phloretic 2
 esculin 2
 STM5 2
 noraucuparin 2
 p_thiocyanatophenol 2
 STM43_165_9795 2
 urallenoside 2
 phloroglucinol 2
 Protocatechuic 2
 STM43_169_0880 2
 kaempferol 2
 unknown_161_0467 2
 STM2 2
 gallocateoylacteoside 2
 STM6 2
 flavaglycosideUnk2 2
 catechin 2
 procyanidin_6_55 2
 procyanidin_b2 2
 epicatechin 2
 procyanidinC1Tri 2
 procyanidin_21_48 2
 STM8 2
 STM9 2
 avicularin 2



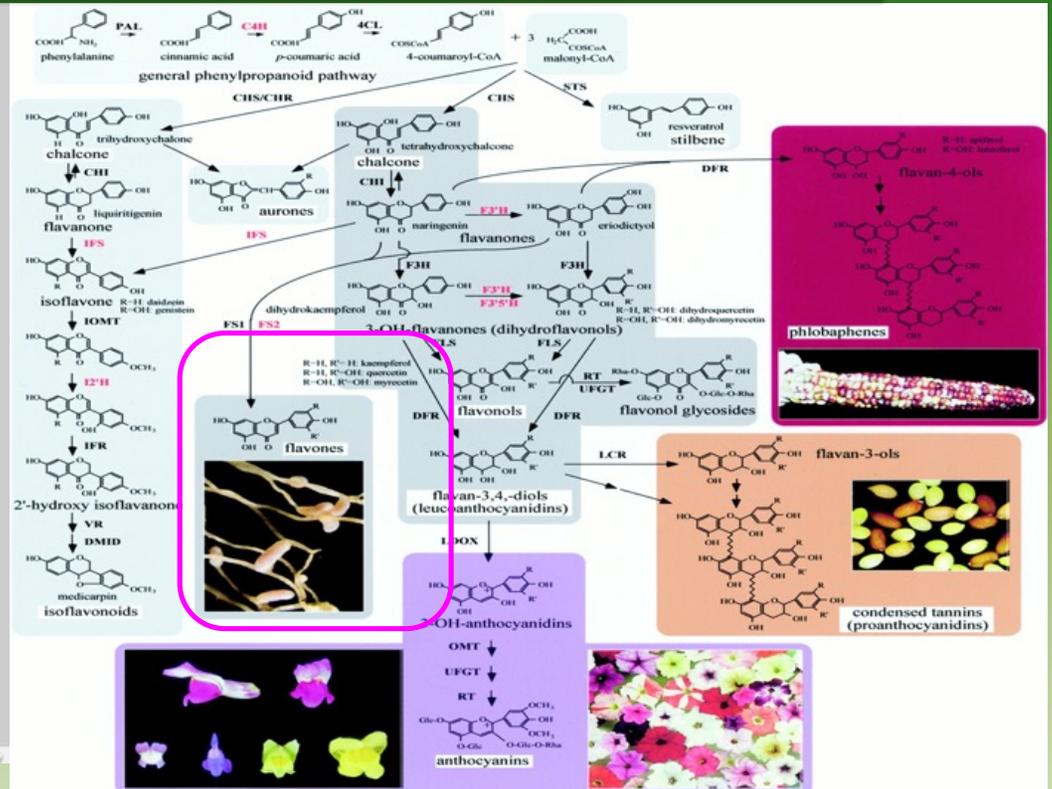
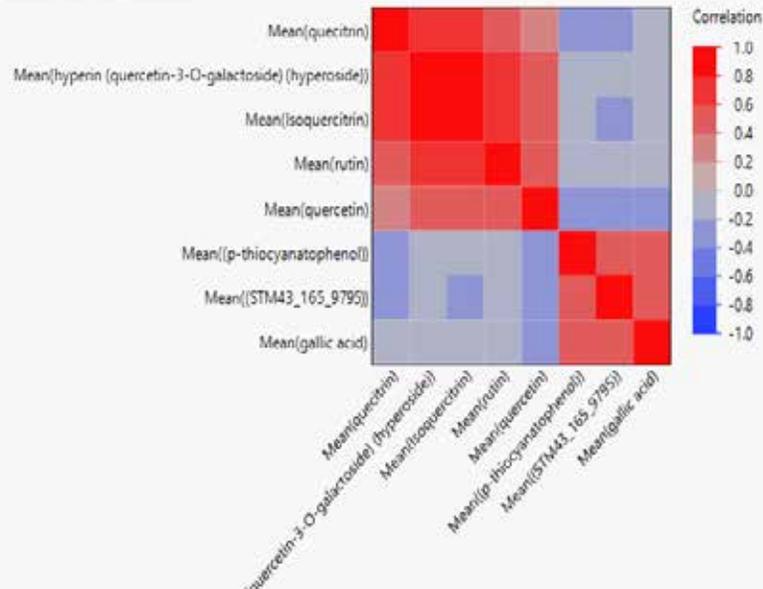
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Root Isoquercitrin relationships and pathways

Cluster the Correlations



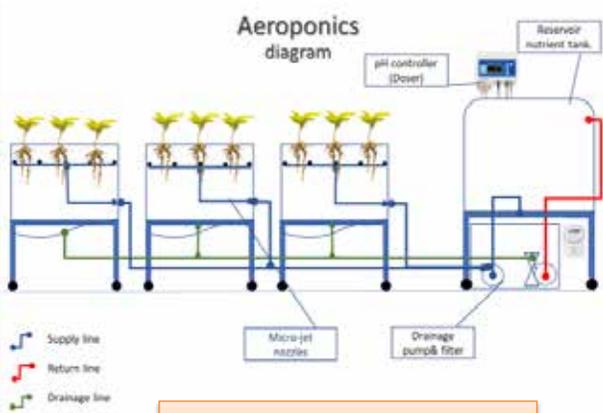
Quercetin 3-O-beta-D-glucofuranoside is a quercetin O-glucoside in which a glucofuranosyl residue is attached at position 3 of quercetin via a beta-glycosidic linkage. It has a role as a metabolite. It is a beta-D-glucoside, a quercetin O-glucoside, a monosaccharide derivative and a tetrahydroxyflavone.

Reaction: $\text{UDP-}\alpha\text{-D-glucose} + \text{quercetin} \rightarrow \text{H}^+ + \text{UDP} + \text{quercetin-3-glucoside}$

Source: <https://pubchem.ncbi.nlm.nih.gov/compound/5484006>

Plant Physiology June 2001 vol. 126 no. 2 485-493

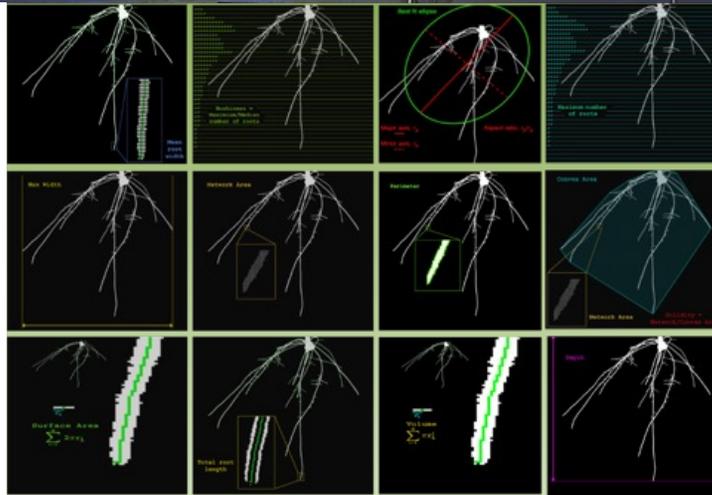




Dr. Ali Farqani PhD work



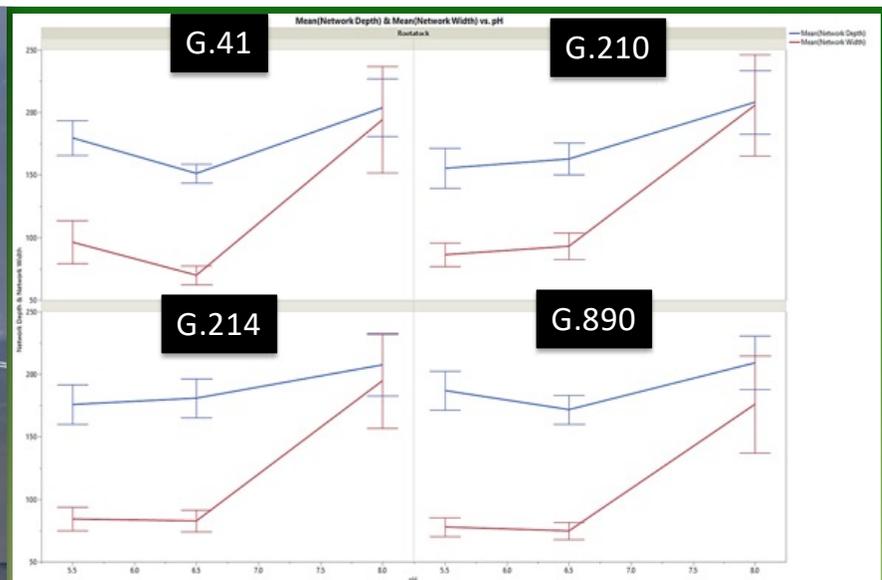
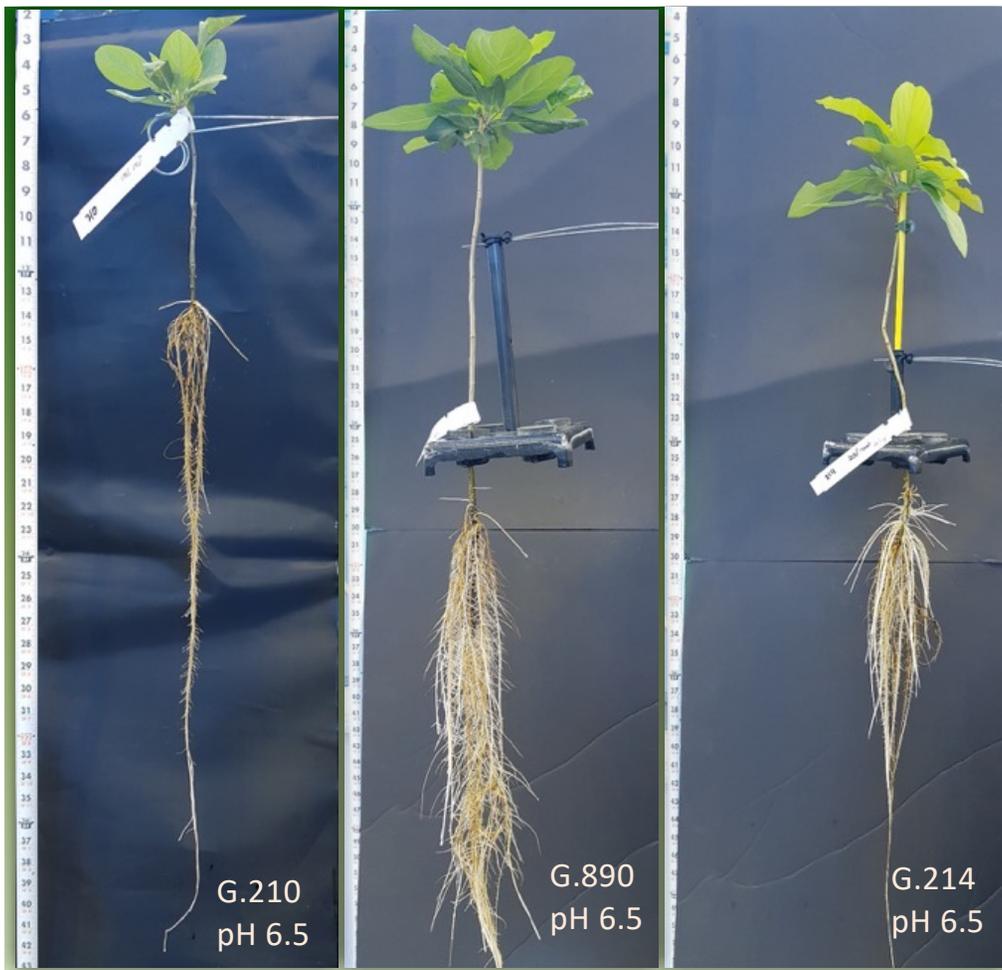
Images processing steps by Gialfoot software from rootstocks (M9, G41, G214, G890, G210) grown in aeroponics with adjusted solution's pH.



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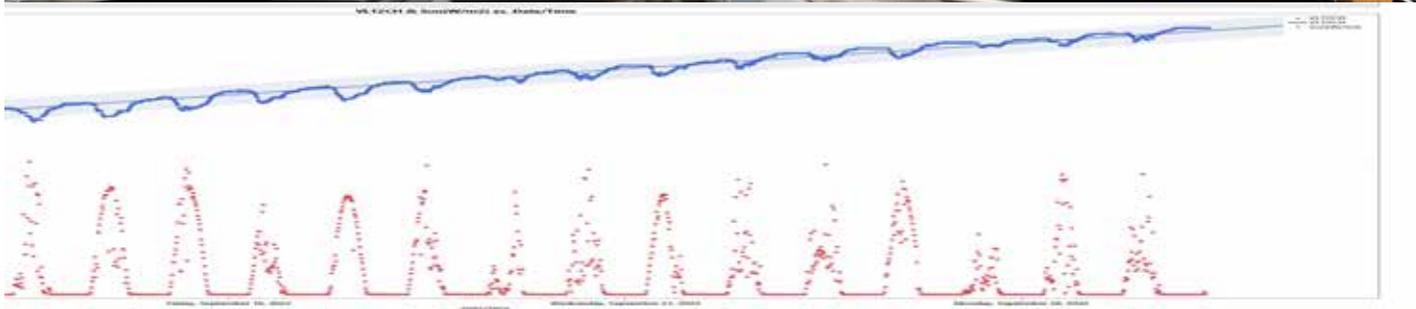
Effect of solution pH on network depth and width in (mm) on four Geneva rootstocks grown in aeroponics system in 2018.



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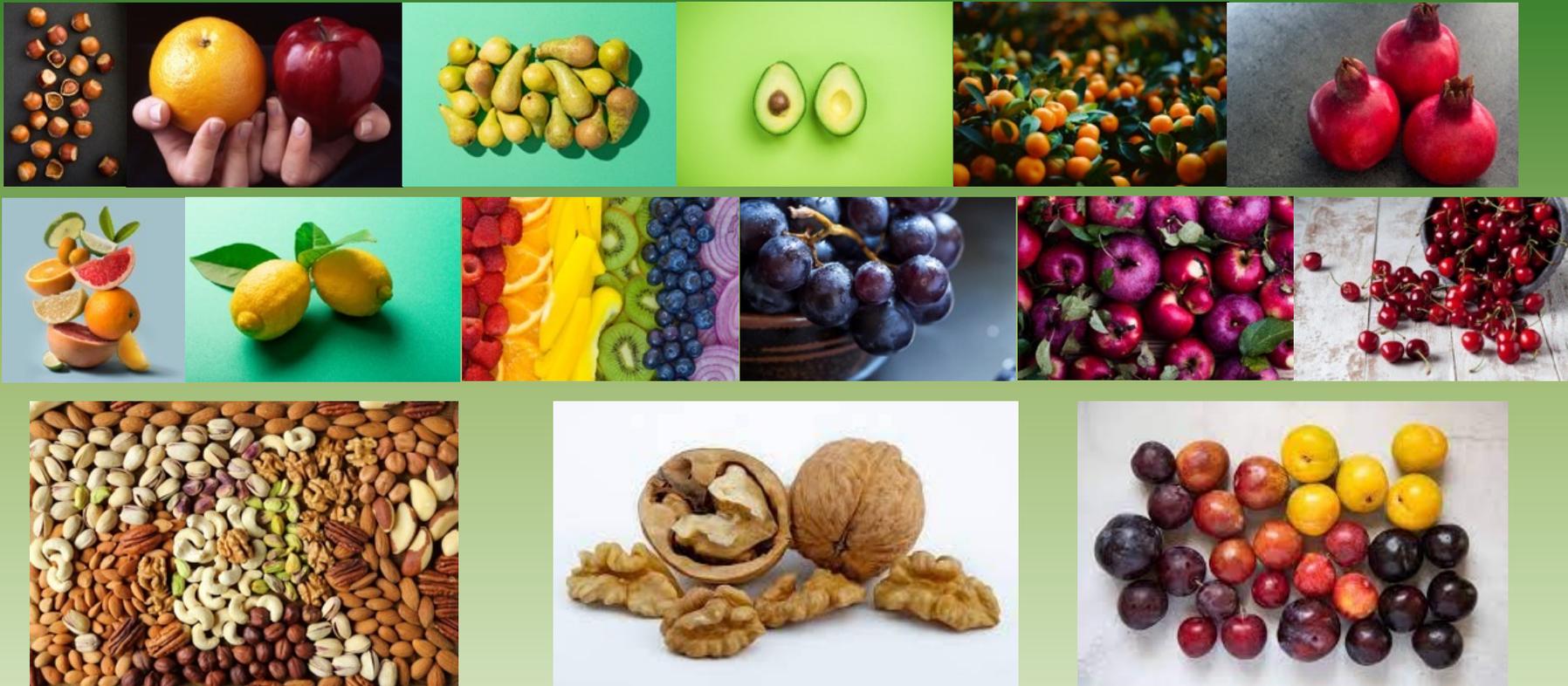


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Is the rootstock revolution limited to Apples?



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Thank You!



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