



Is there useful resistance/tolerance to Huanglongbing within citrus?

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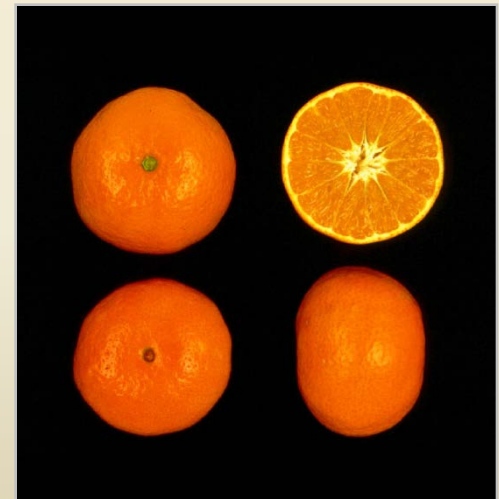
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ARS Citrus Improvement

The oldest citrus breeding program in the world?



W. T. Swingle

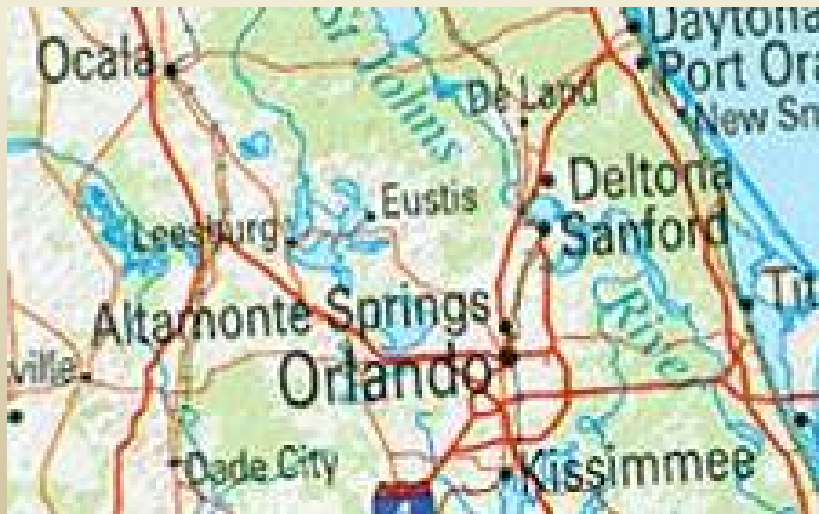


H.J. Webber



**Sub-Tropical Laboratory
Eustis, Fla.**

**Swingle and Webber
1893-1897**



**>75% of US citrus industry has
rootstock and/or scion from USDA
program.... mainly Carrizo/Troyer
and Swingle rootstocks!**

USDA Citrus Scion Breeding Program- more than 100 years of effort

- Orlando Tangelo
- Minneola Tangelo
- Nova Tangelo
- Lee
- Osceola
- Robinson
- Page
- US Early Pride
- Sunburst Tangerine
- Fallglo Mandarin
- Sunstar Orange
- Midsweet Orange
- Gardner Orange
- Flame Grapefruit
- Ambersweet Orange
- US Seedless Pineapple

Breeding program continues to evolve to keep it vital and productive.... for another 100+ years!!

Huanglongbing, AKA Citrus Greening

- First identified in Florida in August 2005, now found in all citrus producing counties
- Estimated that ~40% of FL citrus trees are infected, but some groves no longer productive
- Associated with a bacterium, *Candidatus Liberibacter asiaticus*, vectored by the Asian Citrus Psyllid (in TX, AZ, CA), phloem limited
- Within a few years of infection, many citrus trees become weak, have poor quality fruit, with lots of fruit drop, and trees may die or become useless



Photos Bove, 2006

Reports of Citrus Resistant to HLB

- Numerous reports that sometimes limes, lemons or pummelos are tolerant or resistant to HLB from various parts of the world.
 - Differences between reports may be due to genotypic differences alone or strain x genotype differences
- Fred Gmitter of UF- Has project to ID and evaluate survivors in HLB-ravaged areas of China – material under test

Resistance to HLB in Citrus gene pool?

- Folimonova et al. (Dawson group) tested 30 genotypes for HLB response
- Severity of symptoms varied greatly, with sweet orange in the most sensitive, highest titer group
- *Poncirus trifoliata* and *Citrus latipes* displayed the greatest resistance in symptoms and low/no titer

N USDA Ft Pierce FL Farm with Endemic HLB



Poncirus and hybrids in USHRL variety collection Picos Farm, Ft. Pierce:

- Clear that HLB is present in large majority of USHRL Picos trees
- Poncirus and hybrids are maintained as scions in variety block, all on unifoliate stock, SCS
- Apparent that unifoliate rootstock suckers had much greater HLB symptom expression
- Sampled four quadrants of “scions” plus a diagnostic “worst sample”, and both random and “worst sample” for unifoliate suckers



Sampling of leaves of Poncirus and its hybrids from USHRL variety collection, along with unifoliolate rootstock suckers. **Relative abundance of Las 16s sequence vs. citrus dehydrin** in each sample.

Stover, Shatters, Duan, Hall, McCollum

	Scion N quadrant	Scion E quadrant	Scion S quadrant	Scion W quadrant	Scion most HLB sympt	Rootstock sucker: random	Rootstock sucker: worst
Argentina trifoliolate	0	0	0	0	0	na	na
Flying Dragon trifoliolate	0	0	0	0.4	6.4	5	4.8
Norton Citrange	0	0	0	0	19.6	17.4	19.3
Uvalde Citrange	0	0	0	lost	20.6	7.4	22.7
Willits Citrange	0	17	1.8	9.8	21.3	20.7	20.9
C-35 Citrange	3.1	8.6	9.6	14.8	19.9	19.3	18
Rusk Citrange	3.7	17.8	0	1.2	21.3	16.8	22.7
Cunningham Citrange	20	19	19.7	20.5	19.7	17.8	20.3
Troyer Citrange	20.7	22.3	20.1	3.7	24.1	17.5	22

HLB-resistance from distant citrus and relatives

Lee, Stover, Hall, Keremane, Halburth

- 85 accessions: planted June 2009
- Seed from Riverside repository
- 8 plants each randomized in Ft Pierce
- In this experiment, *Poncirus* most resistant to ACP colonization in citrus gene pool (Westbrook et al.) and was among slowest to develop CLas (Lee et al.)
- New (CRB funded) experiment to look more deeply into more resistant germplasm



Using trifoliolate genes for HLB resistance

- U of Florida (Fred Gmitter), UC Riverside (Roose) and USHRL (Stover) are collaborating on a trial of diverse citranges and other trifoliolate orange hybrids, to identify genes associated with HLB-resistance
- When mapped and identified, can use gene markers in conventional breeding and in intragenics

• Includes near commercial quality, advanced Poncirus hybrids



Replicated trifoliolate hybrid trial



Trees have been exposed to ACP/HLB for 13 months



Gnarlyglo-

[F-5-14-96 (FF-6-10-124 x US-119) X
FF-1-11-7 (FF-5-100-47 x FF-6-15-150)]



Sweet Orange like fruit-
Navel in alligator-hide
Apparent tolerance to HLB
1/16 Poncirus
No off-flavor
Being used in many crosses


Significant Resistance to HLB in conventional cultivars?

Survey in groves with multiple cultivars

Liberibacter per sample by cultivar

Stover & McCollum: HortScience 2011

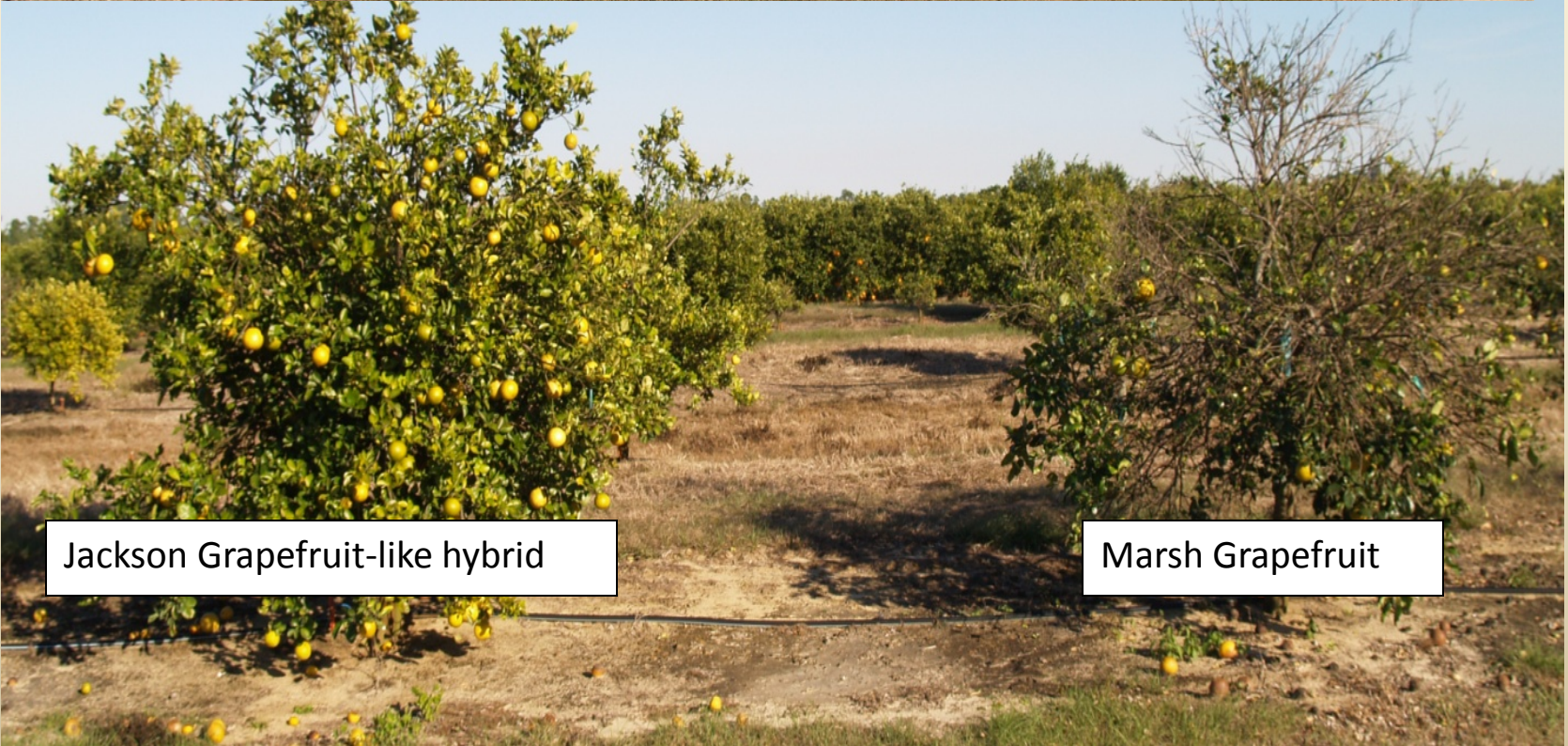
	Mean # CLas genome/sample	% trees “HLB+”
Minneola	304	43%
Murcott	168	44%
Sweet orange	236	31%
Grapefruit	40	20%
Temple	9	15%
Fallglo	13	18%
Sunburst	107	13%



Trial Planting: Grapefruit vs. Near Grapefruit

- Planting established 2003 and 2004 by Jose Chaparro
- Goal was assessment of potential for very early grapefruit for juice and fresh-fruit due to lower bitterness of ‘Triumph’ and ‘Jackson’
- A “discovered experiment” in 2009, as ‘Triumph’ (T) and ‘Jackson’ (J) appeared to be less adversely affected by HLB compared to ‘Marsh’ (M) and ‘Flame’ (F)

Planting in Jan 2010



Two views in May 2012- darker green trees are Triumph and Jackson



May 2012

Typical 'Flame'



Typical 'Triumph'



HLB, Canker and Health all on a 5 point scale such that 1 is healthy and 5 is severely affected

	Health09		HLB09	canker09		Fruit		Fruit	
Cultivar						per tree		drop	
Flame	3.3	b	3.7	3.6	b	62.3	bc	62%	b
Marsh	3.5	b	3.5	3.6	b	42.2	c	69%	b
Jackson	2.1	a	3.6	1.6	a	126.1	a	21%	a
Triumph	2.6	a	3.7	2.0	a	100.2	ab	24%	a
F&M vs. T&J	<0.0001		0.7053	<0.0001		0.0028		<0.0001	
	Health10		HLB10	canker10		Fruit		Fruit	
Cultivar						per tree		drop	
Flame	4.1	b	3.8	2.9	b	22.7	b	5%	a
Marsh	4.6	b	3.7	3.1	b	8.8	b	21%	b
Jackson	3.2	a	3.1	1.5	a	82.4	a	4%	a
Triumph	2.8	a	4.2	1.7	a	94.5	a	5%	a
F&M vs. T&J	<0.0001		0.6663	0.0005		<0.0001		0.0013	

In Press FL State Hort Soc: Stover, McCollum, Ritenour, Chaparro

Responses to HLB?

Good canopy density in T & J despite HLB symptoms



Some “healthy” sectors, even in strongly declining GF



Fruit Quality

- Fruit quality assessments were made each growing season with ‘Triumph’/‘Jackson’ showing generally acceptable commercial fruit quality
- ‘Flame’/‘Marsh’ having low Brix/acid.
- In 2011/2012 many ‘Flame’/‘Marsh’ were small and/or misshapen while ‘Triumph’/‘Jackson’ displayed normal size and shape.

Fruit quality January 2012

Cultivar	Fruit weight (g)	Fruit with canker lesions	Total soluble sol.	Titratable acidity
Flame (F)	275c	22%ab	8.0ab	1.1 b
Marsh (M)	209c	30%b	7.5b	1.3c
Jackson (J)	363b	14%ab	9.2a	0.9a
Triumph (T)	514a	3%a	8.8ab	0.9a
Contrast T&J vs M&F	0.0001	0.0278	0.0112	0.0001
Cultivar	TSS/TA ratio	Weight of juice/fruit (g)	Ratio juice / fruit wt.	Seed per fruit
Flame (F)	7.0b	123b	0.45a	2.6a
Marsh (M)	5.7c	94c	0.43a	2.0a
Jackson (J)	10.6a	149b	0.42a	3.0a
Triumph (T)	9.6a	220a	0.44a	40.5b
Contrast T&J vs M&F	0.0001	0.0001	0.759	0.0001



Flame
VS
Triumph



Triumph
Vs.
Jackson

CLas- assessed random quadrant samples and
most symptomatic diagnostic samples on 5
trees of each on 9/23/09
no significant differences by Kruskal Wallis

<u>Scion</u>	<u>quadSW</u>	<u>quadSE</u>	<u>quadNE</u>	<u>quadNW</u>	<u>mnquad</u>	<u>diagnostic</u>
Flame	33.6	34.2	38.2	33.0	34.8	33.6
Marsh	37.1	32.4	31.9	33.5	33.7	32.8
Jackson	34.7	34.6	33.0	35.2	34.4	31.9
Triumph	37.6	36.5	36.1	38.6	37.2	34.2

Triumph/Jackson clearly have CLas and show HLB symptoms, just less deleterious to the tree= “Tolerance”

Triumph/Jackson a hybrid between GF and Sweet Orange?



Combining genes for resistance or losing genes for susceptibility?

N USDA Ft Pierce FL Farm with Endemic HLB



Some hybrid
populations show
considerable tolerance
to HLB





'Flame'
5/8 Pummelo, 3/8 Mandarin



'Hirado' x 'Ninkat'
1/2 Pummelo, 1/2 Mandarin



5-51-2
Clementine x
Orlando cross by J.
Hearn shows
tolerance to HLB



USDA 5-51-2

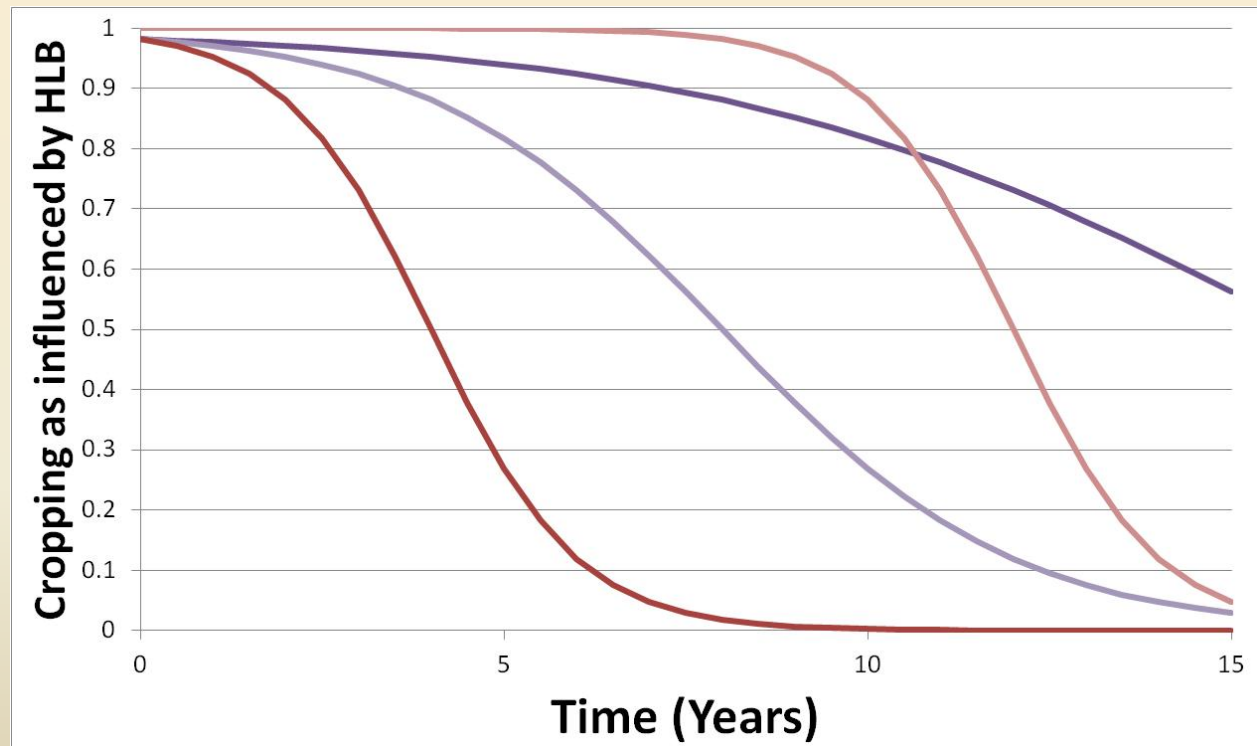
5-51-2 on left- Clementine x Orlando (Duncan x Dancy)

> little evidence of HLB in grove where trees are heavily infected

1-43-21 on right Fortune (Clementine x Dancy) x Encore (King x Willowleaf)



- Appears that there is substantial resistance/ tolerance to HLB in conventional citrus germplasm
- If resistance / tolerance is confirmed, how may this benefit citrus industries?
- How much of a delay in compromise of cropping is needed to be useful?



- Is this of value where HLB is being excluded, or only where the industry is “living with” HLB?

New 3-year Grant from CRDF: Exploiting Tolerance/Resistance to HLB in Citrus

- Postdoc began 10/8/12
- Verifying levels of tolerance/resistance in promising material and testing all advanced selections
- Comparing growth (and ultimately cropping) in susceptible and tolerant material at various CLas titers
- Exploring non-GM methods to enhance resistance



**Susceptible
rootstock
Orange #1**



**Tolerant?
Rootstock
Green #7**

HLB-infected Valencia scion on previously clean rootstocks. Left 3 trees: rootstock Orange # 1 (Nova+HBP x Cleo+trifoliate orange); Right 3 trees: rootstock Green #7 (Nova+HBPummelo x Sour orange+Carrizo)

CREC/McTeer Rootstock Trial with SugarBelle – Haines City
-almost all HLB+ summer of 2011, 3-year old trees.



SugarBelle/Orange #4
2 trees on left



SugarBelle/Orange #19
5 trees on left

Transgenics for HLB- Resistant Citrus

- Tolerance and resistance is great..... IF you have decided to live with HLB
- Transgenics appear to be the most promising solution for strong HLB resistance and perhaps immunity
- Another major advantage is ability to improve an existing cultivar with essentially no other changes: HLB-resistant Tango, Clementine, Navel, Satsuma, Lisbon Lemon etc.

Transgenes we are studying for HLB resistance- Just another source of variability in breeding

- Antimicrobial peptides, including chimeral peptides designed by Goutam Gupta (Los Alamos National Laboratory)
- Transmembrane protein from *Liberibacter* and bacteria-product resistance triggering genes identified from the *Liberibacter asiaticus* genome (working with Duan group at USHRL).
- Phloem-specific protein induced during HLB infection (Bowman data USHRL)
- Dormancy-inducing gene from peach (Bielenberg Clemson U)
- Working with ARS scientists in California to develop constructs so that ALL inserted genes are from Citrus! (Belknap ARS CA)
“**Intragenic**” and possibly “**Cisgenic**”
- New opportunities as they arise.....

Whole Genome Assembly and Annotation of Citrus Sinensis (JGI)

Downloads

Please note: if you download and use the JGI whole genome assembly and annotation please abide by the requirements for this data as specified on phytozome.org's [Citrus sinensis download page](#).

Citrus sinensis Genome Assembly (JGI v1.0)

Scaffolds (FASTA file, 83Mb compressed)	Csinensis_v1.0_scaffolds.fa.gz
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Scaffolds w/ masked repeats (FASTA file, 83Mb compressed)	Csinensis_v1.0_scaffolds_RM.fa.gz
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Scaffolds (GFF3 file, 78 Mb compressed)	Csinensis_v1.0_scaffolds.gff3.gz
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Citrus sinensis Genome Annotation (JGI v1.0)

Coding sequences--CDS (FASTA file, 11 Mb compressed)	Csinensis_v1.0_cds.fa.gz
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Transcript sequences--mRNA (FASTA file, 15Mb compressed)	Csinensis_v1.0_transcript.fa.gz
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Protein sequences (FASTA file, 7Mb compressed)	Csinensis_v1.0_peptide.fa.gz
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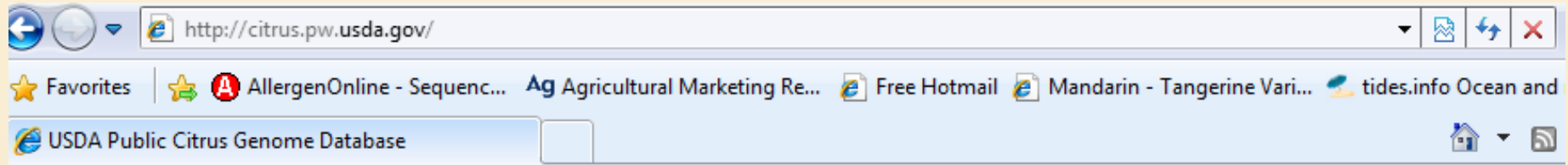
Gene models (GFF3 file, 4Mb compressed)	Csinensis_v1.0_gene.gff3.gz
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Alternate genes (GFF3 file, 3.5 Mb compressed)	Csinensis_v1.0_alt_gene.gff3.gz
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Resources

- [Genome Browser](#)
- [Assembly Details](#)
- [Downloads](#)

Bill Belknap-led Carrizo Sequence Data



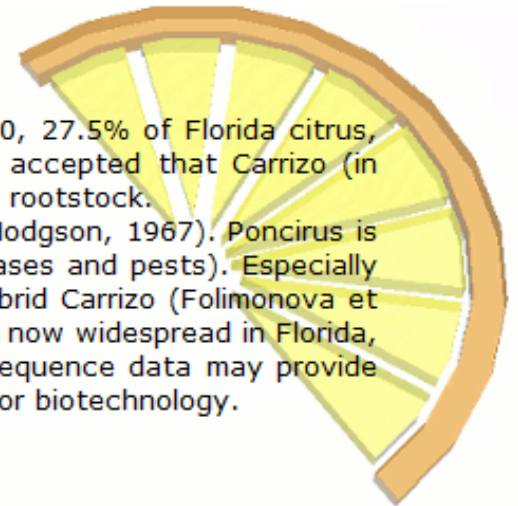
USDA Public Citrus Genome Database

[Overview](#)[Repeats](#)[SSR](#)[Annotation](#)[BLAST](#)[Download](#)[Contact](#)

Overview

Carrizo is likely the single most important rootstock in the US citrus industry. Since 1970, 27.5% of Florida citrus, amounting to 39.5 million trees, have been propagated on Carrizo rootstock. It is widely accepted that Carrizo (in combination with the identical hybrid Troyer) is also the most widely planted California citrus rootstock.

Carrizo (Troyer) is a hybrid from a 'Washington' navel orange x *Poncirus trifoliata* cross (Hodgson, 1967). *Poncirus* is a common source of resistance or tolerance to both biotic and abiotic stresses (cold, diseases and pests). Especially noteworthy is the high degree of huanglongbing tolerance reported for *Poncirus* and its hybrid Carrizo (Folimonova et al., 2009). Huanglongbing is considered to be the most serious threat to citrus worldwide, is now widespread in Florida, and threatens California, Texas and other US citrus growing areas. Availability of Carrizo sequence data may provide researchers the key to developing huanglongbing resistance, through conventional breeding or biotechnology.



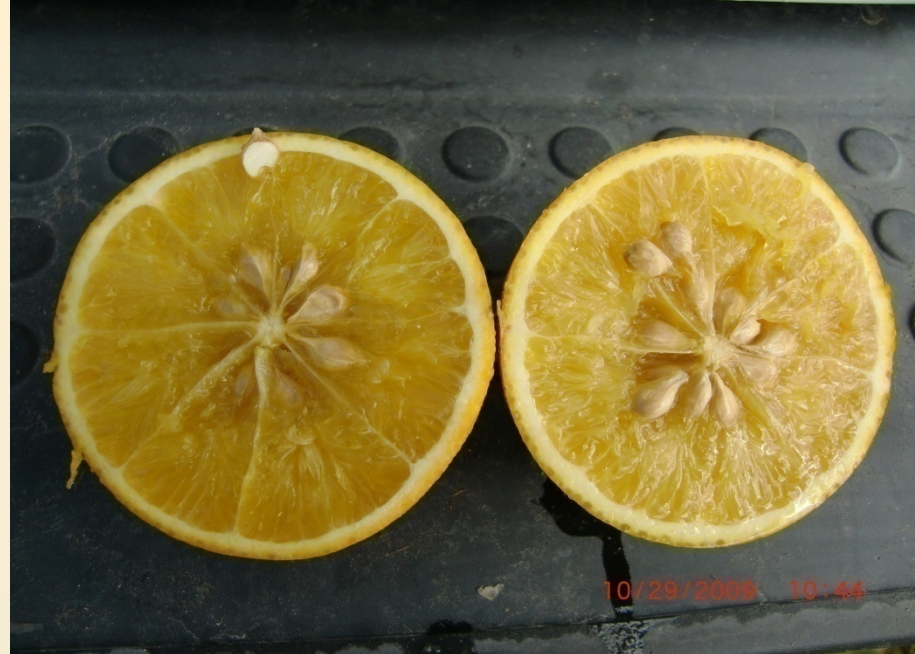
Disclaimer

The Public Citrus Genome Database is made freely available to the citrus research community with no limits on its dissemination. Use of this sequence is not limited by the Fort Lauderdale agreement.



FF-1-75-113 This selection is half orange. It has the most orange-like juice of any hybrid selection we have. Most tasters say it tastes and smells exactly like really good orange juice. Juice quality is extremely high with 14.5 brix, 1 percent acid, and 37.7 color when it ripens around December 1st.

USDA /ARS Citrus Hybrid that is very sweet-orange like and may have different disease resistance since different genetic background



1-4-59 This selection is very orange-like with little trifoliate taste or smell when fully ripe. This selection is highly resistant to tristeza and may have some tolerance to HLB. It may also have some level of cold tolerance acquired from the Poncirus grandparent.

USDA /ARS Citrus Hybrid that is very sweet-orange like and may have different disease resistance since different genetic background

Thanks!

- Florida Citrus Research & Development Foundation
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Diane Helseth

Scott Hyndman

Angel Ledger

Tim Lind

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