

# The Florida Citrus Repository

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Division of Plant Industry,*
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- Winter Haven & LaCrosse, FL



# The Florida Citrus Repository at LaCrosse

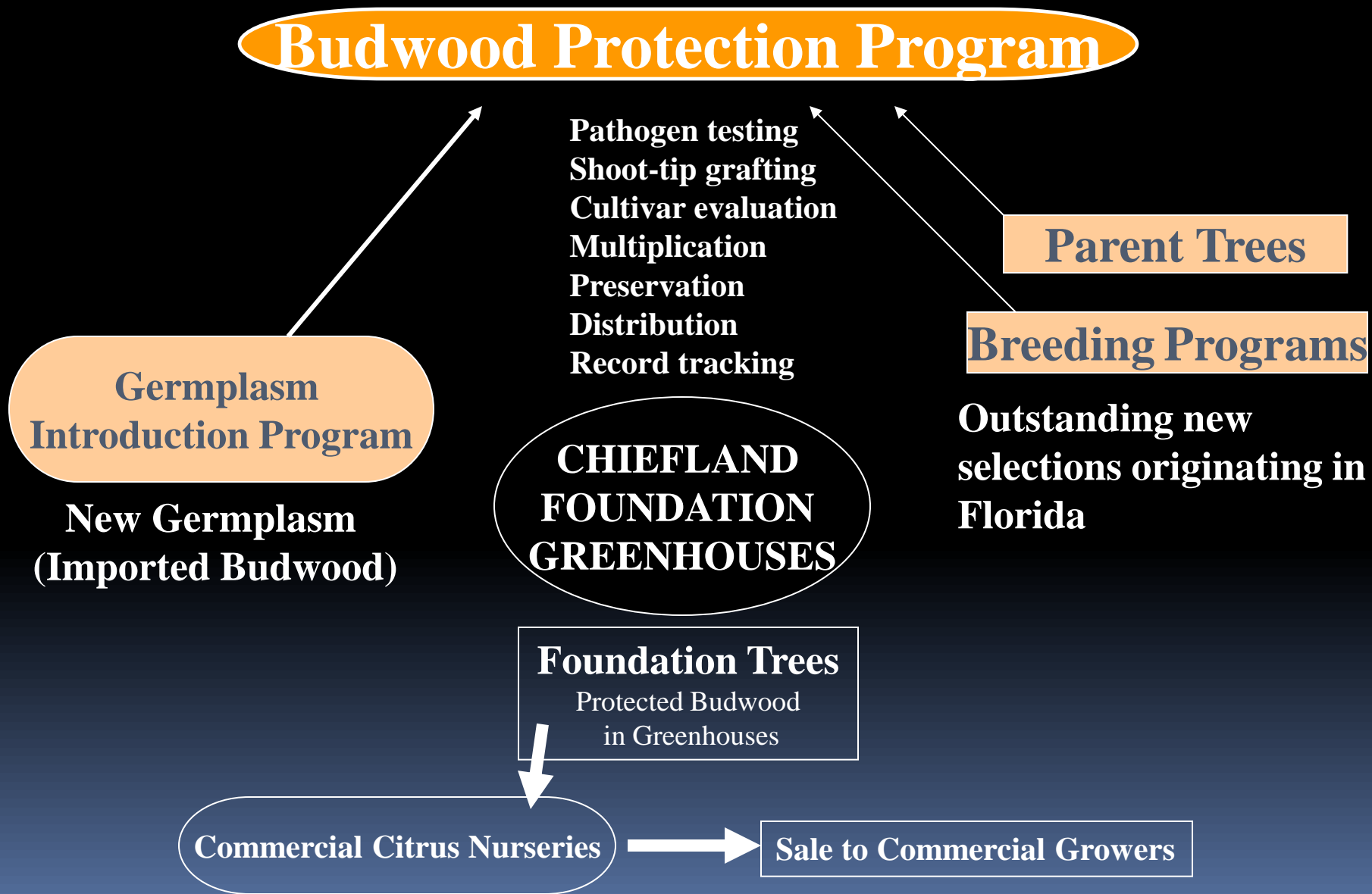
- PHASE ONE:
- Isolated location
- 4 Greenhouse bays
- 5,000 ft<sup>2</sup> of Offices & Labs
- 20,000 ft<sup>2</sup> greenhouse space



# Varieties undergoing pathogen indexing at Gainesville/LaCrosse

- Daisy SL Mandarin CGIP-191
- Atwood Navel CGIP-201
- Nova Mandarin IR CGIP-200
- Sukega Grapefruit CGIP-182
- Cambria Navel CGIP-179<sup>NV</sup> (ZA)
- Hadas CGIP-221
- Odem CGIP-219
- Taylor Lee LS CGIP-185 (AUS)
- Chislett Late Navel CGIP-190 (CA)
- Kinnow CGIP-193, LS Mandarin (CA)
- Ruby Valencia CGIP-195 (ZA)
- Texas Transgenic CGIP-204 thru 218
- C latipes CGIP-184
- Meravit CGIP-220
- Ryan Navel CGIP-186 (AUS)
- Wheeny Grapefruit CGIP-183
- Clementine Haploid CGIP-180<sup>BP</sup> (ESP)
- Natal Sweet Orange CGIP-138 (BRA)
- Setoka Man CGIP-187<sup>NV</sup> (JPN)
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# CITRUS BUDWOOD FLOWCHART



# OUR VISION

- The Bureau of Citrus Budwood Registration will be diligent in providing high yielding, pathogen tested, quality budlines that will positively impact the productivity and prosperity of our citrus industry.



# OUR MISSION

- The Bureau of Citrus Budwood Registration administers a program to assist growers and nurserymen in producing citrus nursery trees that are believed to be horticulturally true to varietal type, productive, and free from certain recognizable bud-transmissible diseases detrimental to fruit production and tree longevity.



# Practical

❁ What point is the logical place to test?

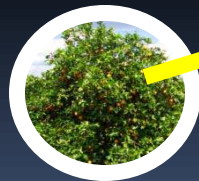
- 📁 Trees coming out of the nursery?

  - 📁 6,000,000 trees

- 📁 The source trees?

  - 📁 3,000 trees

TEST



Source trees



Larger  
population of  
nursery trees



# Facilities Made Obsolete by Poor Location

Shaw Building



Florida Citrus Arboretum



Dundee Foundation Grove



Winter Haven  
Greenhouses



Immokalee Foundation  
Screenhouses

*The Florida Citrus Budwood  
Protection Program*



# Pathogens tested for

## Viruses

- Citrus tristeza virus
- Citrus psorosis virus
- Concave gum
- Citrus tatter leaf virus
- Citrus leaf blotch virus

## Citrus viroids

- Citrus exocortis viroid
- Cachexia
- Citrus viroids I, II, III, IV, V

## Bacteria

- Citrus greening



Psorosis



Exocortis



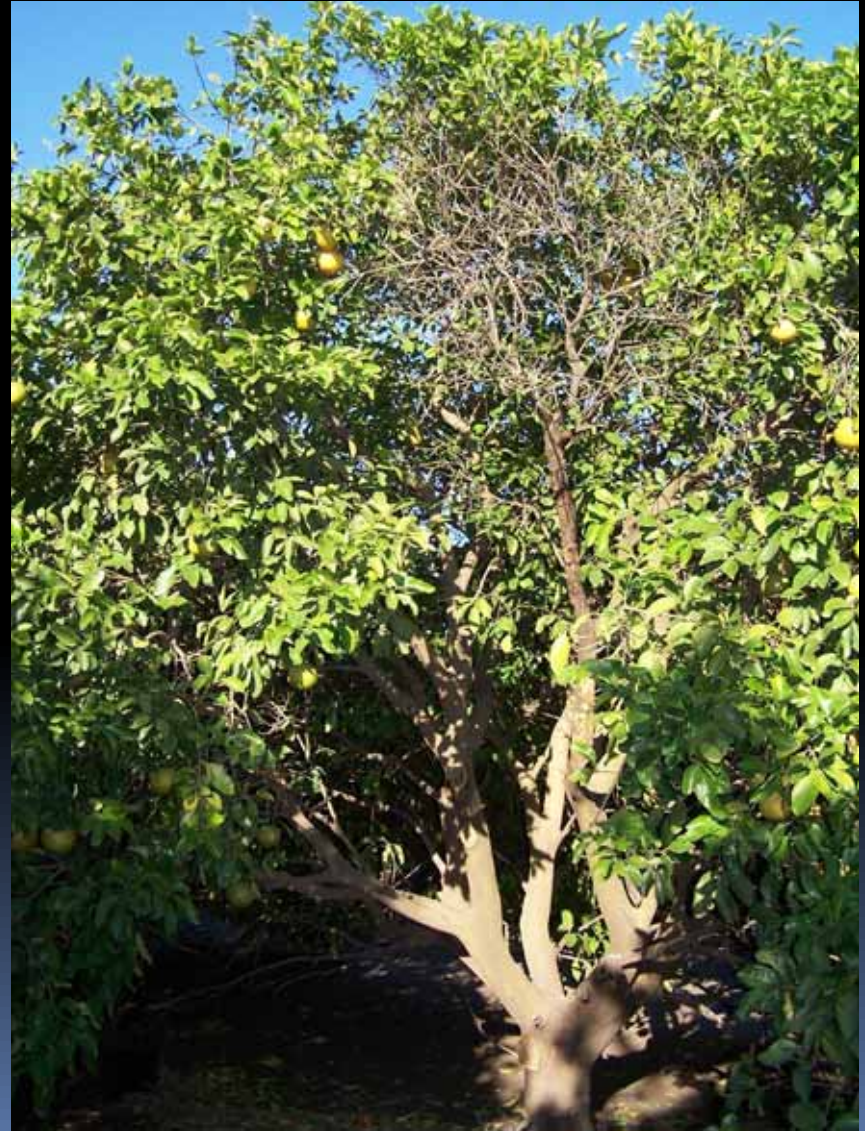
Cachexia



Tristeza stem pitting

# Psorosis Virus

- Citrus psorosis virus spreading in the Texas foundation block





# Tahitian/Persian Lime Success Story

- Wood Blotch is a genetic condition that results in die back of branches, bark cracking in trunk, and staining of underlying wood.
- Other symptoms include fruit sectoring and leaf mottle.
- Time until tree death increases with increased temperatures 2 – 8 years



# Wood Pocket

- In 1956 -57, DPI worked with industry to examine 100,000 trees and find 10 free of Wood Pocket symptoms.
- Ultimately 2 selected clones were used to bring back the Florida/World lime industry and we still use one of these clones today.





Mexico  
2004



# Parents Selected or Bred in Florida

<b>Year</b>	<b>Candidates Entered</b>	<b>Parents Released</b>	<b>Cultivars being Shoot-tip Grafted</b>
2014	110	69	121
2013	42	103	81
2012	72	61	138
2011	72	51	119
2010	34	48	107
2009	24	27	99
2008	16	14	111

# Entries by Agency

Entries by Agency	2014	2013	2012	2011
IFAS CREC	48	26	30	32
IFAS Gainesville	9	0	4	10
USDA	51	13	24	0
Bureau / Participant	1	0	3	2
Private / Proprietary	1	3	11	28





# Winter Haven Laboratory





# Pathogens Tested for by qPCR

Pathogen	Test Type	Tests run			
		2014	2013	2012	2011
HLB	qPCR	8,410	7,307	6,481	7,348
Tristeza	qPCR	7,657	7,727	7,554	7,744
Psorosis	Biological	104	122	58	126
Psorosis	qPCR	2,281	1,523	1,589	1,713
Tatter Leaf*	qPCR	6,599	1,501	1,970	4,546
Leaf Blotch*	qPCR	6,690	1,523	1,970	4,546
Viroid	(Biological)	159	87	83	89
Viroid	CVd I – V, CEVd (qPCR)	12,977	14,755	8,255	9,953
Total		44,877	34,545	27,960	36,065
Testing Frequency	HLB & CTV – PCR Annual; * Seed Source every 3 years Psorosis/Viroids/Leaf Blotch – every six years (PCR).				

# Laminar Flow Biosafety Cabinet for Clean PCR Set-up



# i-Pipette Pro: 96-channel Liquid Handling



# Maintaining Identity

- Access Database to track
- Variety and clone
- ID number for all propagations
- STGs all receive unique number (STG-13001)
- No hand writing of labels
- Computer printed labels for budsticks and STGs
- Stem tags for propagations





# Stream-Lining Testing

- Incoming Parents simultaneously budded
  - Preserve germplasm
  - Viroid biological indexing
  - Indexing for Psorosis and concave gum
  - Incoming Parents simultaneously budded
- Outgoing STGs tagged for testing
- Lab testing all one type of test, one extraction



# STERILIZATION

- 20% Household bleach
  - Adequate for dropping seed and setting up budsticks
  - Need to use stainless steel tools
- Flame
- Bead Sterilizers
  - Can use carbon steel blades





# Disposable Teabags Replace cheese cloth for Sterilizing Seed and Tips



- It does not absorb contaminants or sterilizing solution, very quick to add seeds, tips, then tear open.
- No tying of strings, making cheese cloth bundles or cutting open.

# Other Equipment

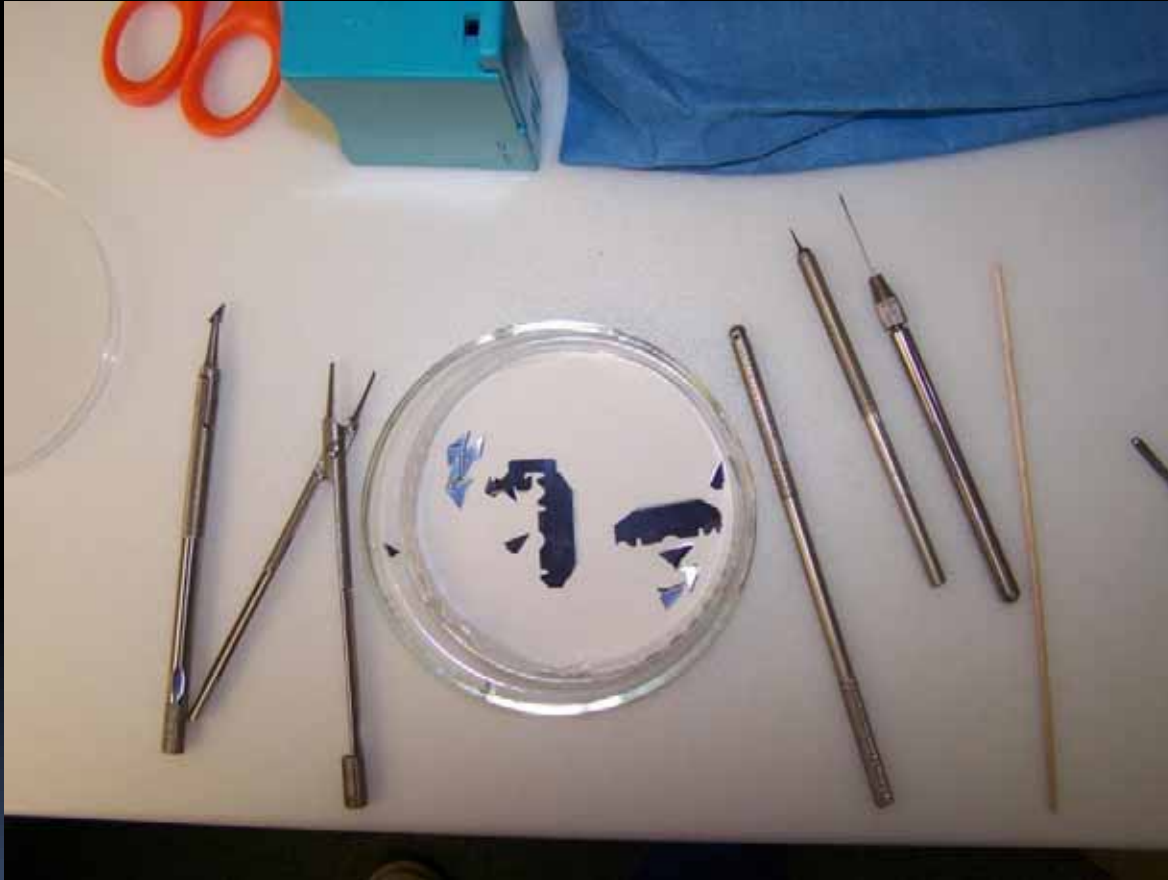


- Good quality microscope for removing tips from budsticks, STGing and trimming
- Cooler for media incoming
- Bread box to hold seedlings in the dark
- Teaching microscope





# Blade Breaker to Make and Hold Microblades



- A medical tool that is now more readily available.
- It precisely breaks carbon steel blades and then holds them in place easier than using a needle holder.

# Media

- Seed and Budstick media

- Agar
- MS salts
- No sucrose



- STG Media

- Gelrite (Phytigel)
- MS salts
- sucrose
- Thiamine, inositol, nicotinic acid, pyridoxine



# Without Reliable source of correct seed types, you cannot have a program

- Proper treatment, drying, redrying without over drying.
- Sterilize seeds in 20% bleach for 10 min before peeling
- In addition to sterilize after peeling
- Use a good dark incubator for precise seed growing
- Store in heavy duty black gift bags at 4°C for up to 1 month



# Harvest Tips from Greenhouse trees and budsticks

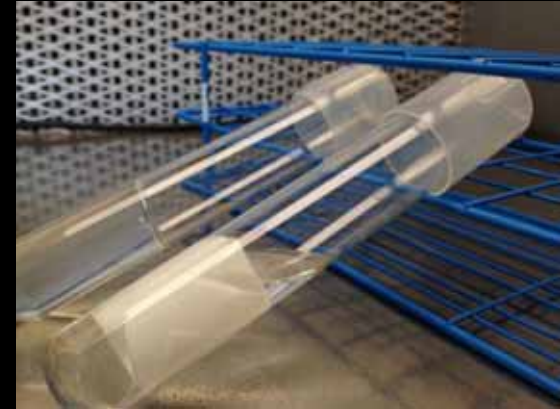


- All tips in 5% house hold bleach.
- There might be mold not visible to the eye in tubes.

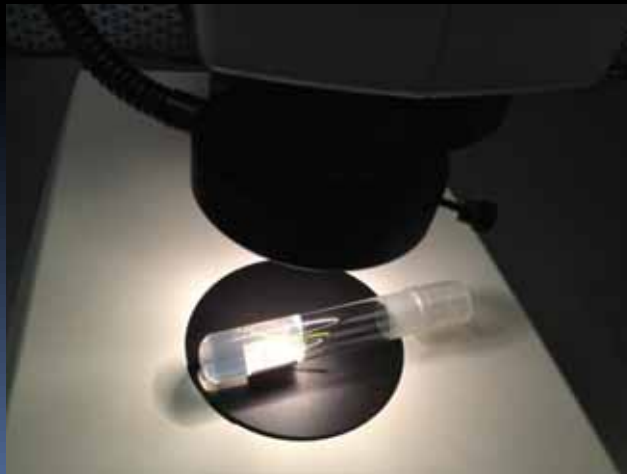




# Solid Media for STGs Allows Precision Trimming



- Softer Gelrite or Phytigel allows examination of STGs under the dissecting microscope
- Easy removal and insertion back into the same tube
- Precision trimming is done by removal of STG to sterile small paper plate and removal of all unwanted growth with scalpel.



# Length of Time in Weeks on P tri foli ata

How long should you keep a STG before giving up?

	STGing to "Shooting"	STGing to Grafting
Mean	3	5
SD	2	2
Range	1 - 13	3 - 15

Thirty sweet orange and mandarins selections set up on P tri in 2013

# Right size to graft in the greenhouse

- Too small



- Just Right



- Too big/Old



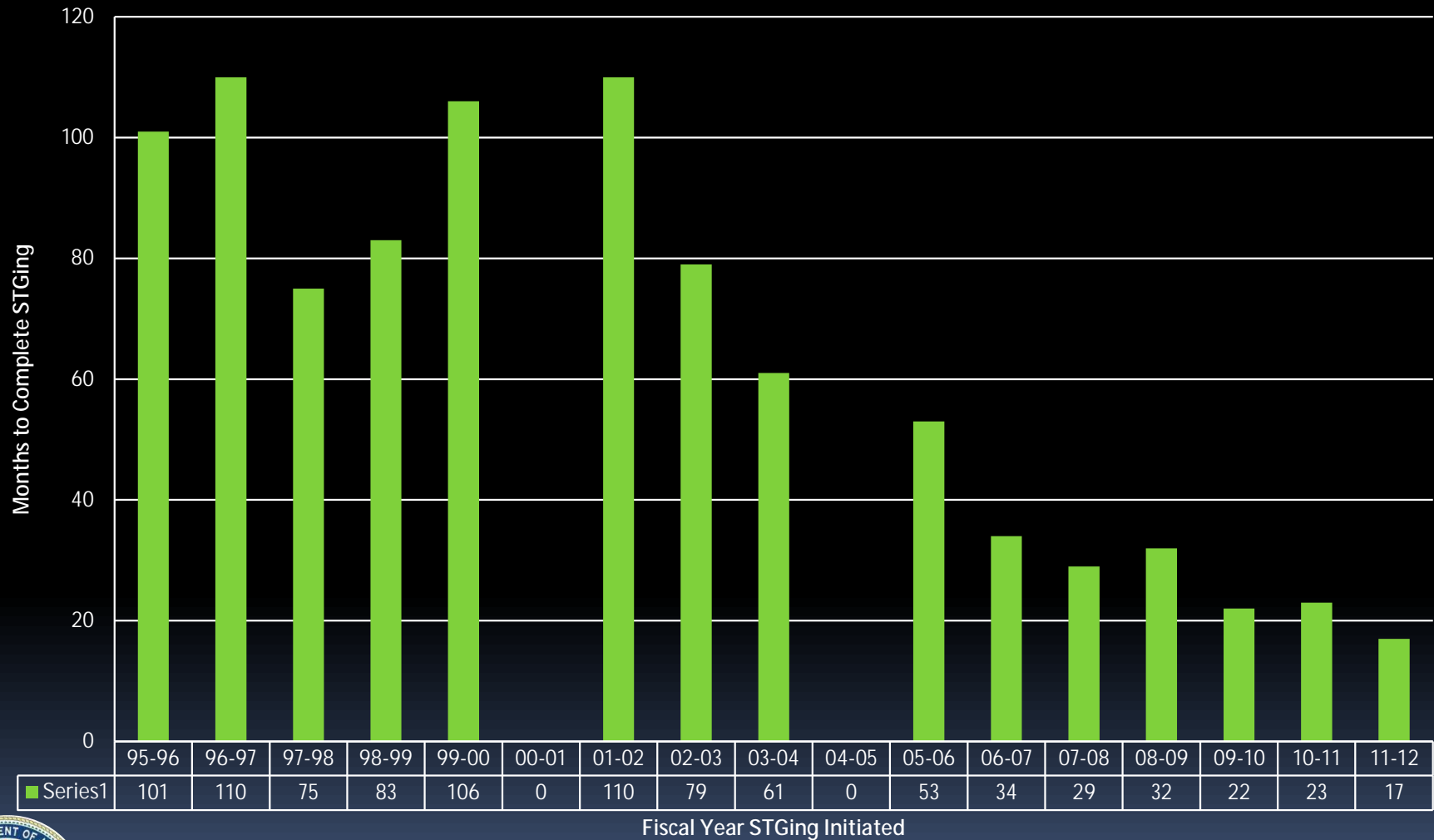
# Live in the greenhouse



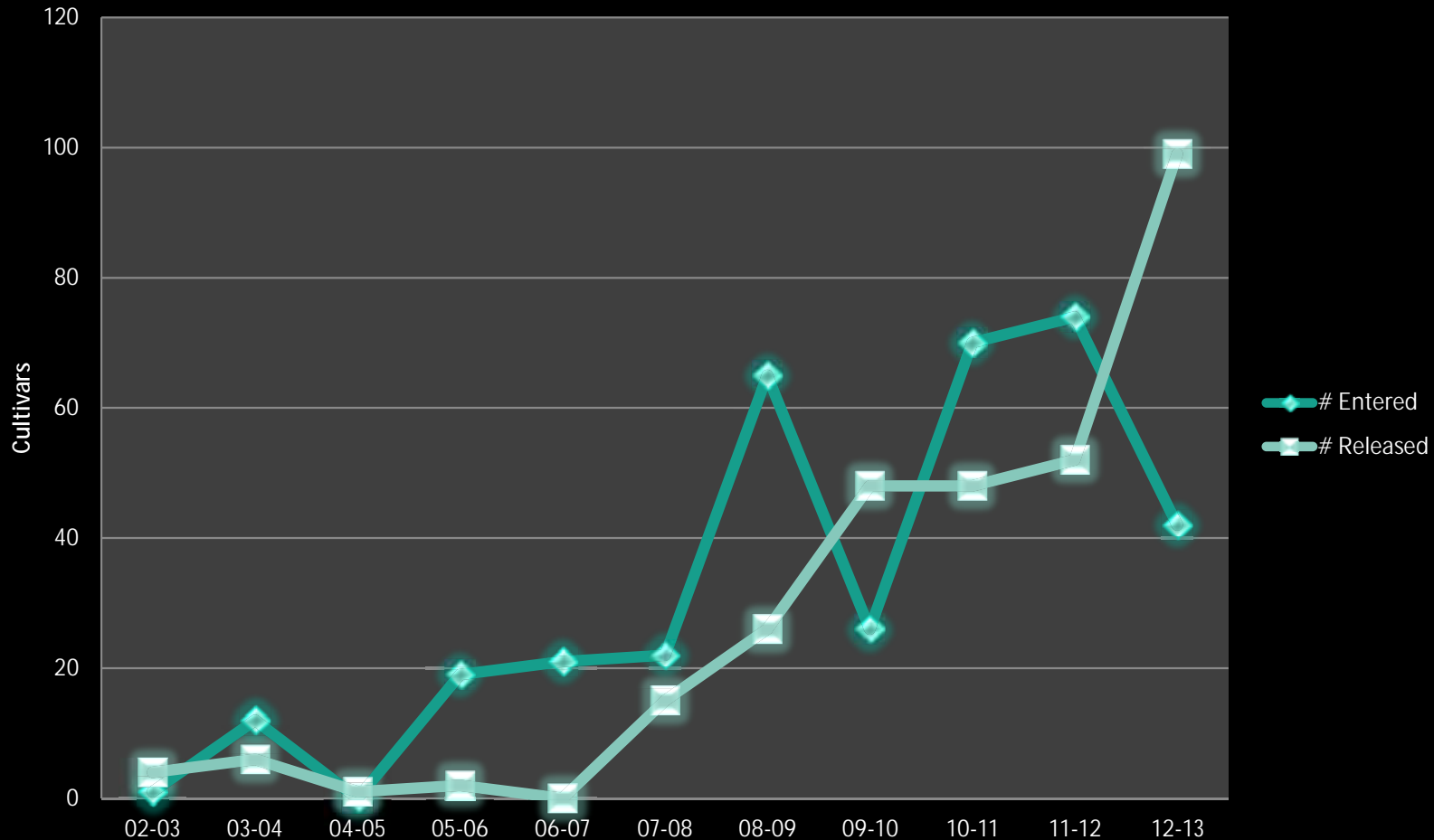
- Should be close to 100%
- Bud into Kinkoji, *Citrus obovoidea*



# Average Duration for STGing by Year Entered



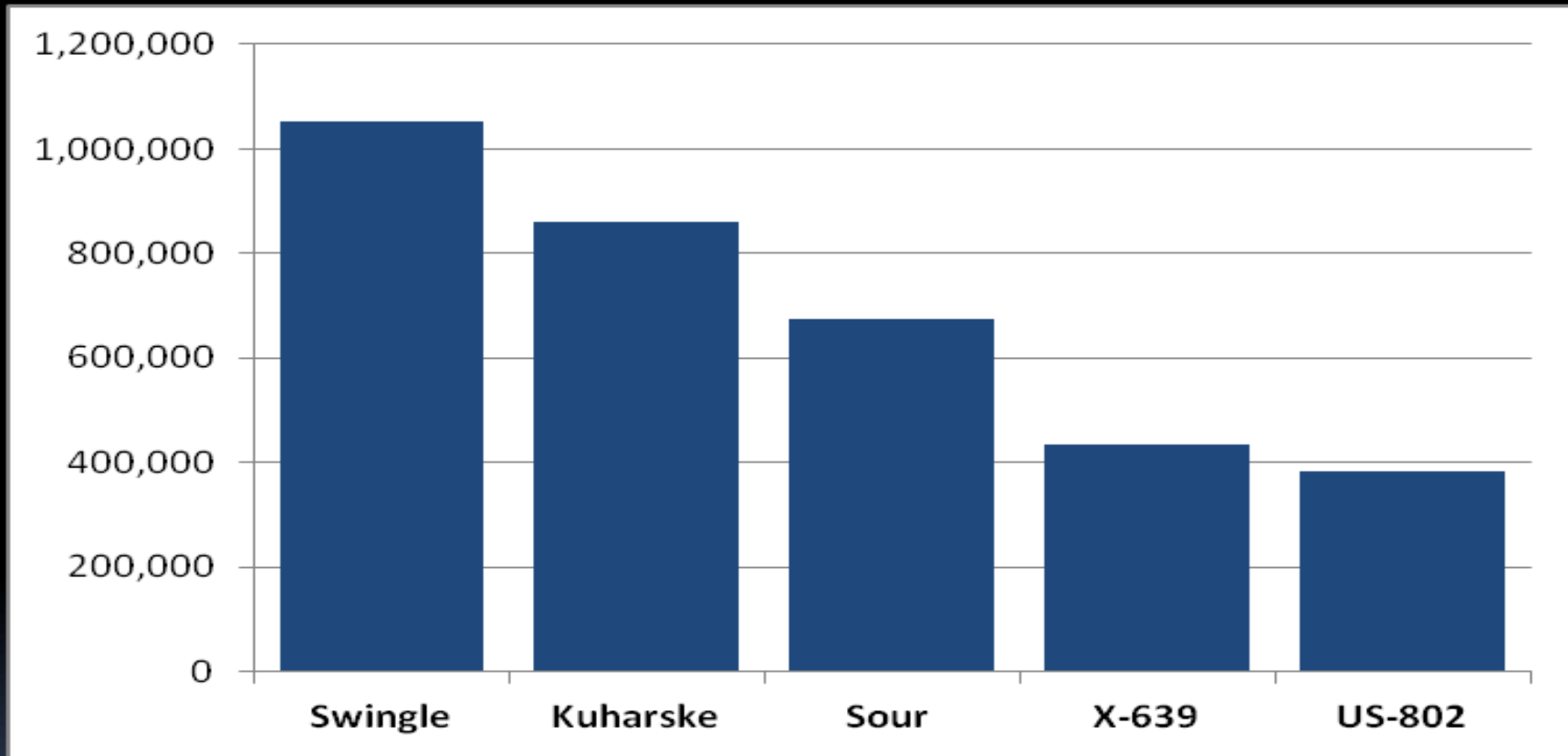
# Number of Selections Per Year



# Our Most Dramatic Improvements

- Lighting in incubator
- Blade breaker tool
- Semi-solid media (Gelrite)
- *Poncirus trifoliata* as seedling for STGing
- Kinkoji for rootstock for grafting incoming parents from the field and STGs from lab to GH
- Decent microscope
- Teaching Microscope
- Long budsticks (in Jumbo Tubes)
- Precision trimming
- Real time PCR to replace ELISA and conventional PCR
- Increase in greenhouse space
- Improvements in Psorosis biological indexing (from three year test to a 6 month test).

# Rootstock Usage 2014





# Rootstock Use by Citrus Type

Rootstock	Grapefruit	Kumquat	Lemon	Lime	Mandarin	Pummelo	Sweet
Carrizo	1.5	0.0	0.0	0.0	0.1	0.2	4.2
C-35	0.0	0.0	0.0	0.0	1.1	0.0	1.4
Cleopatra	0.1	3.8	0.6	0.1	14.3	0.2	3.1
Kinkoji	0.0	6.6	0.0	0.0	1.8	19.4	0.1
Kuharske	1.6	31.3	24.4	37.0	6.7	4.8	20.2
Rough Lemon	0.1	0.0	0.7	1.0	0.2	1.2	0.6
Sour Orange	83.2	8.1	12.9	17.4	25.2	12.8	9.0
Sun Chu Sha	0.0	0.0	0.0	0.0	0.3	0.0	0.2
Swingle	5.6	0.4	18.6	32.5	14.6	13.3	24.3
US-802	2.4	0.0	0.0	0.0	8.5	0.2	8.8
US-812	2.0	0.0	0.8	0.0	2.3	0.0	7.0
US-852	0.0	0.0	0.0	0.0	0.0	0.0	0.0
US-897	0.7	0.0	3.6	2.9	6.9	28.8	5.7
US-942	0.2	0.2	0.4	0.1	0.9	0.4	1.8
Volkamer	0.0	46.0	33.9	6.0	1.4	10.5	1.1
X-639	0.3	0.0	0.0	0.0	6.3	2.2	10.4
Other Stocks	2.4	3.6	4.2	3.0	9.4	5.8	1.9



# New Rootstocks

- University of Florida released 4 in April.
- USDA released 5 rootstocks in October with improved tolerance to HLB.
- Currently 79 Florida breeding program rootstocks being Shoot-tip grafted and tested.



# Rootstock Micropropagation

- North American Plants – Agromillora in Oregon
- Agromillora in Florida
- Agristarts
- Phillip Rucks Citrus Nursery
- Citrus Repository – Phase II



# The Florida Citrus Arboretum

- 250 Cultivars and relatives
- Come and visit (863) 298-3041





# Ravages of HLB

- Declining Trees are Being Removed
- New trees planted
- Arboretum is being actively conserved.



# Antibiotic Trials

- Penicillin
- Streptomycin
- Larger Trials in Dundee
- Injected
- Sprayed





# Psyllid Trap Trials

- 3-D Printer Made
- Streptomycin
- Larger Trials in Dundee



# LACROSSE PHASE TWO

- This year, the Florida Legislature allocated \$2 million to build phase two at LaCrosse
- Phase two will consist of additional laboratory and greenhouse space for the bureau to back up part of the Chiefland Foundation collection
- grow off clean shoot-tip grafted material
- Rootstock micro-propagation
- Multiply new introductions for industry release.





# ACKNOWLEDGEMENTS

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