

Programas de cuarentena, saneamiento y certificación para prevenir plagas cuarentenarias de los cítricos en EUA



Quarantine, sanitary and certification programs to prevent citrus quarantine pests in the USA

Georgios Vidalakis

Universidad de California, Riverside
Programa de Protección a los Clones de
Cítricos, EUA



Robert Krueger

USDA-ARS Repositorio Nacional de
Germoplasma de cítricos y palmas datileras
Riverside, California, EUA



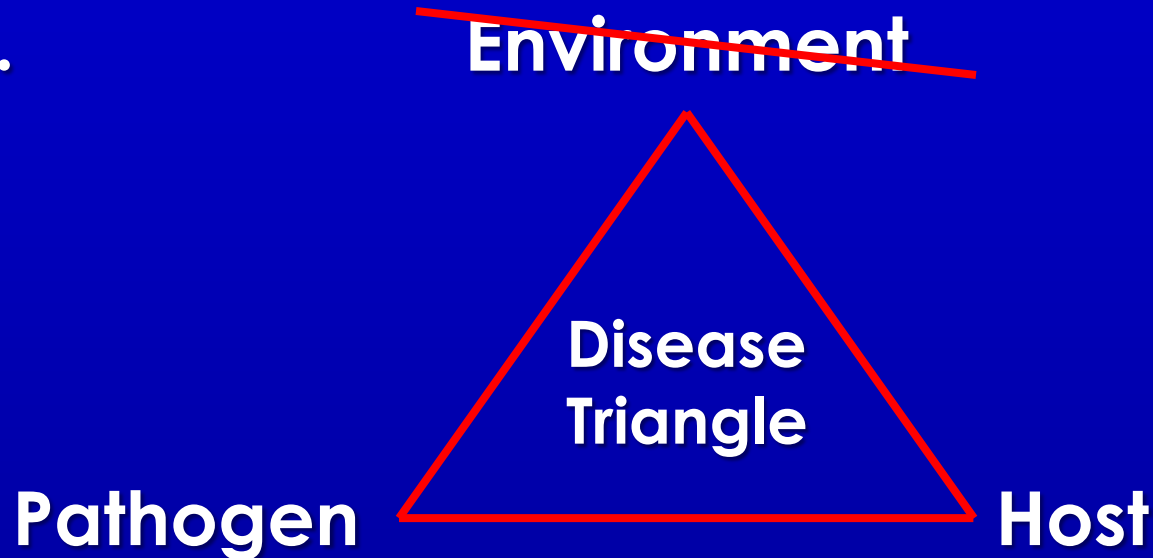
Villahermosa, Tabasco , Mexico, July 27-31, 2009

The Principal Introduction

Management of Graft Transmissible Diseases of Citrus

#1. Exclusion: Quarantine Programs for Safe Introduction of Citrus Germplasm-Varieties

#2.



- Elimination
- Maintenance of tested sources of germplasm

- Availability of different disease tested citrus varieties

A Continues Battle

Psorosis → Nucellar seedlings



Sour orange ← Fruit quality Phytophthora



CTV quick decline → Trifoliate & hybrids



????????????????????????????????????

Huanglongbing ← Citrus Exocortis Viroid

Variegated Chlorosis

????????????????????????????????????

Citrus Exocortis Viroid

Tatter leaf

Citrange stunt

USA Citrus Germplasm Programs

Quarantine
Variety
Introduction

Commercial
Budwood
Distribution

California

Citrus Clonal Protection Program (CCPP) Yes Yes

Arizona

Certified Budwood Program No Yes

Texas

Texas A & M University-
Kingsville Citrus Center Program No Yes

Florida

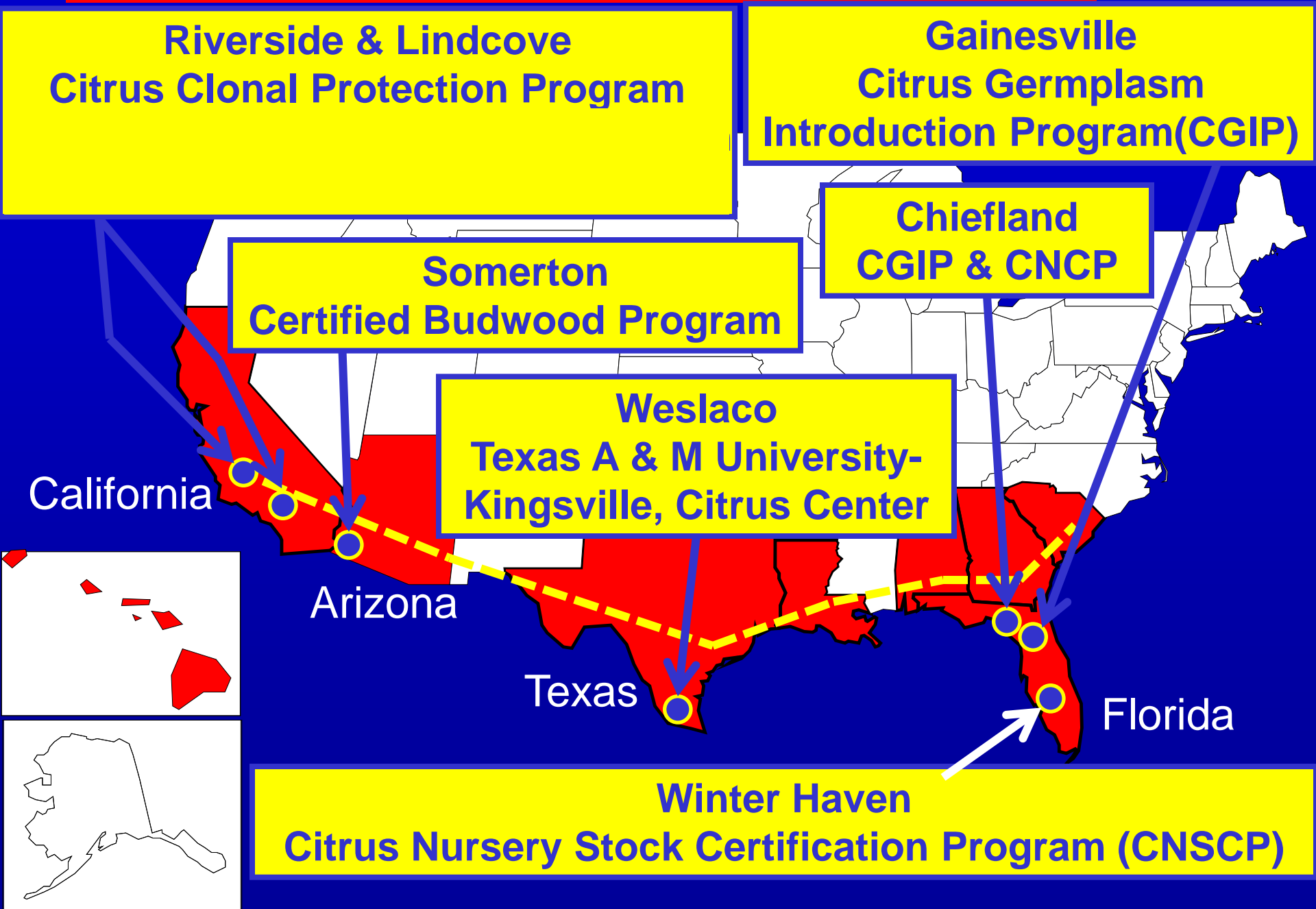
Citrus Germplasm Introduction Program
(CGIP) Yes No

Citrus Nursery Stock Certification Program No Yes

National

National Clonal Germplasm Repository for
Citrus & Dates (NCGRCD) Yes No
(Free worldwide)

USA Citrus Germplasm Programs-Geography



The California Citrus Clonal Protection Program-CCPP

**Quarantine - Variety Introduction
&
Commercial Budwood Distribution**

Citrus Clonal Protection Program-History

1937: Psorosis Free Program

- Voluntary, periodic inspection for Ps free trees
- Cal. Dept. of Agriculture & Nursery Services
- Dr. Fawcett & Wallace

1957: Citrus Variety Improvement Program

- Source of true to type & disease free budwood
- Dr. Calavan & Reuther
- PLPTH, BPSC, CDFA, UCAES, USDA, CCNS, CRB

1977: Rename to Citrus Clonal Protection Program

- Dr. Gumpf
- USDA-APHIS permit for budwood importation directly to CA
- PLPTH, CDFA, UCAES, CCNS, CRB

Citrus Clonal Protection Program

- **To provide a safe mechanism of introduction of citrus into California**
- **To maintain primary source of disease tested & true to type citrus material**

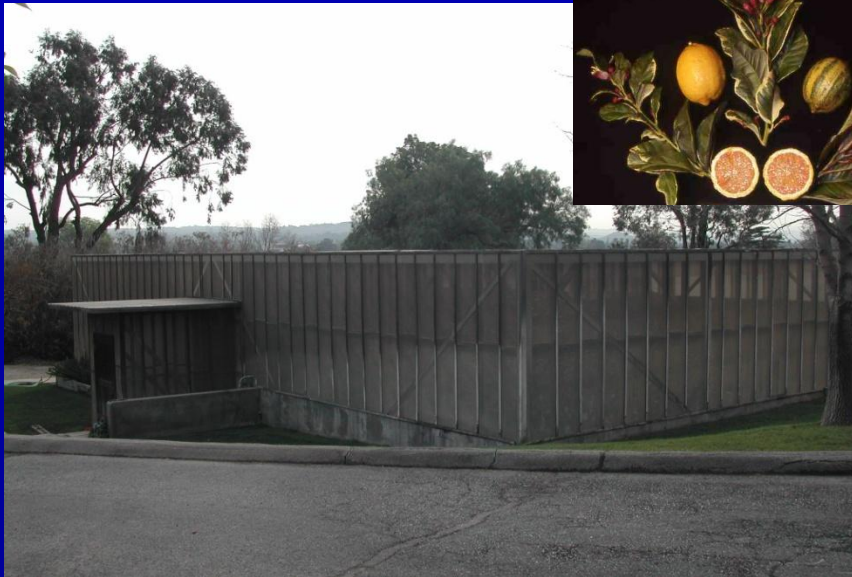
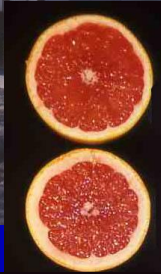
Collaboration:

- **Citrus Research Board & California Citrus Nurserymen Board**
- **University of California**
- **CDFA & USDA-APHIS**

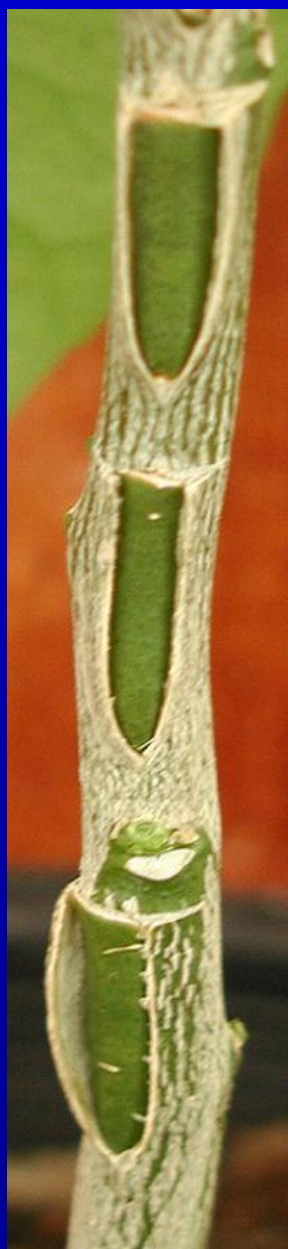
CCPP Functions

- 1. Introduction of Citrus Propagative Material**
- 2. Disease Diagnosis**
- 3. Pathogen Elimination**
- 4. Maintenance (retesting & evaluation) of
Primary Budwood Sources**
- 5. Distribution of Citrus Propagative Material**
- 6. Outreach and Extension**

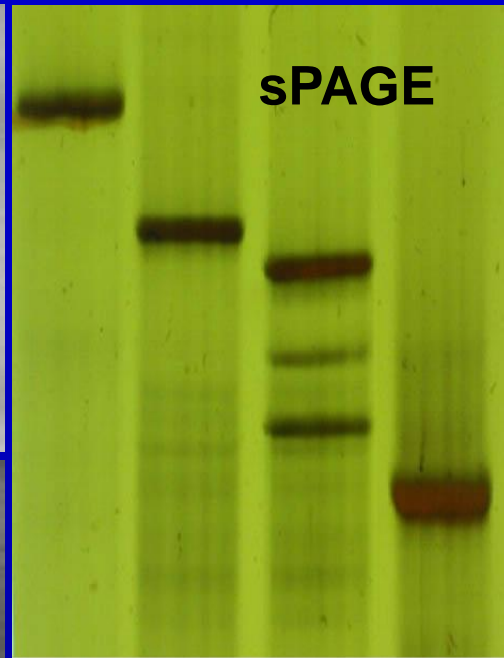
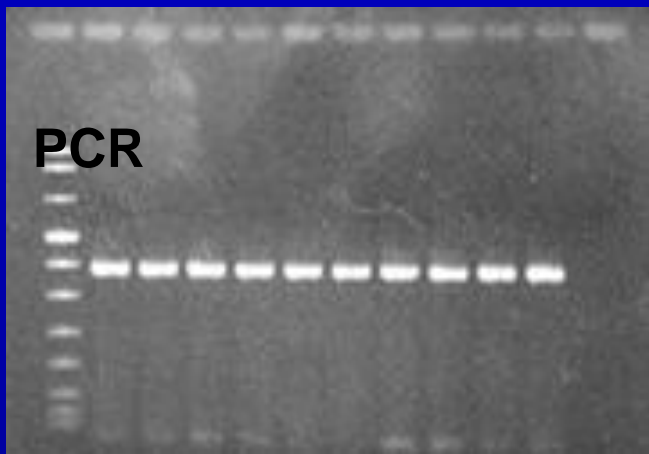
1. Introduction-Rubidoux Quarantine Facility



2. Disease diagnosis –Rubidoux Glasshouse

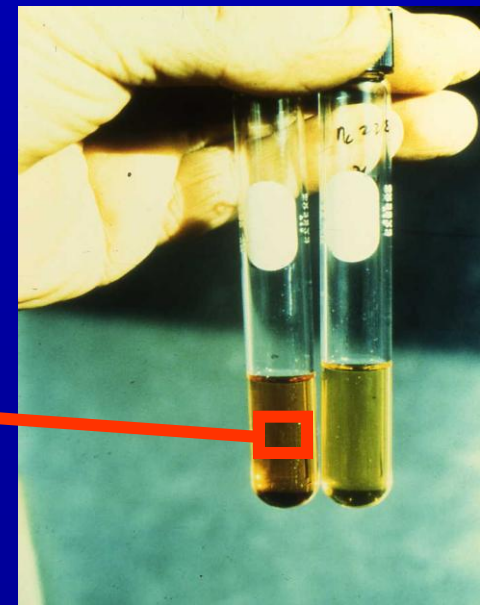
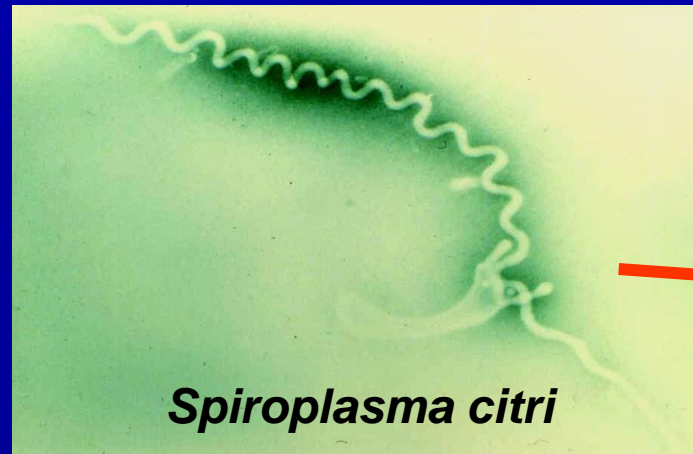


2. Disease diagnosis – Rubidoux & UCR Lab



IMMUNOLOGICAL

MOLECULAR



CULTURE &
MICROSCOPIC
OBSERVATION

3. Pathogen elimination– Rubidoux Facility

A. THERMOTHERAPY



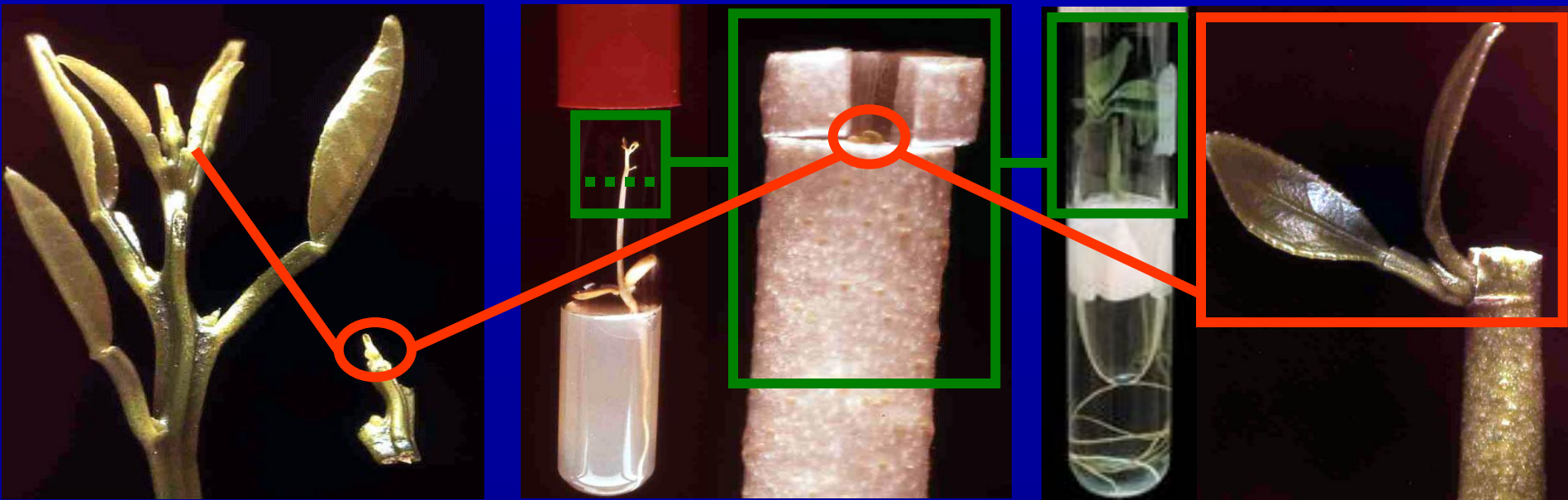
CITRUS GROWING

IN CHAMBERS

40 °C DAYS

35 °C NIGHTS

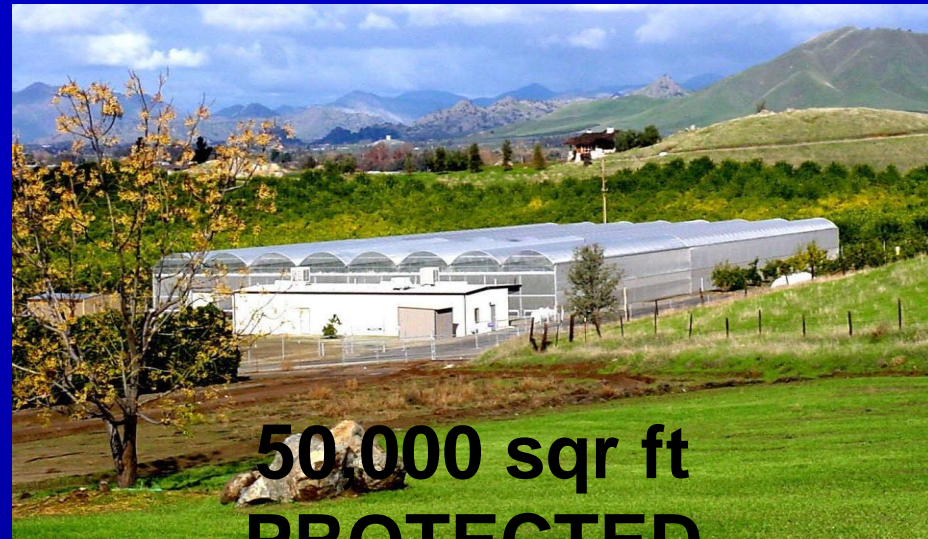
B. SHOOT-TIP MICROGRAFTING



4. Maintenance

U.C. Lindcove Research & Extension Center

**~300 COMMERCIALY IMPORTANT VARIETIES
DISEASE RETESTING & EVALUATION**

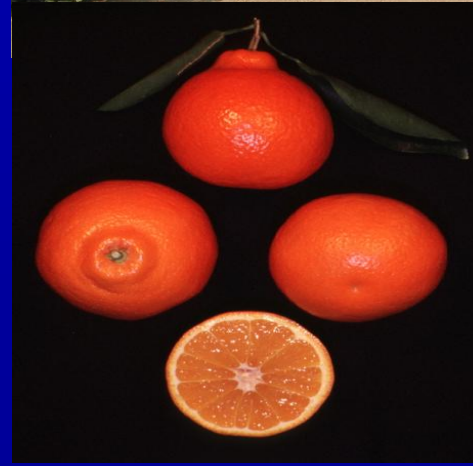


**50 000 sqr ft
PROTECTED**

FOUNDATION BLOCK

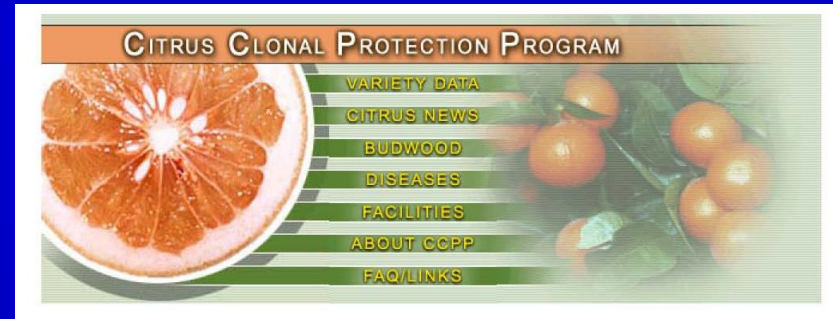


**~2000 TREES
FOUNDATION &
EVALUATION BLOCK**



6. CCPP Outreach

On line:
www.ccpp.ucr.edu



Agricultural Shows:
AgExpo, Citrus Mutual



Foundation Block Walkthrough:
Every winter



National & International Visitors

USDA & CDFA

QUARANTINE RELEASE



REGISTERED BUDWOOD
creation

-Horticu

-Historic

-Selected Periodic re-indexing for:

-Virus

-Tristeza (every year)

-Viroids (every 5 years)

-Psorosis (every 5 years)

-Stubborn (every ? years)

PRE-INDEX

-Bud



CLEAN UP B

-shoot tip s

and/or

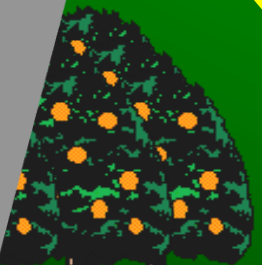
-heat treatment

CULTURE SPACE BLOCK-ISA, FOR COMMERCIAL CITRUS GROVE
on INCREASE BLOCK-ISA, FOR COMMERCIAL CITRUS GROVE
18 MONTHS LIMIT

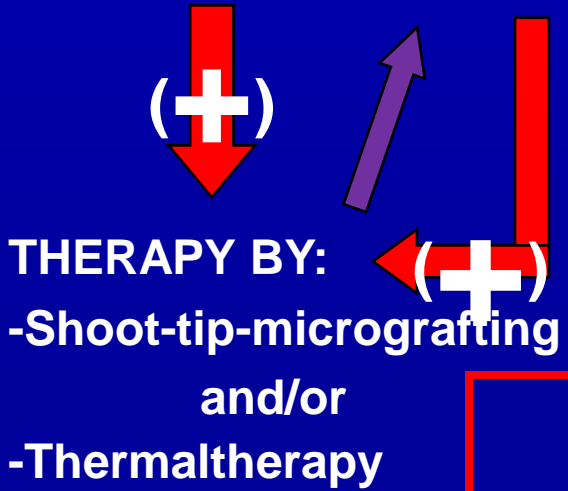
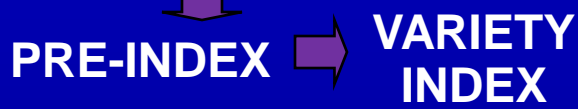
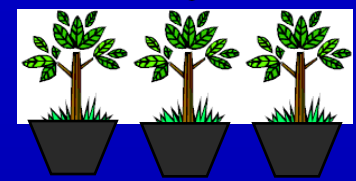
ation

TREES

on for:



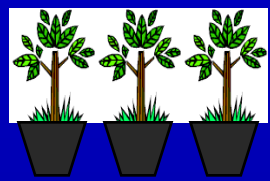
QUARANTINE-INTRODUCTION



QUARANTINE RELEASE-BUDWOOD DISTRIBUTION



EARLY
RELEASE



18 MONTHS LIMIT – NO TESTING



- DISEASE TESTING:
- Every year, Tristeza
 - Every 5 years, Psorosis & Psorosis like and Citrus viroids



Mandatory:

Annually Tristeza

Approved test: ELISA

Voluntary:

Every 5 years Psorosis & Viroids

Approved Test:

Dweet tangor

Etrog citron

Imprint Hybridization

• Custom

• CDFA

Citrus S

• Custom

Arizona & Texas

Do Not Have

**Quarantine - Variety Introduction
Programs**

Florida

1. Quarantine - Variety Introduction

**Citrus Germplasm Introduction Program
Gainesville**

2. Commercial Budwood Distribution

**Citrus Nursery Stock Certification Program
Winter Haven**

Florida's Citrus Germplasm Introduction Program (CGIP)

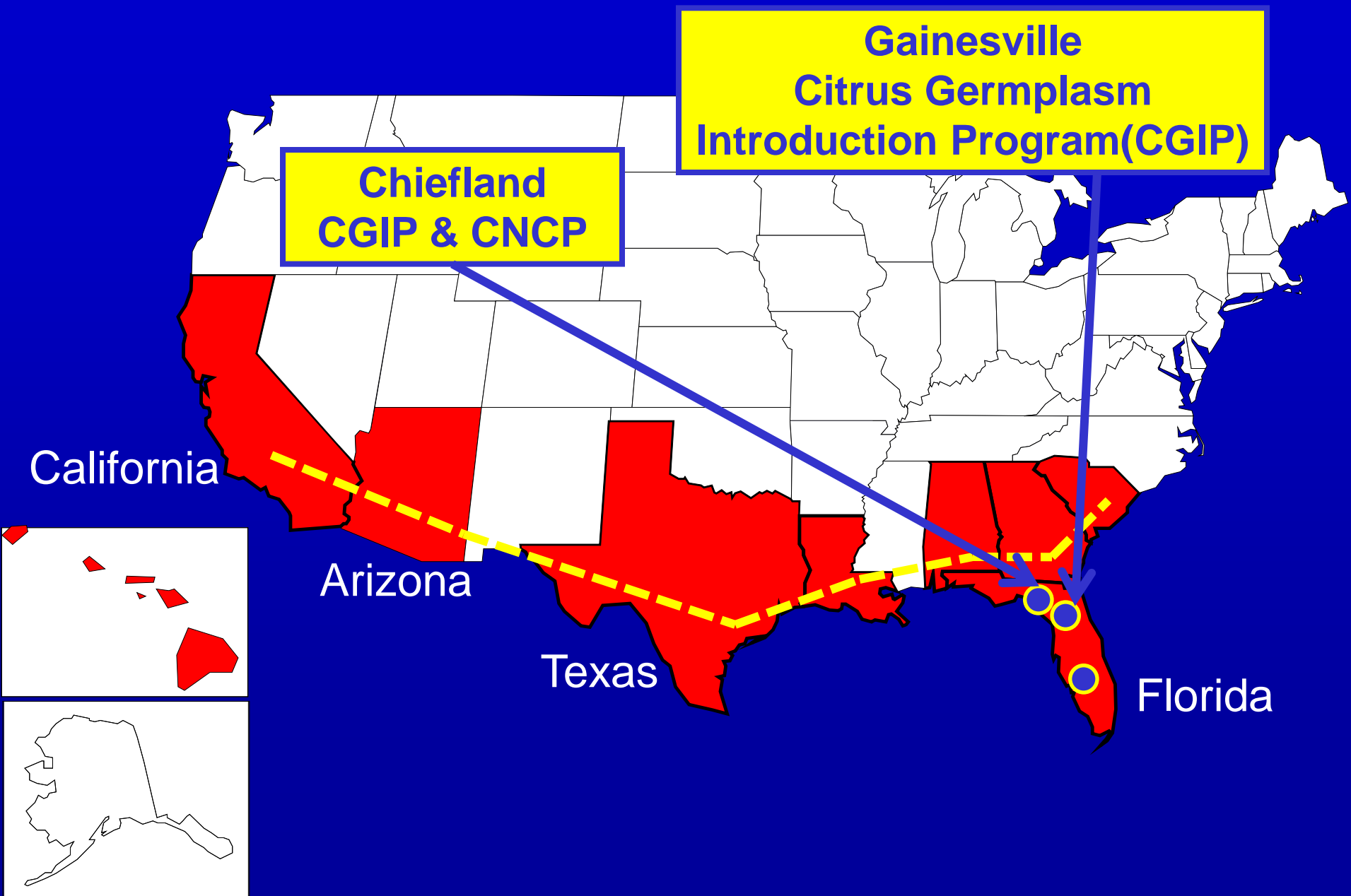
Lisa Williams,
Tim Schubert,
Wayne Dixon,
Richard Gaskalla,



Florida Department of Agriculture & Consumer Services
Division of Plant Industry



USA Citrus Germplasm Programs-Geography



Three Levels of Quarantine

Each level is based upon a different stage of plant material:

- * New Germplasm
- * Shoot-tip Grafted Plant
- * “Thermal” Plant



Preliminary Bio-indicators

Citrus

- * *C. excelsa*
- * Citron
- * Dweet
- * Eureka Lemon
- * Key Lime
- * Parson's Special
- * Rusk
- * Sour orange
- * Sweet orange

Herbaceous

- * *Chenopodium quinoa*
- * *Crotolaria spectabilis*
- * *Gomphrena globosa*
- * *Nicotiana glutinosa*
- * *Phaseolus vulgaris*
- * *Sesamum indicum*
- * *Vigna unguiculata*

Preliminary Laboratory Tests

* ELISA

- Citrus Psorosis Virus
- Citrus Tristeza Virus
- Citrus Variegation V
- Spiroplasma citri
- Tatterleaf CS Virus
- Xylella fastidiosa

* PCR

- Citrus Greening
- CVd II
- CVd III
- Exocortis Vd

* sPAGE viroid*

* Electron Microscopy



Three Levels of Quarantine

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Final Bio-indicators

Citrus

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- * Citron
- * Dweet
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- * Key Lime
- * Parson's Special
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- * *Nicotiana glutinosa*
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Final Laboratory Tests

* ELISA

- Citrus Psorosis Virus
- Citrus Tristeza Virus
- Citrus Variegation V
- Spiroplasma citri
- Tatterleaf CS Virus
- Xylella fastidiosa

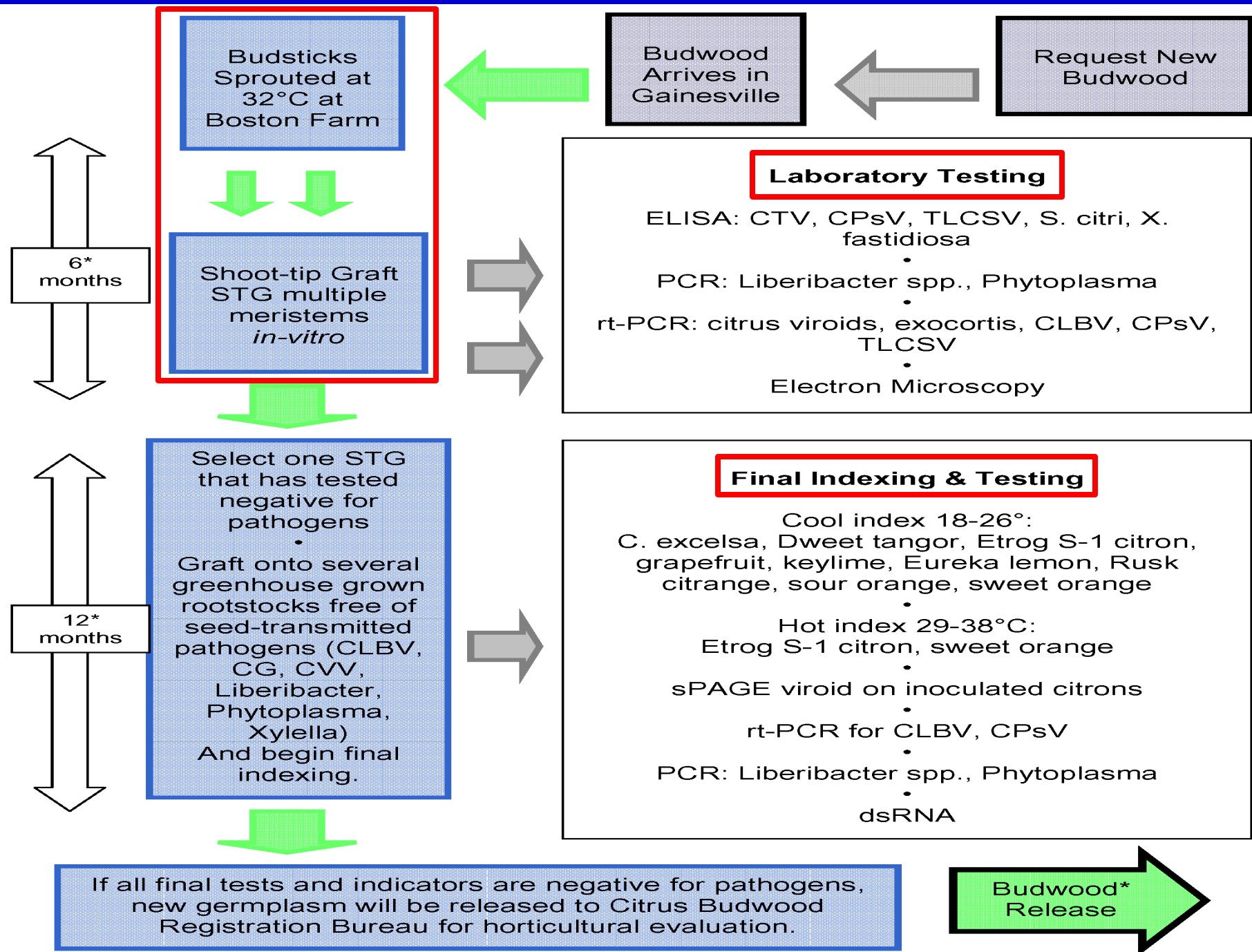
* PCR

- Citrus Greening
- CVd II
- CVd III
- Exocortis Vd

* sPAGE viroid*

* Electron Microscopy



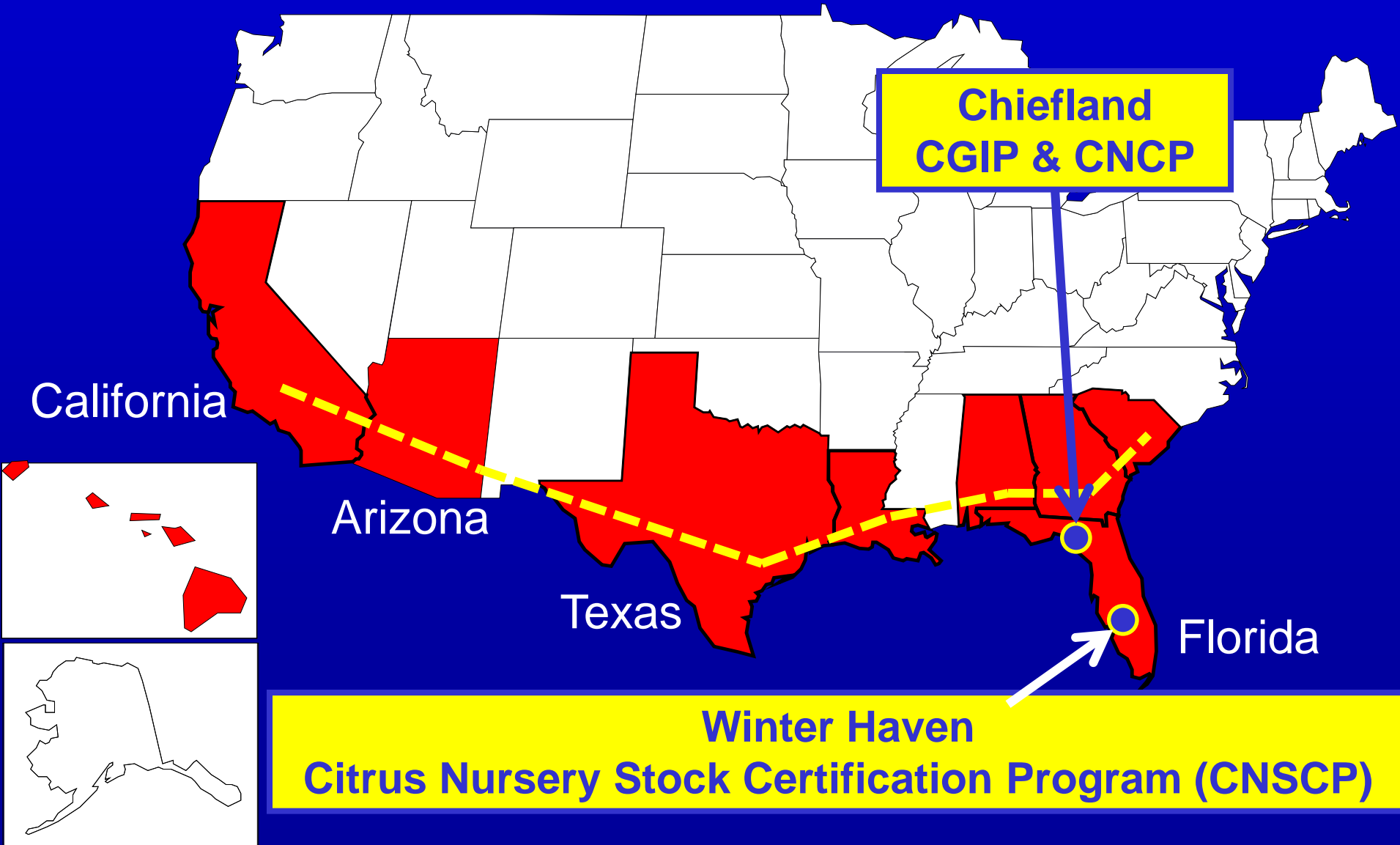


Florida

2. Commercial Budwood Distribution

**Citrus Nursery Stock Certification Program
Winter Haven**

USA Citrus Germplasm Programs-Geography



CITRUS BUDWOOD FLOWCHART

Budwood Protection Program

Michael Kesinger
Peggy Sieburth

Germplasm Introduction Program

New Germplasm (Imported Budwood)

- Pathogen testing
- Shoot-tip grafting
- Cultivar evaluation
- Multiplication
- Preservation
- Distribution
- Record tracking

CHIEFLAND FOUNDATION GREENHOUSES

Foundation Trees
Protected Budwood in Greenhouses

Parent Trees
Breeding Programs

Outstanding new selections originating in Florida

Commercial Citrus Nurseries

Sale to Commercial Growers

Parent Candidate Indexing

PARENT CANDIDATE

INITIAL INDEX
CTV – lab test
Viroid biological
Psorosis biological
Viroid PCR

INFECTED- Viroids

STG

Test again

INFECTED- Not Viroids

STG-with heat therapy
to budsticks

Test again
STG-again without heat therapy
if multiply infected with viroids

Not-INFECTED

Test CTV-lab

Budwood Program Facilities

Shaw Building



Florida Citrus Arboretum



Dundee Foundation Grove



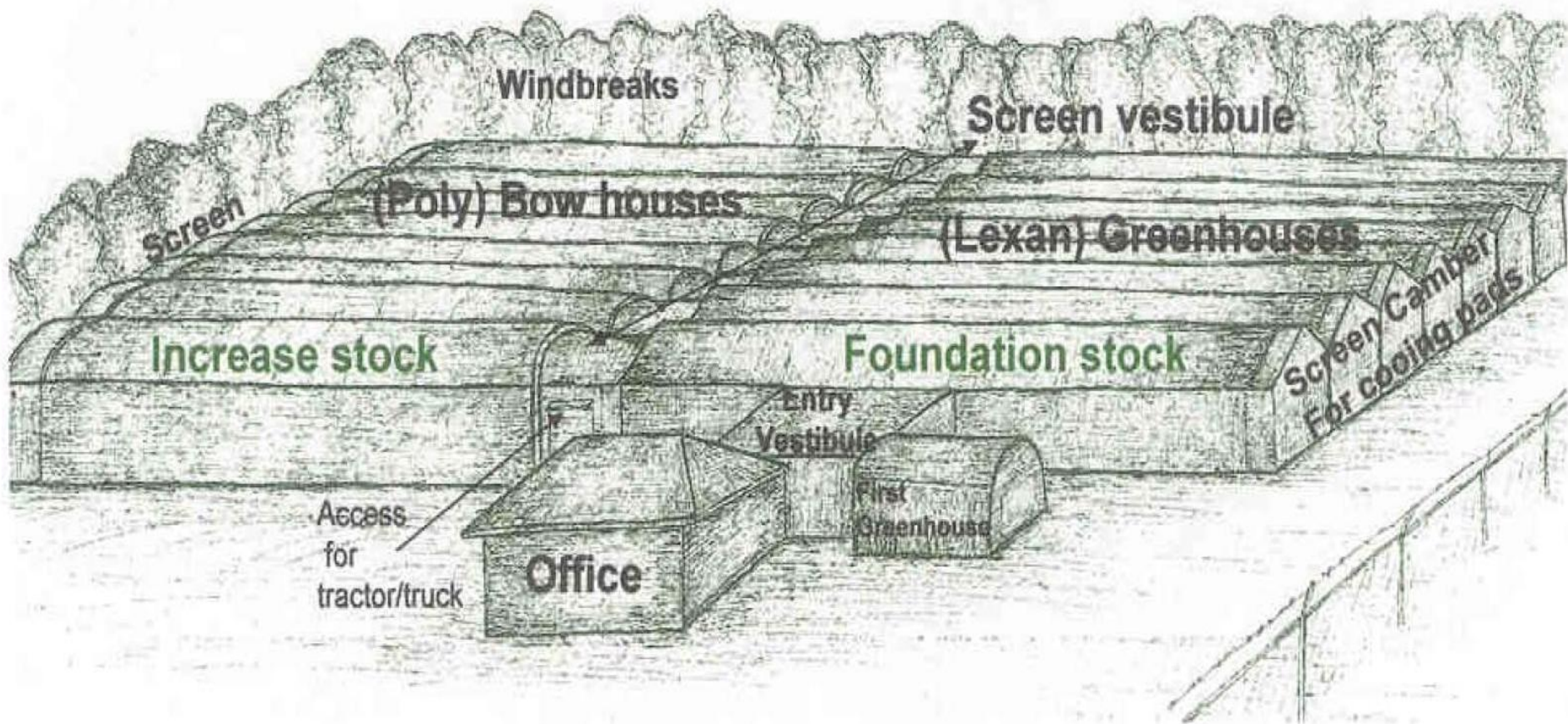
Winter Haven
Greenhouses



Immokalee Foundation
Screenhouses

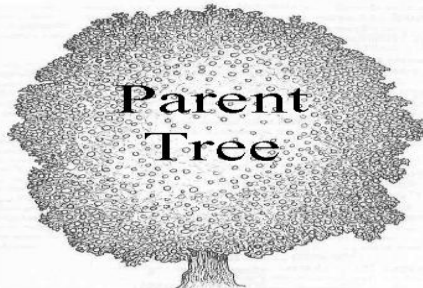
*The Florida Citrus Budwood
Protection Program*

Chiefland Budwood Complex



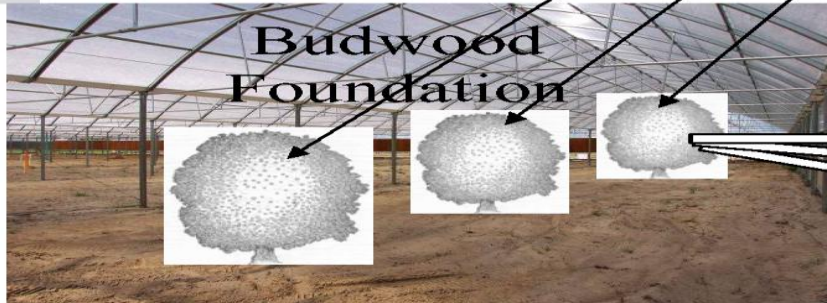
Designed to protect and multiply over 400 different varieties and clones of citrus

The Florida Citrus Budwood Registration Program



1 Candidate Tree Selected

2 Pathogen Detection



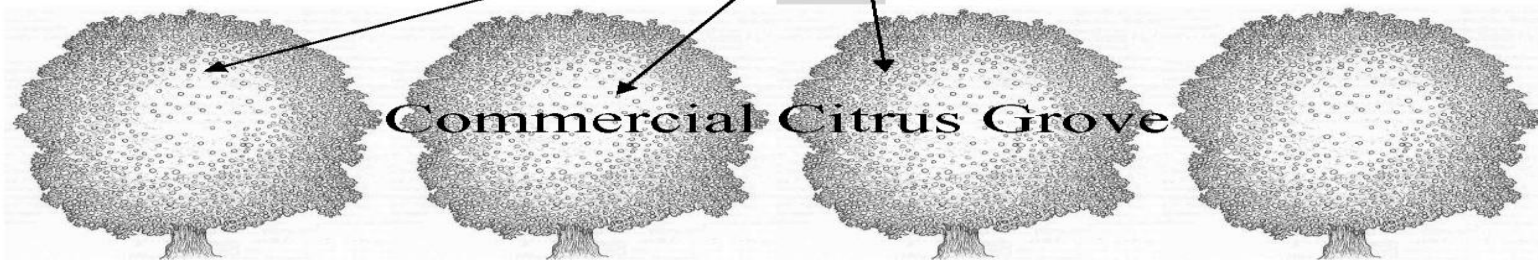
3 Foundation Trees planted of each Pathogen free selection

4 Foundation budwood used to start Scion Planting in enclosed greenhouse at nursery site



5 Increase Block started from Scion Trees

6 Commercial trees propagated from Increase Block



7 Sale of trees to Commercial Groves

Texas

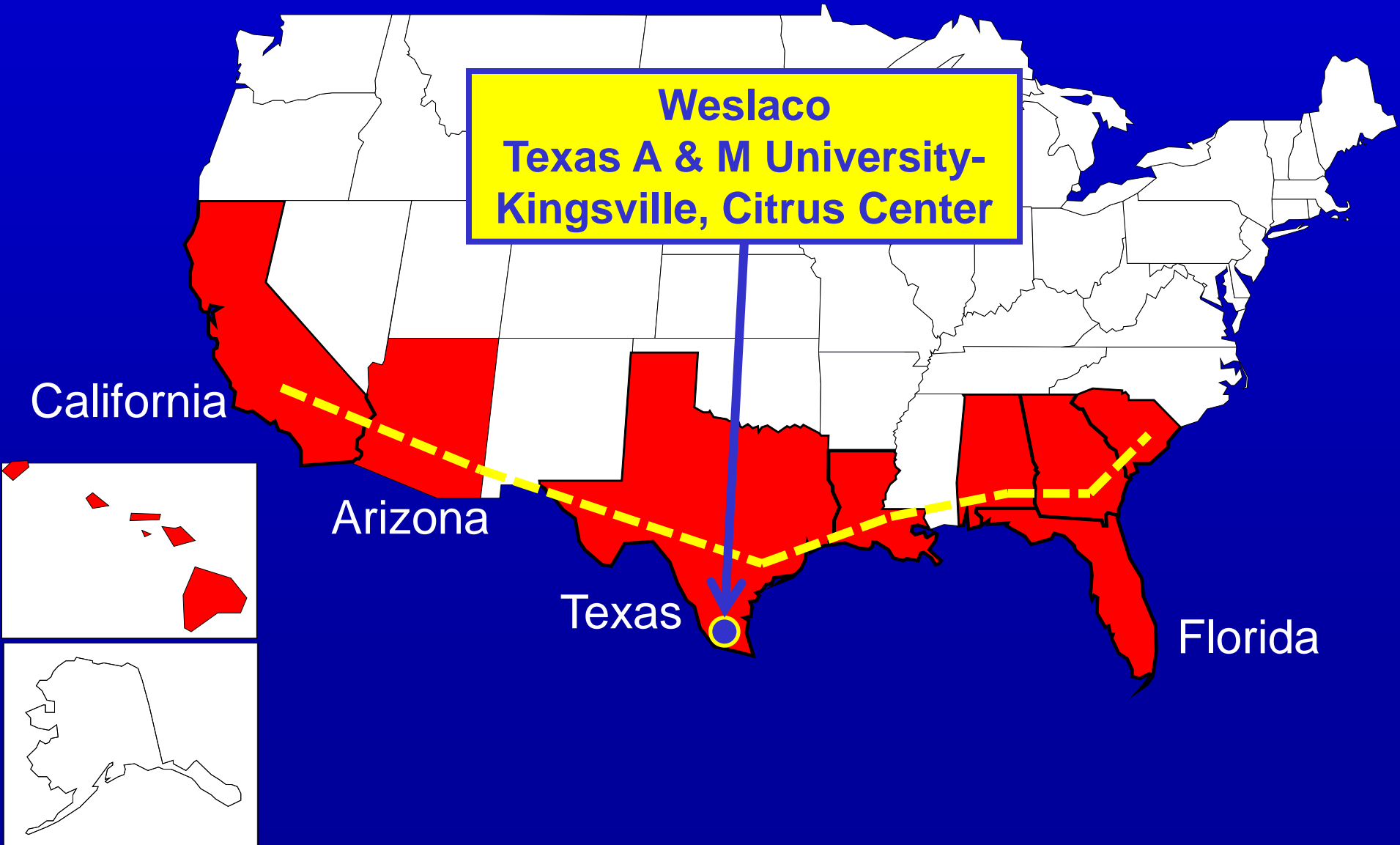
Commercial Budwood Distribution

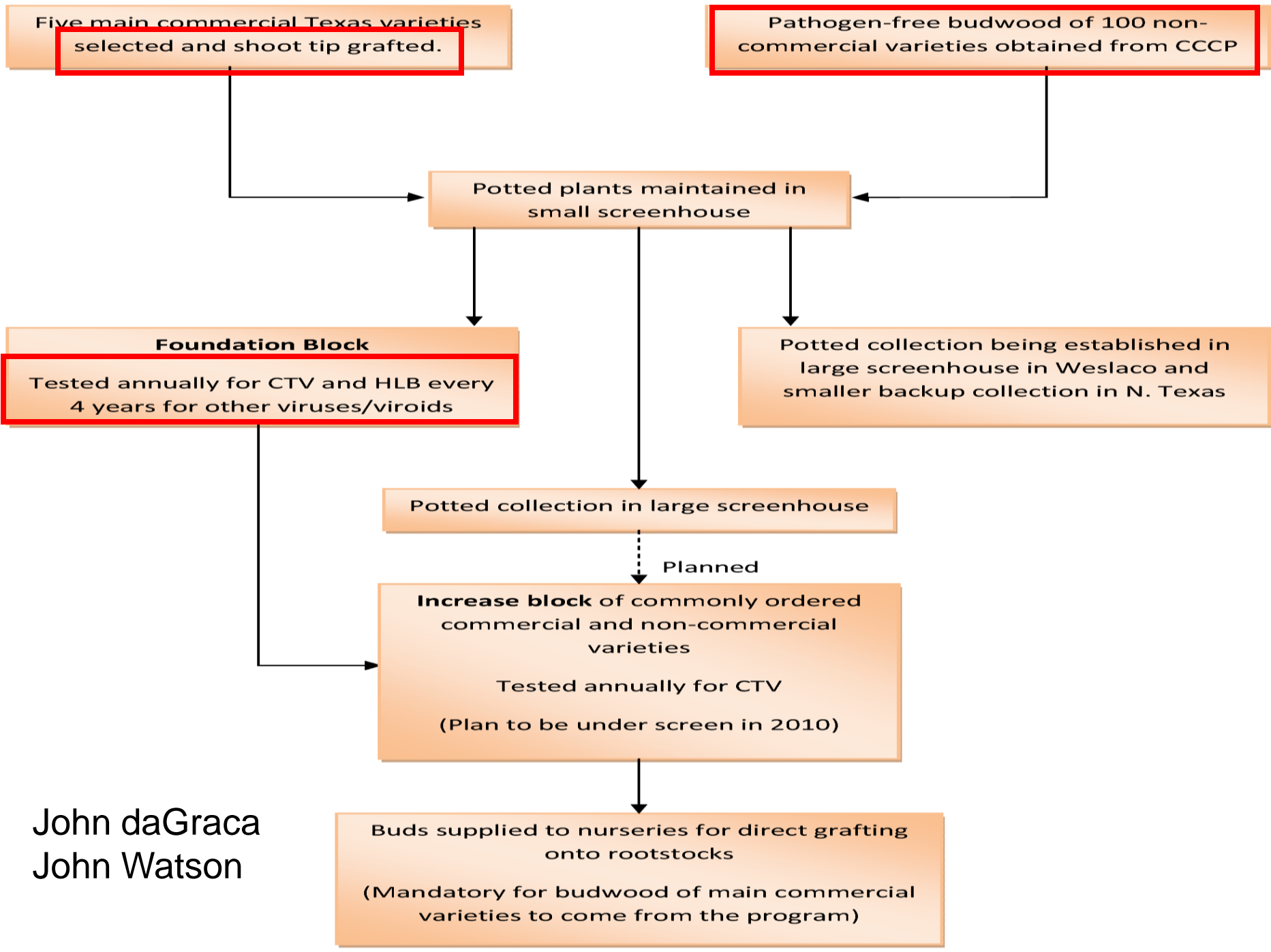
&

Local Selections Clean Up

**Texas A & M University-Kingsville
Citrus Center Program**

USA Citrus Germplasm Programs-Geography





John daGraca
John Watson

Arizona

Commercial Budwood Distribution

Certified Budwood Program

USA Citrus Germplasm Programs-Geography

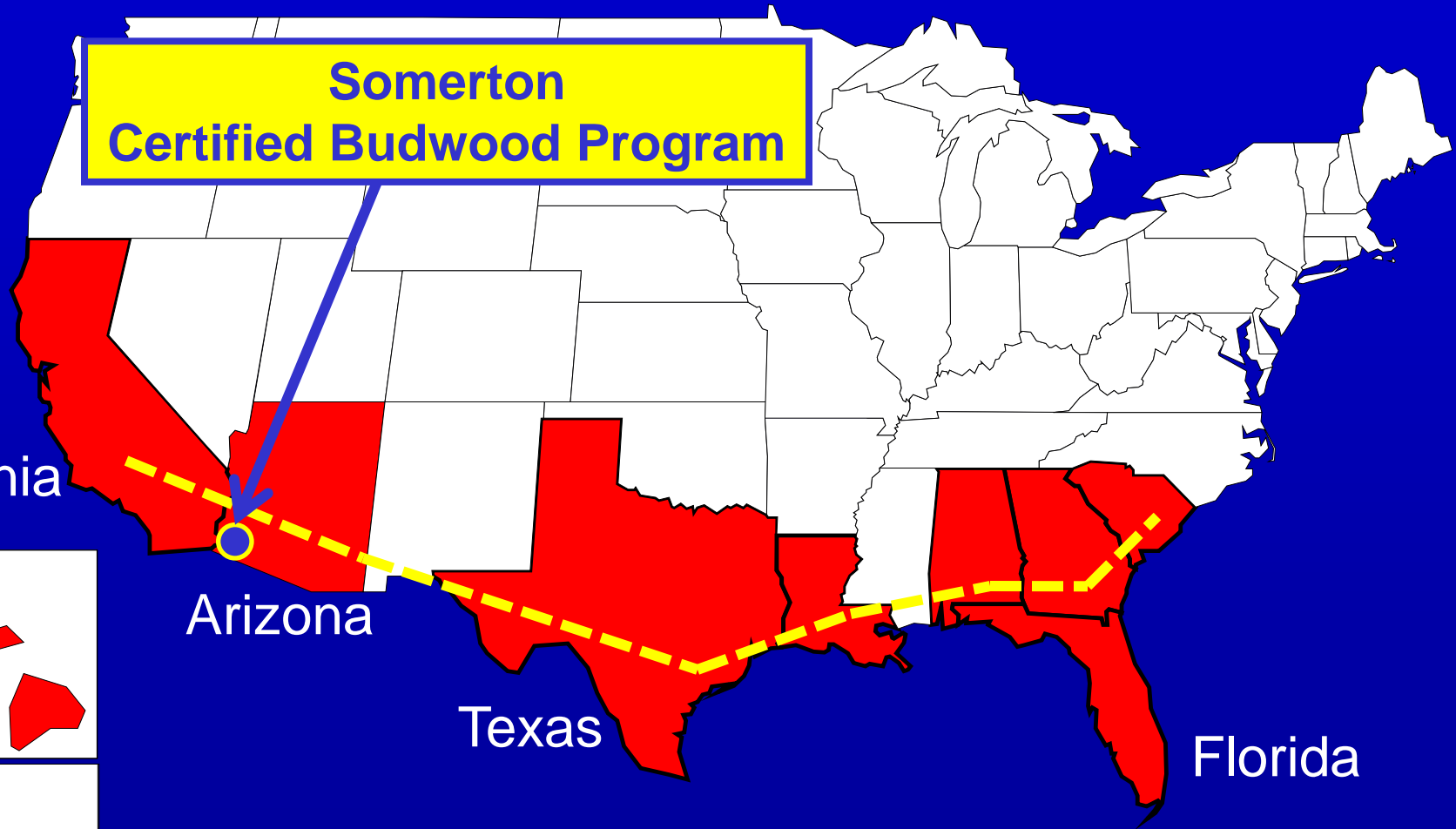
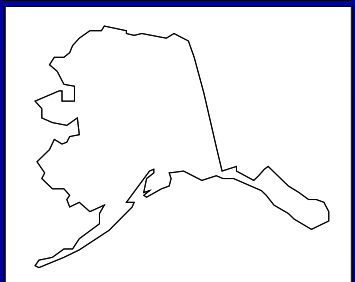
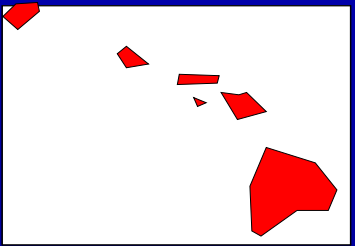
**Somerton
Certified Budwood Program**

California

Arizona

Texas

Florida



Arizona

- Arizona has a very small number of nurseries-4 in total
- The majority of the budwood needed for Arizona comes from California and the CCPP
- Most nurseries collect their budwood from their own increase trees
- The University budwood block distributes approximately 500 buds per year
- The block is occasionally tested for tristeza and citrus viroids, and so far there have been no positive findings..

John Loghry
Glen Wright

National

1. National Clonal Germplasm Repository For Citrus & Dates

**Quarantine - Variety Introduction
&
Budwood Distribution Free of Charge**

2. National Citrus Germplasm Passport Program

3. National Clean Plant Network

USA Citrus Germplasm Programs-Geography

Riverside: National Clonal Germplasm Repository Citrus & Dates

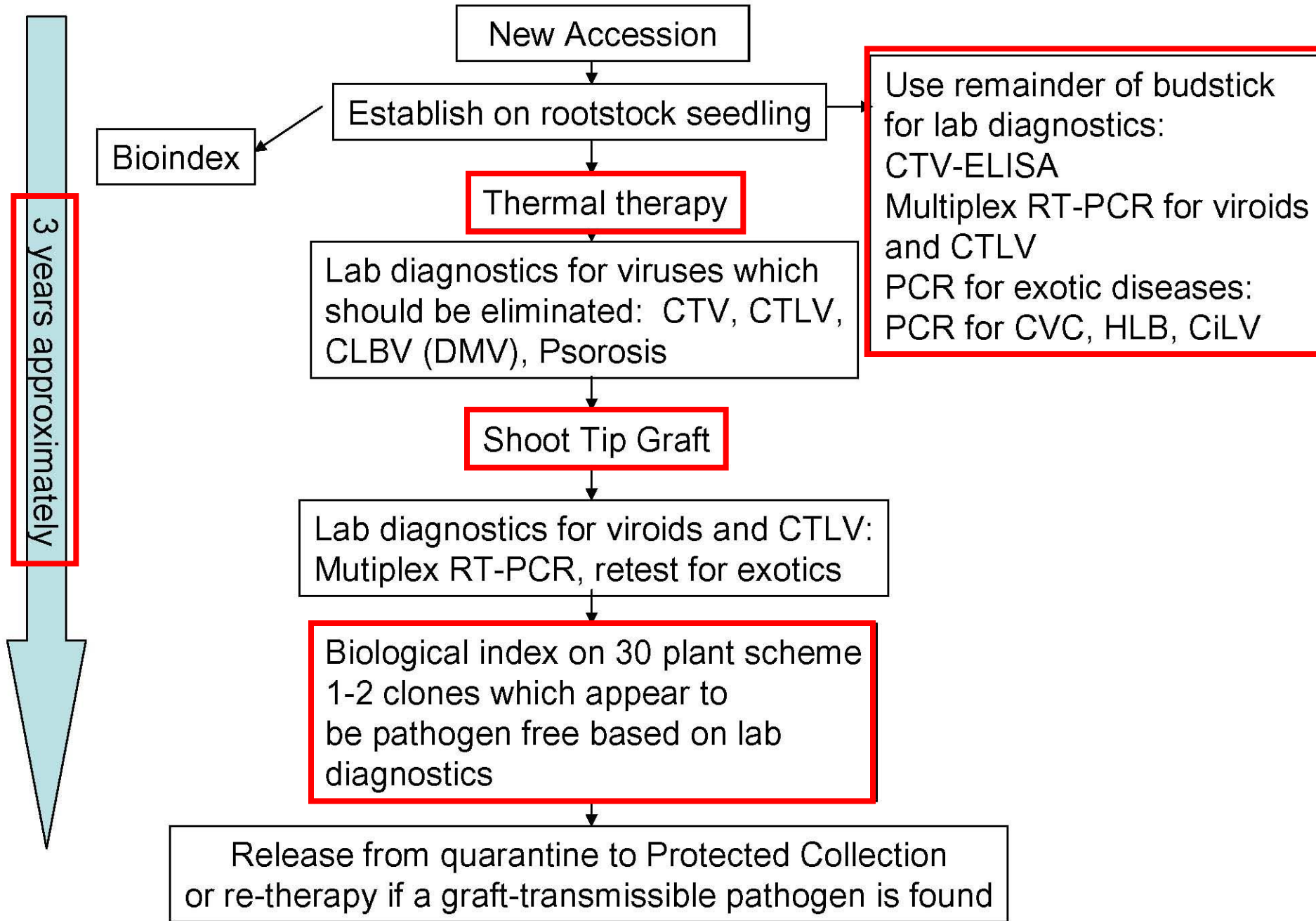


UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
IN COOPERATION WITH
UNIVERSITY OF CALIFORNIA, RIVERSIDE

**NATIONAL CLONAL
GERMPLASM REPOSITORY
FOR CITRUS AND DATES**

Mission

- Access, evaluate, maintain, preserve, and distribute germplasm of Citrus, Citrus relatives, and date palms
- Maintain informational files on accessions
- Conduct research that is compatible with primary mission



New Accession

Establish on rootstock seedling

Thermal therapy

Lab diagnostics for viruses which should be eliminated: CTV, CTLV, CLBV (DMV), Psorosis

Shoot Tip Graft

Lab diagnostics for viroids and CTLV: Mutiplex RT-PCR, retest for exotics

Biological index on 30 plant scheme 1-2 clones which appear to be pathogen free based on lab diagnostics

Release from quarantine to Protected Collection or re-therapy if a graft-transmissible pathogen is found

Bioindex

Use remainder of budstick for lab diagnostics:
CTV-ELISA
Multiplex RT-PCR for viroids and CTLV
PCR for exotic diseases:
PCR for CVC, HLB, CiLV

3 years approximately

Pathogen testing of accessions

- All *Citrus* accessions in the collection undergo indexing for these virus and viroid diseases:
 - Tristeza and seedling yellows Mexican Lime, ELISA
 - Exocortis Citron, seqPAGE, multiplex RT-PCR
 - Cachexia Parson's special mandarin, Citron-seqPAGE, multiplex RT-PCR,
 - Other viroids Citron-seqPAGE, multiplex RT-PCR
 - Psorosis MV Swt Orange, Dweet tangor, RT-PCR
 - Tatterleaf virus *C. excelsa*, Rusk, RT-PCR
 - Concave gum complex MV Swt Orange, Dweet tangor
 - Vein enation virus (woody gall) Mexican lime, Sour orange
 - Infectious variegation virus Sour orange, Mex. lime
 - Citrus leaf blotch virus [Dweet mottle virus] Dweet tangor, RT-PCR
 - Stubborn (Index, culture, PCR assay) MV Swt Orange, Culture-PCR, PCR directly
 - Huanglongbing (Greening)* PCR, use cloned PCR product as +
 - Leprosis* RT-PCR, use cloned PCR product as +
 - Citrus variegated chlorosis* PCR, use cloned PCR product as +
 - Phytoplasmas (several) PCR, sequence product
- Accessions with positive symptoms are treated by thermotherapy and/or shoot tip micrografting to eliminate the pathogen(s), then reindexed prior to repropagation.

* Lab-based tests on these exotic pathogens

NCGRCD Distributions

- Domestic & international
- Gratis to qualified requestors
- Utilization
 - Research
 - Certification
 - Other

NCGRCD & Certification

- Distribution of pathogen-tested propagative material
 - Programs
 - Nurseries
- Recognized by FAO as a source of high-health citrus propagative materials
- Non-tested materials (specific cases, eg Spain)
- Technical assistance: training, consultation, positive controls, etc



Riverside-California

USDA ARS National Clonal Germplasm Repository for Citrus and Dates

Richard Lee, Research Plant
Pathologist

Robert Krueger, Curator

Preserving Citrus Germplasm for Future Generations

USDA ARS National Clonal Germplasm Repository for
Citrus and Dates, Riverside



The National Citrus Germplasm Passport Program

National Citrus Germplasm Passport Program 2006

Citrus Germplasm Programs:

1. Citrus Clonal Protection Program (California)
2. Citrus Germplasm Introduction Program (Florida)
3. National Clonal Germplasm Repository for Citrus and Dates (National).

Other participants and supporters:

1. Florida Department of Agriculture and Consumer Services
2. California Department of Food and Agriculture
3. California Citrus Research Board
4. USDA APHIS Plant Protection and Quarantine

National Citrus Germplasm Passport Program

- Minimal standard of indexing and therapy procedures
- Transparency for movement of citrus germplasm among states
- Reduce time of introduction (typical time of variety introduction two or three years)
- Reduce the risk of pest and pathogen introduction
- Will help to set national standards for the U.S.A. citrus germplasm programs

National Citrus Germplasm Passport Program (NCGPP)

Example of Core Tests

DISEASE	PATHOGEN	CORE TEST
Tristeza	<i>Citrus tristeza virus</i>	Mexican lime, ELISA
Psorosis A & B	<i>Citrus psorosis virus</i>	Sweet orange, Dweet tangor
Concave gum	Unknown	Sweet orange, Dweet tangor
Infectious Variegation	<i>Citrus variegation virus</i>	Sour orange, Etrog citron, Eureka lemon
Tatter leaf	<i>Apple stem grooving virus</i> syn. Citrus tatter leaf virus	Rusk citrange/RL, <i>Citrus excelsa</i>
Leaf blotch	<i>Citrus leaf blotch virus</i>	Dweet tangor, RT-PCR
Dweet mottle	Dweet mottle virus	
Exocortis	<i>Citrus exocortis viroid</i>	Etrog citron, Arizona 861-S1/RL, sPAGE
Cachexia	<i>Hop stunt viroid</i>	
	HSVd citrus variants: CVd-IIb & -IIc: Cachexia	
and various citrus growth abnormalities	Five citrus viroids species	
Huanglongbing	<i>Candidatus Liberibacter</i> sp.	Sweet orange, PCR, Source plant observation
Variegated chlorosis	<i>Xylella fastidiosa</i>	PCR
Stubborn	<i>Spiroplasma citri</i>	Culture

The National Clean Plant Network

Will incorporate existing programs such as:

Citrus Clonal Protection Program

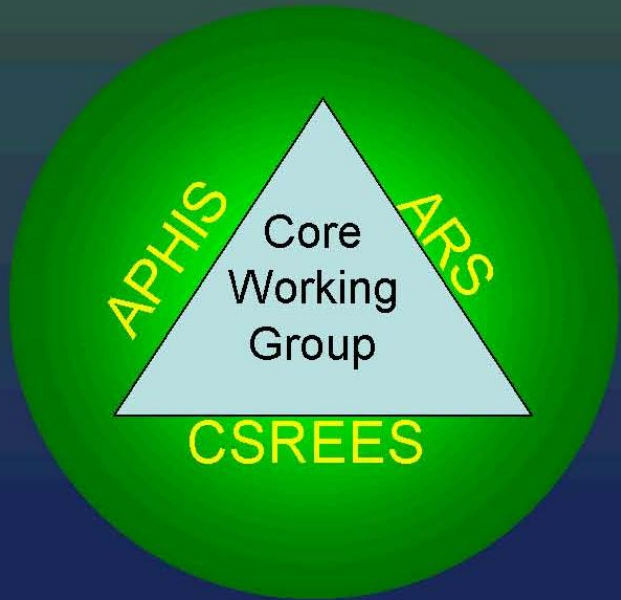
Citrus Germplasm Introduction Program

**National Clonal Germplasm Repository for Citrus &
Dates**

NCPN – A Brief History > 2005 - 2009

- **5 Existing U.S. Clean Plant Programs met at UC-Davis in 2005 to Discuss Early NCPN goals**
 - Establish Regional Clean Plant Centers
 - Initially focus on fruit crops
 - Secure sustainable funding
- **NCPN Steering Committee formed in 2006**
 - Identify and draft initial NCPN goals/objectives
 - Report on history of existing clean plant programs
 - Propose national workshop with a broader audience
- **NCPN Workshop – May 2007**
 - Discuss/refine goals and objectives
 - Draft Mission and Vision Statements
 - Draft a Strategic Plan
- **NCPN Implementation 2008**
 - NCPN 'Federal' MOU Developed
 - Farm Bill 2008
 - Stop-Gap Agency Funding Sources
- **NCPN Governance Formation 2008-2009**
 - Core Working Group and Tier 1 National Body
 - Tier 2 Commodity Bodies Chartered
 - Grapes
 - Tree Fruits (Pomes and Stone Fruits)
 - Tier 3 Regional Bodies Chartered
 - Grapes
- **NCPN General Meeting – March 2009**
 - National Governance Body Roll-Out
 - Develop and Ratify a Business Plan
 - NCPN Cooperative Agreement Funding Program

March 2009, Washington DC



NCPN MOU Signatory Parties

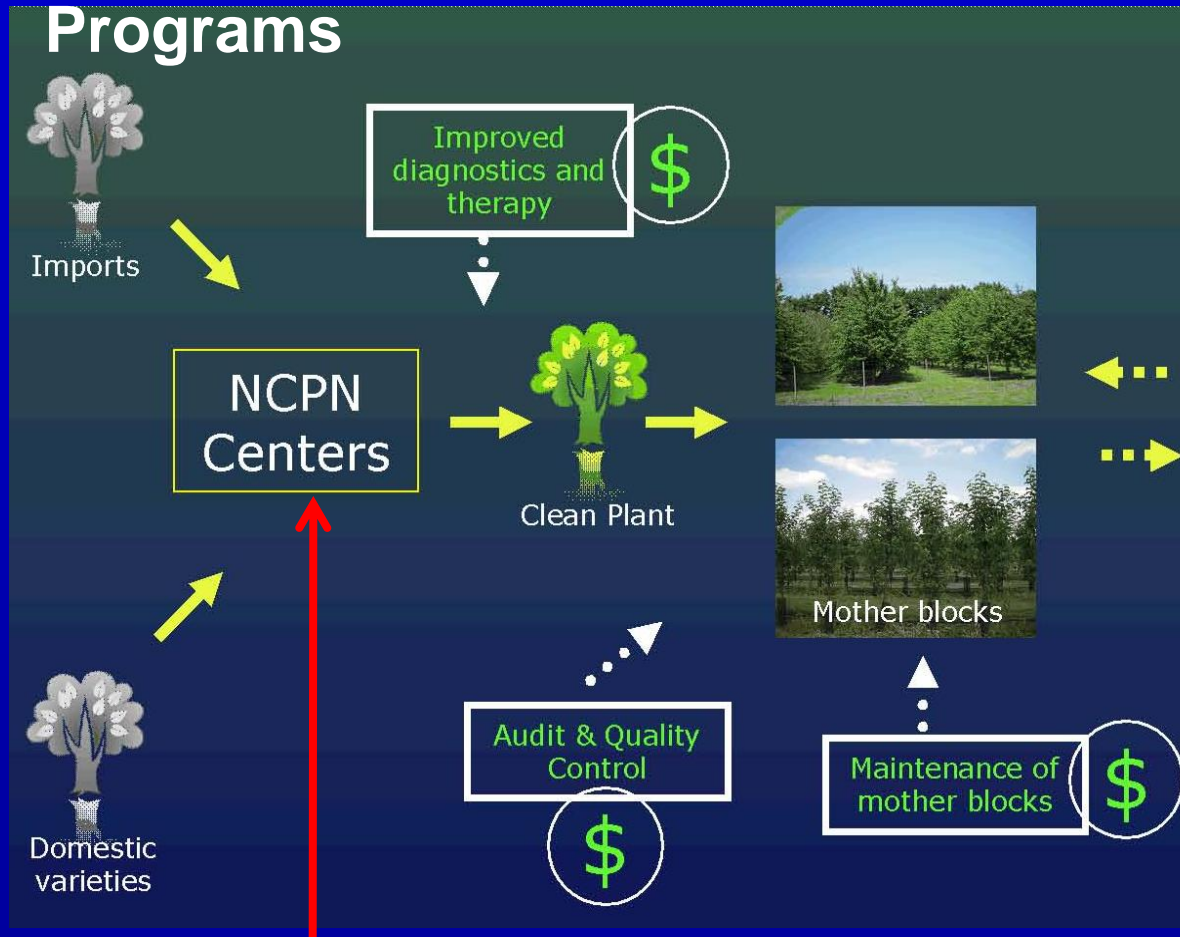
- Agricultural Research Service (ARS)
 - Dr. Edward Knipling; Administrator
- Cooperative State Research, Education, and Extension Service (CSREES)
 - Dr. Colien Hefferan; Administrator
- Animal and Plant Health Inspection Service (APHIS)
 - Mr. Kevin Shea; Acting Administrator

The National Clean Plant Network

	2009	2010	2011	2012
1. Grapes	✓	✓	✓	✓
2. Fruit tree	✓	✓	✓	✓
3. Citrus		✓	✓	✓
4. Berries		✓	✓	✓
5. Other Crops				✓

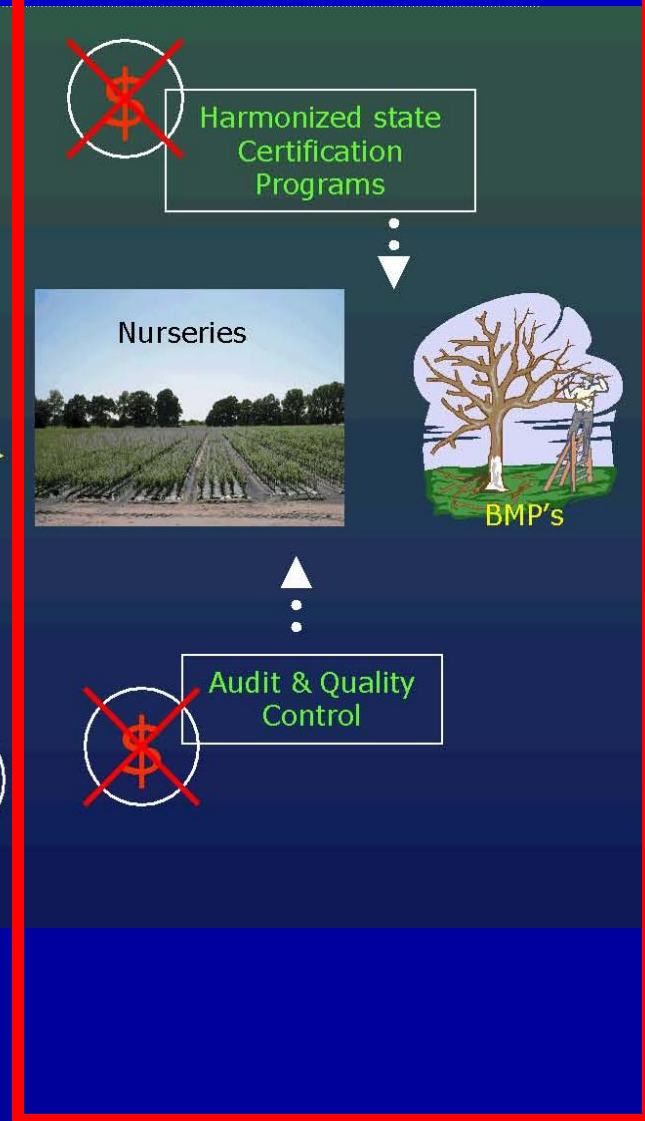
**\$5,000,000 per year for 4 years
(2009-20012)**

Existing G1 Programs



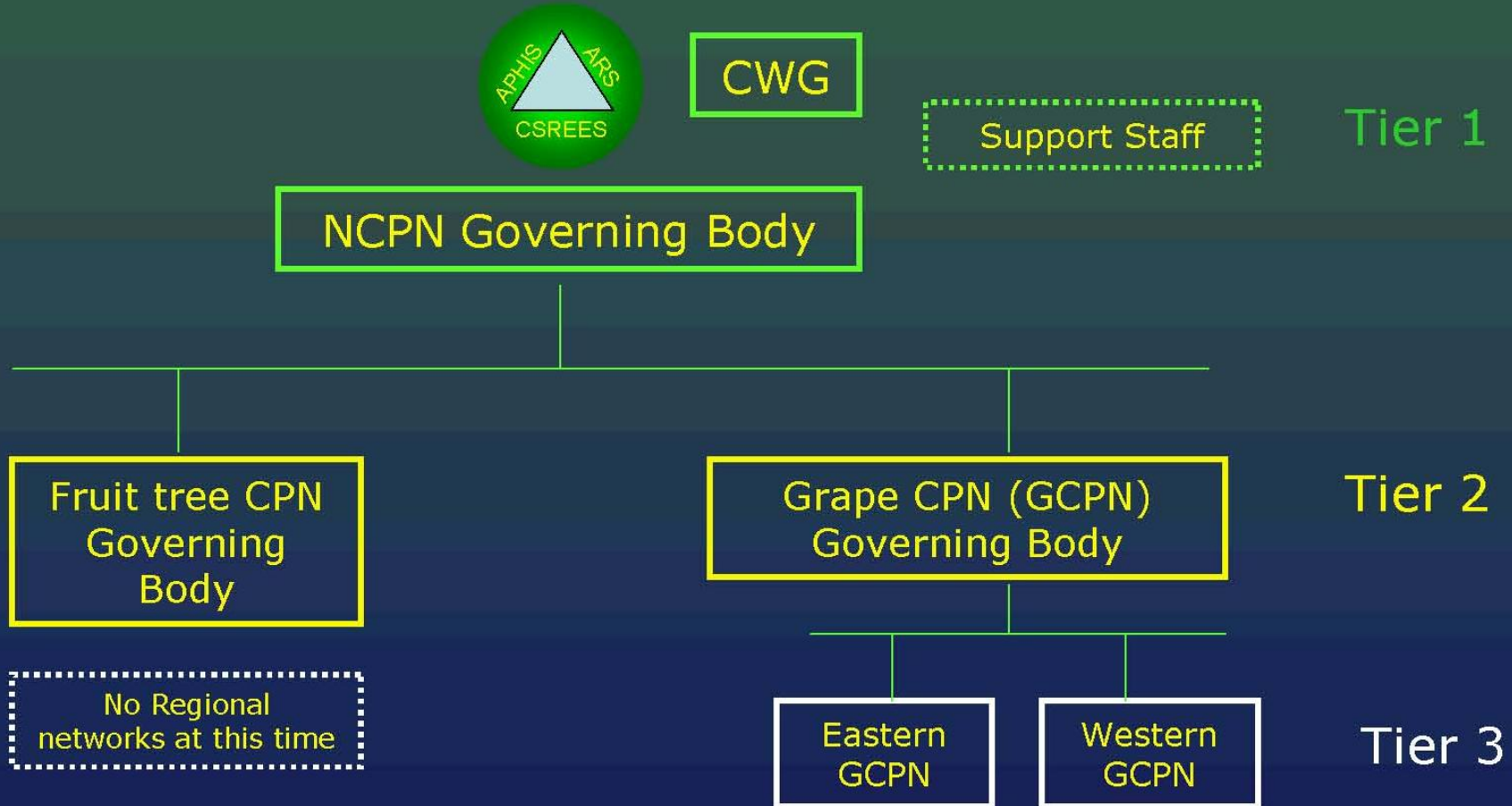
Quarantine Citrus Programs

G2 & G3 Programs



The National Clean Plant Network

Summary of NCPN Governance – Through March 2009



Tier 2
National Group for Citrus
California, Arizona, Texas, & Florida

Met June 30, 2009 in Florida to Elect Tier 2 for Citrus

The Principal Conclusion

Importance of Citriculture

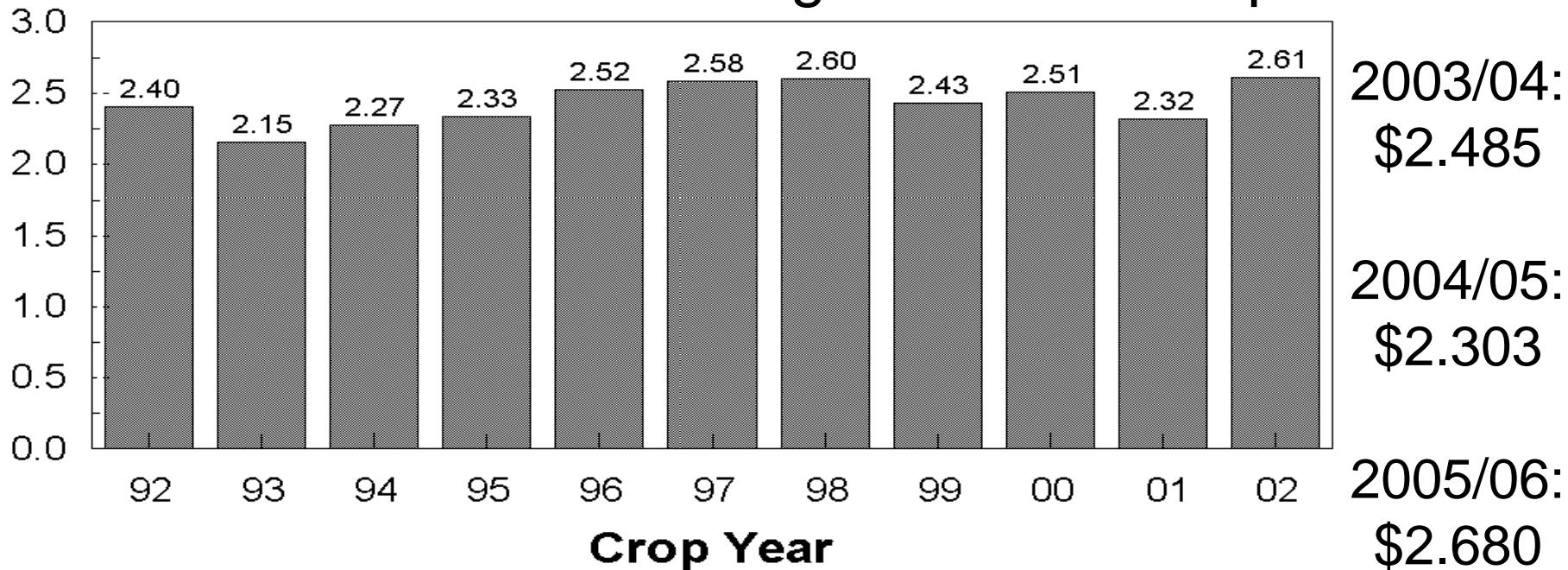
Human Nourishment, Numerous Products & Byproducts, Employment, & Revenue

USDA - National Agricultural
Statistics Service

U.S. Citrus

Value of Production

Billion Dollars Fresh Fruit Packinghouse-Door Equivalents



Why Only Disease Tested Budwood

- **Citrus diseases are not native, were introduced to the citricultural areas with infected propagative material**
- **Citrus viral diseases are not treatable at the field**
- **Citrus is a perennial crop**
 - >**different from annual crops - seed**
 - >**chronic infections**

Losses from Graft Transmissible Pathogens of Citrus

Citrus Tristeza alone :

50 millions trees dead world wide

- 1930s Brazil & California**
- 1940s Australia & New Zealand**
- 1960s Spain**

Loss:

- Value of the tree +**
- + Time lost from interrupted production**
- + Cost of replacement**
- + Cost of diagnosis**

Losses from Citrus Viruses

Dr. Reuther estimated:

- **More than 30 disorders of citrus caused by viruses**
- **25 - 100% yield restraint of CA orchards**
- **If not contained it will grow steadily worse**

- **Reduction of tree productive life**
 - i.e. Florida replanting <20 years**
- **Limitation of cultivation choices**
 - i.e. Japan primarily mandarins**

Rootstocks

Diseases & Pests

Citrus canker



Brown citrus aphid



Huanglongbing (HLB)



Asian citrus psyllid





Citrus sweet orange scab



Citrus leprosis



Citrus variegated chlorosis

Brazil: Estimated damage = US\$ 150.000.000/year



Sudden death

Brazil: Estimated damage = US\$ 40.000.000/year

Black spot

Brazil: Estimated damage = US\$ 10.000.000/year

Thank You



University of California, Riverside



Contact Information

- California, Citrus Clonal Protection Program
Georgios Vidalakis: vidalg@ucr.edu
- Arizona, Yuma Agriculture Center
Glen Wright: gwright@ag.arizona.edu
- Texas, Citrus Center
John daGraca: JDaGraca@ag.tamu.edu
- Florida, Citrus Germplasm Introduction Program
Wayne Dixon: dixonw@doacs.state.fl.us
- Florida, Bureau of Citrus Budwood Registration
Michael Kesinger: kesingm@doacs.state.fl.us
- National Clonal Germplasm Repository for Citrus & Dates
Robert Krueger: robert.krueger@ars.usda.gov