

Citrus Greening Research: the Value of Partnerships

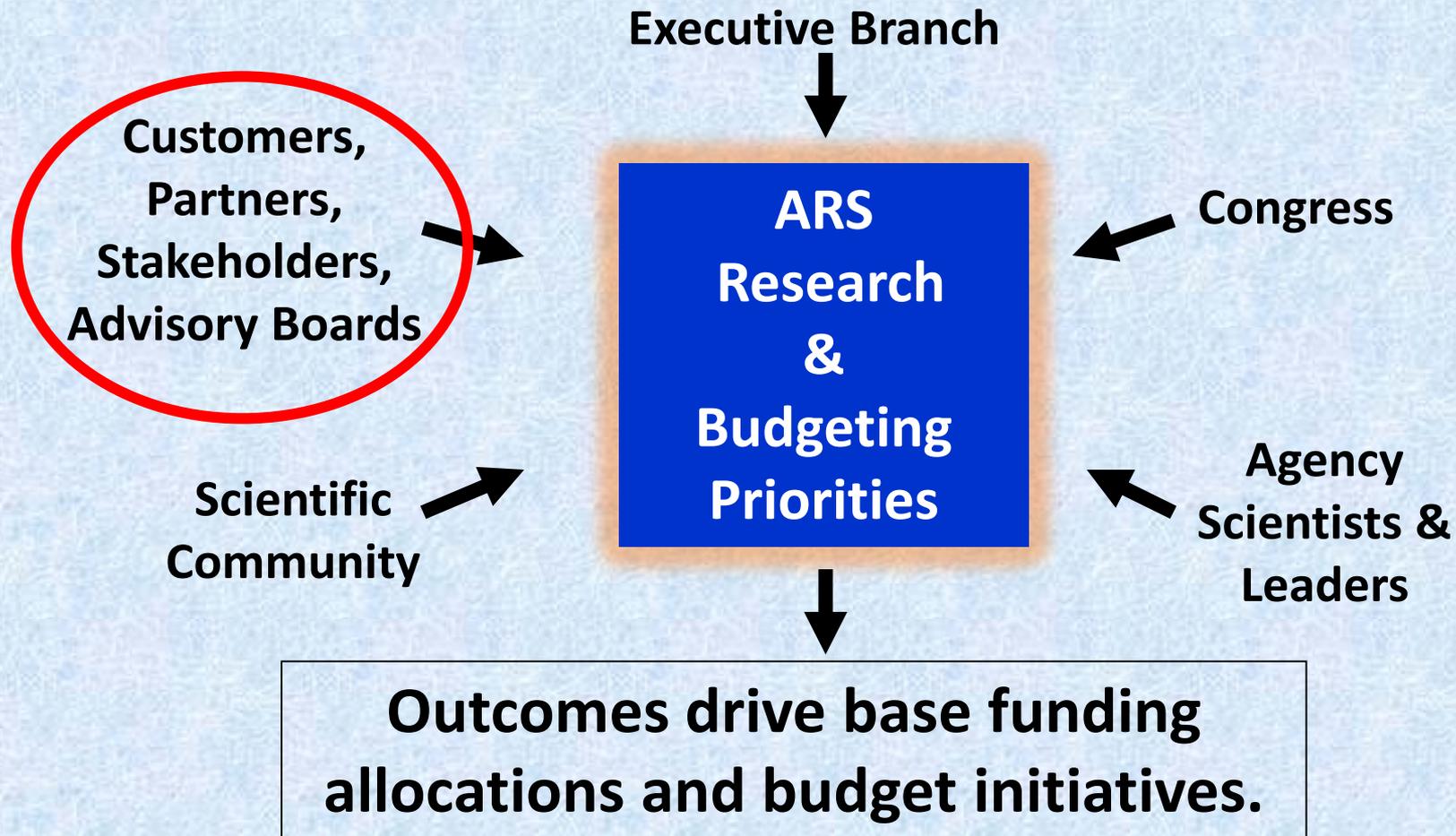


**Gail Wisler, National Program Leader
Crop Production and Protection
USDA-Agricultural Research Service**

Partnerships Essential for HLB Management

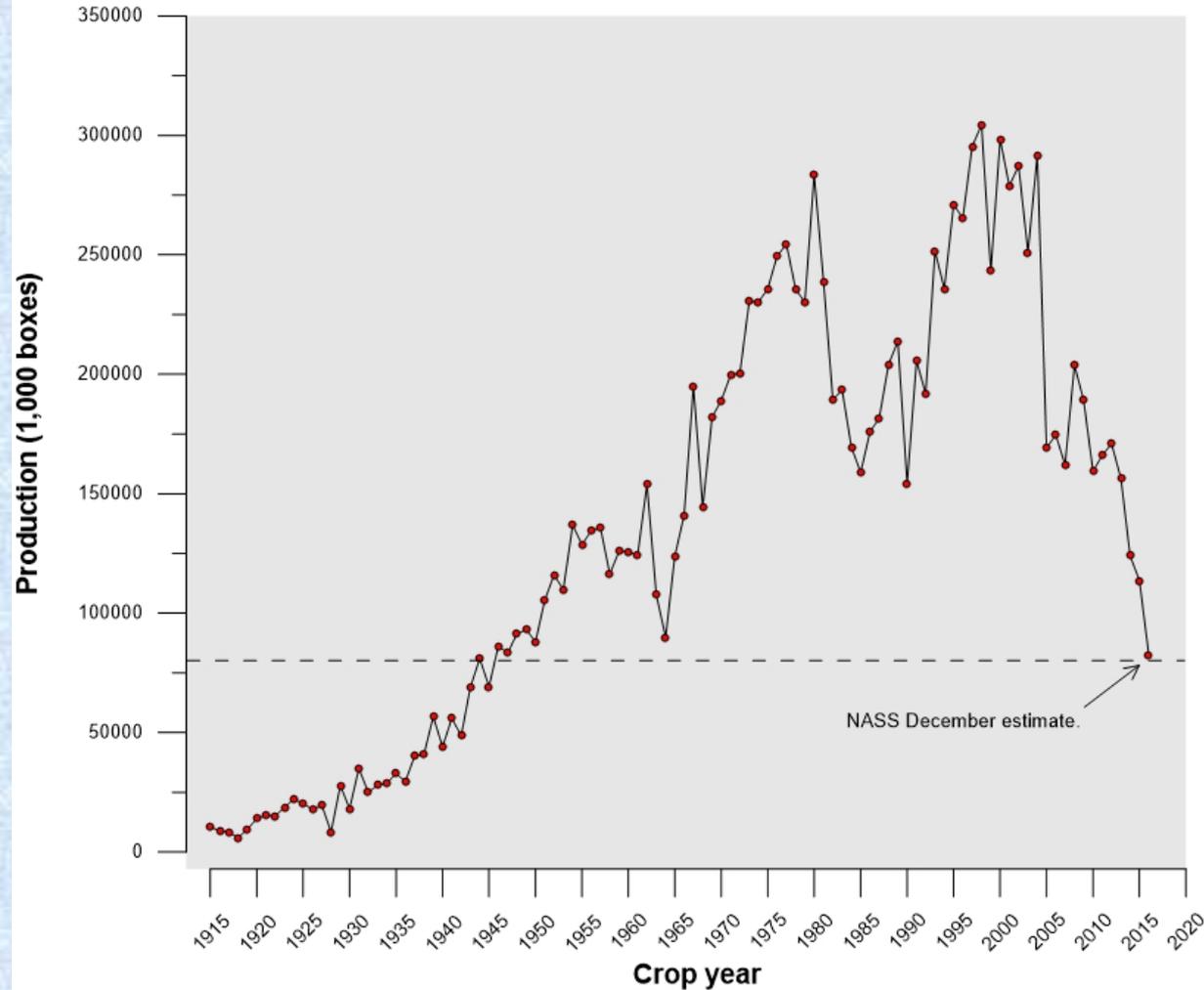
- 2008; ARS, APHIS, NIFA, citrus representatives from FL, CA, and TX joined forces against this disease.
 - Four consecutive years of Citrus Research Forum
 - Enhanced national collaboration
 - Resulted in NuPssyllid project funding (NIFA)
- Modest increases to ARS base funds received 2009
- Significant funding from CRDF and CRB
- HLB-MAC funding (APHIS)
- NIFA funds

Setting Research Priorities through Customer/Stakeholder Input



Current situation in Florida

Florida Total Citrus Production



- **ACP detected;
1998**
- **canker eradication
program stopped;
2006**
- **HLB detected;
2005**

This should never happen again!

Current tools available to growers:

- Insecticides; Area-wide control of vector (CHMA's)
- Antibiotics, emergency label expected **Spring, 2016**
 - Fireline®
 - Firewall®
- Infected tree removal
 - Not mandatory
 - HLB-MAC funded abandoned tree removal in selected CHMAs
- Early detection of “hot” psyllids; used by State and Federal regulatory
- “Nutritionals”

ARS Response to Citrus Greening

All Hands on Deck

Albany:

- Intragenic citrus

Wooster:

- Intelligent sprayer

Ithaca:

- Block ACP transmission*

Beltsville:

- antibodies to Liberibacter
- Citrus disease collection

Ft. Pierce (The Hot Zone)

- Block ACP transmission*
- Tolerant/resistant trees
- Biocontrol
- RNAi for ACP control
- Antimicrobials

Mayaguez:

- Vector not found above 600m



Parlier:

- Sequences of CLas

Riverside:

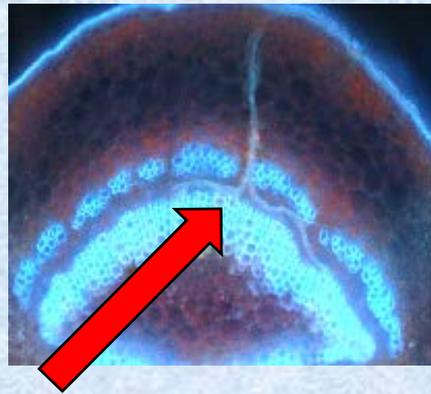
- Germplasm screened
- new assays developed



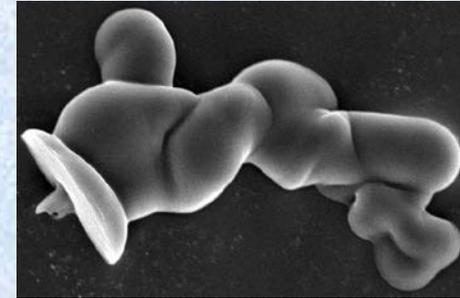
Block Psyllids from Feeding: Inhibiting Stylet Sheath Formation



Asian Citrus Psyllid (ACP)



Salivary sheaths in citrus leaves.



Isolated salivary sheath
from ACP fed on artificial diet

Proprietary materials used to treat leaf surfaces prevent polymerization of stylet sheath: psyllids cannot feed/transmit. R.Shatters.

New assay development



SmartDART and Visual PCR On-site Detection Systems for Testing Psyllids

Smart-DART™ platform with handheld hardware device to conduct diagnostic assays using isothermal nucleic acid amplification, under wireless control of user interface on a mobile Android computer.

Bluetooth

Smart-DART interface showing a graph with 'D&A' logo and assay results:

- Device RNB1-6649 running: test
- Reaction: 65°C 30 min, Denature: 95°C 5 min
- Vertical Scale Adjust: In Out
- Check wells to include on plot:
 - ✓ F1 (T = 16.5; F2 (T = 16.0; Well 1: plot A-14 tomato
 - ✓ Well 2: Negative Control
 - ✓ F1 (T = 16.0; F2 (T = 15.5; Well 3: plot B-46 habanero
 - ✓ Well 4: Negative Control
 - ✓ F1 (T = 15.5; F2 (T = 15.5; Well 5: Positive Control

SmartDART technology is being currently being used for testing of psyllids in some citrus groves in Riverside

M. Keremane, C. Ramadugu, R. Lee,
D. Jenkins, R. Kubota, D. Hall

Visual PCR Under Development



As compared to SmartDART with an initial investment of about \$3000, visual PCR requires an initial investment of less than \$300. Suitable for home owners and small growers

FireLine® & FireWall® Field Trials for HLB

Results from all Trials:

- **Two seasons** across three growing regions in the state
- With foliar applications of FireLine® 17 WP and FireWall® 50 WP – used alone or as a tank-mix combination
- Demonstrate these bactericides:
 - **Can significantly reduce CLas bacteria levels** in HLB-infected trees. (2 seasons includes: 37 field sites and 2,459 trees)
 - **Can significantly improve tree health metrics**, including reduced fruit and leaf drop, decreased dieback and Las titer, new growth
 - **Most Pronounced Effects:** Both products show **effectiveness against canker** in multiple metrics (5 sites, 550 trees)
 - Need to understand the factors effecting variability in response
 - Need evaluate effectiveness on new plantings.

Improved Citrus Biotechnology

Jim Thomson, ARS-Albany, funded by CRB

- **Site specific recombination systems for targeted gene integration and excision in plant cells.**
 - gene integration and gene stacking
 - marker gene removal to prevent gene flow
- **Citrus-derived gene expression control elements** (promoters/enhancers/terminators/insulators) that facilitate trait development in crop plants
- Isolate and characterize novel **tissue-specific promoters** and transcription terminators
- 123 transgenic lines from Carrizo and all 15 sweet orange lines were confirmed to each contain a single copy of the transgene; J. Thomson, E. Stover.

Heping Zhu; Wooster, OH

Intelligent Sprayer



Living with HLB through IPM

- **ACP management**
 - Biocontrol
 - RNAi
 - Block transmission
 - Lures, attract and repel
- **Crop improvement**
 - Commercial cv evaluation
 - Conventional breeding
 - Biotech approaches
 - Trans- and intragenics
 - Sharing pollen btw/ UF and ARS
- **Soil quality, Phytophthora**
 - (J. Graham, UF)
- **Therapy for infected trees**
 - Thermal
 - Antimicrobials
- **Early detection, eradication**
 - Direct assay from ACP*
 - PCR derivations, SmartDart, etc.
 - Canines
 - Infected tree removal
- **Juice and fruit quality**
 - Blending
 - ID off-flavors
- **Optimized spray technology**
 - Reduce cost by 50-70%