Stover 18-058C

# Fort Pierce Field Test Site for Validating HLB and/or ACP Resistance

PI: Ed Stover
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Collaborators: Jude Grosser, David Hall, Fred Gmitter, Kim Bowman, TAMU (was E. Mirkov), Mike Roose, Chandrika Ramadugu, Malcolm Smith, Gloria Moore, Jeffrey Jones, Zhonglin Mou, Tim McNellis, 2Blades Foundation

Duration of Requested Funding: 3 years
Total Requested Budget: $287,950

Annually:
$64,458 one tech
$31,525 farm acreage charges for ten acres

Project justifications:

- There is no HLB- resistance within cultivated *Citrus*
- Transgenic citrus offers the greatest potential for HLB-immunity/strong resistance, and has the huge benefit that:
  - transgenics can be created from high-demand cultivars
  - CLas-killing rootstocks MAY provide protection to conventional susceptible scions
- Research has shown that there is resistance in other genera in the citrus gene pool
- This project provides a controlled research site for testing HLB/ACP resistance/tolerance with high exposure to CLas and ACP.
- Provides care of plants, help with planting, liaison with all programs using the site, and some sample collection
- When desired, inclusion on Stover BRS permit with associated reporting, compliance visits, and BRS negotiations.

# Transgenics

- Over the previous iterations of this project, transgenics from five different research programs have been maintained, with UF and ARS dominating area.
- Total area of transgenics had reached 5 acres
- 1.75 acres have been pushed for new plantings
- Five more researchers have requested that I add their material to our permit and plantings will occur when this project is approved
- ARS transgenics have evolved to point where we have material that virtually eliminates CLas, some newly in field and some propagated in GH for field next year.  Budwood of best at DPI


USDA, ARS, USHRL

# UF Grosser transgenics on left, ARS Bowman on right

# UF Gmitter & Grosser, ARS-Stover transgenics

# Open rows ready for planting

The collection of this information is authorized by the Plant Protection Act of 2000. The information will be used to determine eligibility to receive all types of permits. No permit will be issued until this application has been approved.

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
BIOTECHNOLOGY REGULATORY SERVICE

# APPLICATIONS FOR PERMIT OR COURTESY PERMIT UNDER 7 CFR 340

*(Genetically Engineered Organisms or Products)*

## 1. NAME, ADDRESS, TELEPHONE, AND EMAIL OF APPLICANT

**Name:** Eddie Stover
**Position:** Research Horticulturist
**Organization:** USDA / ARS
**Organization Unique ID:**
**Address:** 2001 S Rock Rd
USDA/ARS US horticultural
Research Lab
Ft. Pierce, FL 34945
**County/Province:**
**Township/Island:**

**Day Telephone:** 772-462-5951
**FAX:** 772-462-5961
**Alternate:** 772-971-8990

**Email 1:** ed.stover@ars.usda.gov
**Email 2:**

## 2. INTRODUCTION TYPE

- [ ] Importation
- [ ] Interstate Movement
- [ ] Interstate Movement and Release
- [x] Release

## 3. PERMIT TYPE

- [x] Standard Permit
- [ ] Courtesy Permit

## 4. PURPOSE OF PERMIT

- [ ] Industrial Product
- [ ] Pharmaceutical Product
- [ ] Phytoremediation
- [x] Traditional

## 5. CONFIDENTIAL BUSINESS INFORMATION VERIFICATION (CBI)

**Does this application contain CBI?** [ ] Yes [x] No
**CBI Justification:**
N/A

## 6. REQUEST TYPE

[x] New [ ] Amendment [ ] Renewal [ ] Variance [ ] Amendment, Renewal and/or Variance
**Amendment/Renewal Description:**
**Previous Permit Number(s):**

## 7. MEANS OF MOVEMENT

Transgenic citrus plants in 4-inch citripots will be taken from a certified greenhouse and transported to the field site (from University of Florida 230 miles, or Ft. Pierce 2 miles) in a closed truck. Transgenic plants will be planted and maintained by standard practices.

## 8. VARIANCE VERIFICATION

**Have you previously applied for variance(s) that you wish to apply to this permit?** [ ] Yes [x] No

**10. ARTICLE SUPPLIER AND/OR DEVELOPER**

| Name | Location | Contact Information |
|---|---|---|
| Dr. Jeffrey Jones | U of Florida Dept of Plant Pathology<br>2550 Hull Rd<br>U of Florida Dept of Plant Pathology<br>Rm. # 2553 Fifield Hall<br>Gainesville, FL 32611 | **Day Telephone:** 352-273-4673<br>**FAX:**<br>**Email:** jbjones@ufl.edu |
| Dr. T. Reuber | Two Blades Foundation<br>1630 Chicago Avenue,<br>Suite 1901<br>Evanston, IL 60201 | **Day Telephone:** 847-425-1277<br>**FAX:**<br>**Email:** tlr@2blades.org |
| Dr. T Mirkov | 2415 E. Hwy 83<br>Weslaco, TX 78596 | **Day Telephone:** 956-969-5628<br>**FAX:**<br>**Email:** e-mirkov@tamu.edu |
| Dr. Ed Stover | USDA/ARS, US Horticultural Research Laboratory<br>2001 S. Rock Rd.<br>St. Lucie<br>Ft Pierce, FL 34945 | **Day Telephone:** 772-462-5951<br>**FAX:** 772-462-5961<br>**Email:**<br>ed.stover@ars.usda.gov |
| Dr. Randall Niedz | USDA/ARS, US Horticultural Research Laboratory<br>2001 S. Rock Rd.<br>St Lucie<br>Ft Pierce, FL 34945 | **Day Telephone:** 772-462-5919<br>**FAX:** 772-462-5961<br>**Email:**<br>randall.niedz@ars.usda.gov |
| Dr. Kim Bowman | USDA/ARS, US Horticultural Research Laboratory<br>2001 S. Rock Rd.<br>St. Lucie<br>Ft Pierce, FL 34945 | **Day Telephone:** 772-462-5920<br>**FAX:** 772-462-5961<br>**Email:**<br>kim.bowman@ars.usda.gov |
| Dr. Timothy McNellis | 318 Buckhout Lab<br>Plant Pathology & Environmental Microbiology Department<br>University Park, PA 16802 | **Day Telephone:** 814-863-7646<br>**FAX:** 814-863-7217<br>**Email:** twm4@psu.edu |
| Dr. Zhonglin Mou | University of Florida<br>Rm. # 1249 Microbiology Building 981<br>Gainesville, FL 32611 | **Day Telephone:** 352-392-0285<br>**FAX:**<br>**Email:** zhlmou@ufl.edu |
| Dr. Yongping Duan | USDA/ARS, USHRL<br>2001 S Rock Rd<br>Fort Pierce, FL 34945 | **Day Telephone:** 772-462-5840<br>**FAX:**<br>**Email:**<br>yongping.duan@ars.usda.gov |

# Negotiating with BRS

## Their initial conditions for new permit

- (1) A. **Flowering regulated GE citrus trees must not be located within 1 mile of nonregulated citrus seed breeding where sexual seed is saved**, except as provided in (1) A.i. and (1) A.ii.
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- i. **Nonregulated citrus may be used to produce zygotic seed if it is at least 500 ft. from the GE citrus (as measured from outside of tree canopy); only if all flowers are bagged where the seeds are saved**. During the days these flowers are receptive to pollen the bags will be checked daily to ensure there are no breaches. All nonregulated citrus fruit not bagged must be treated as regulated and destroyed as provided under conditions No. 2 or No. 3 AND No. 4 AND No. 5.
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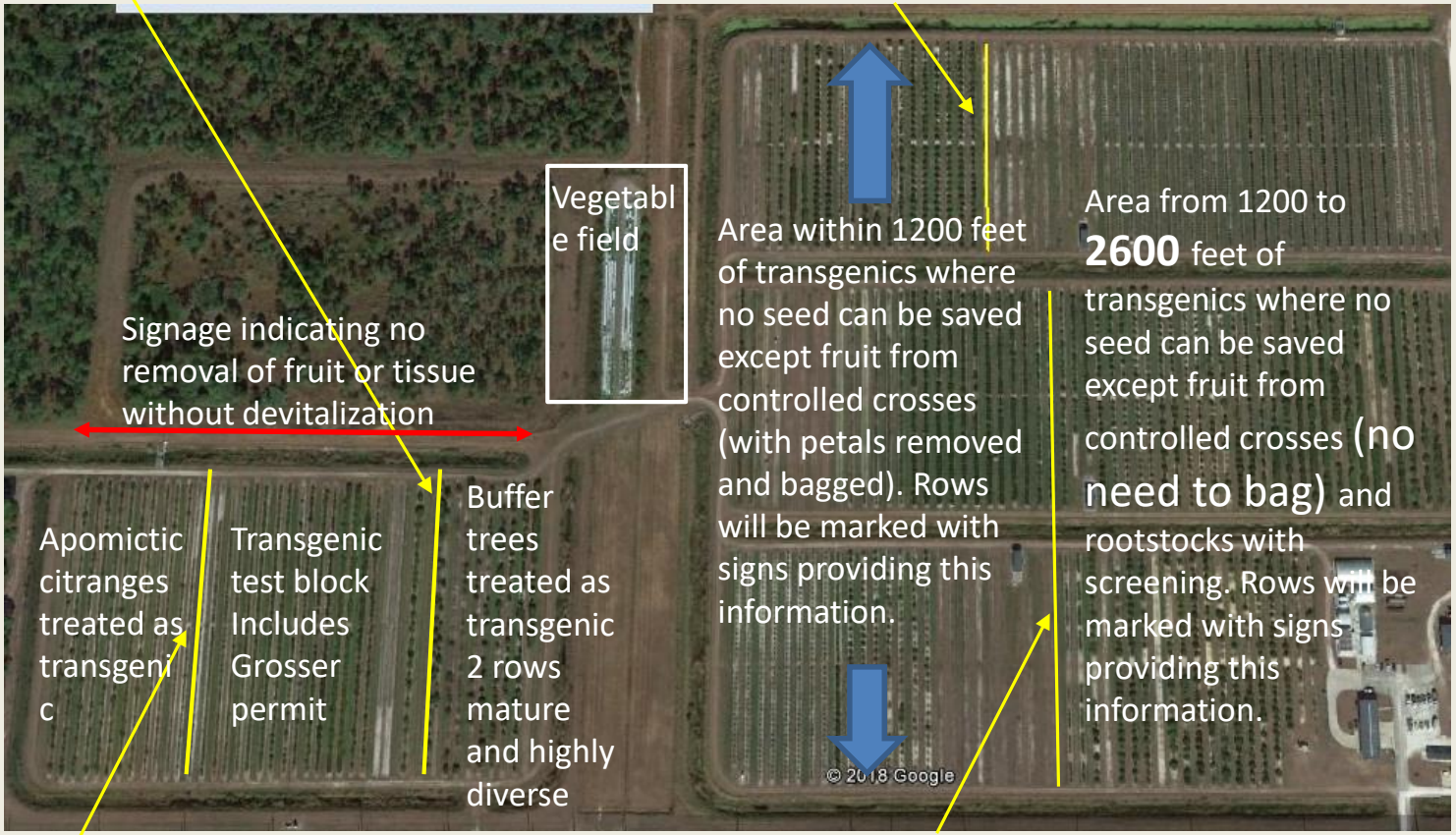
- ii. **Nonregulated citrus may be used to produce seed for rootstock breeding without bagging flowers if it is at least 1,200 ft. from the GE citrus (as measured from outside of tree canopy); only if seeds that are saved are screened to determine that they are devoid of transgenic material.**

Row 10 from east Block 8
N 27.435162 -80.432834 S 27.433628 - 80.432834

Row 38 from east Block 1
N 27.437729 -80.429466 S 27.436469-80.429466




**Changed to allow no restrictions beyond 2600 ft and after this year all rootstock seed trees will be beyond this distance**

Row 31 from east Block 8
N 27.435162 -80.434318 S 27.433480 -80.434318

Row 33 from east Block 2; Row 21 from east block 3
N 27.436232 -80.429087 S 27.433515 -80.429087

**Effect of different isolation distances on breeding at Picos Farm**

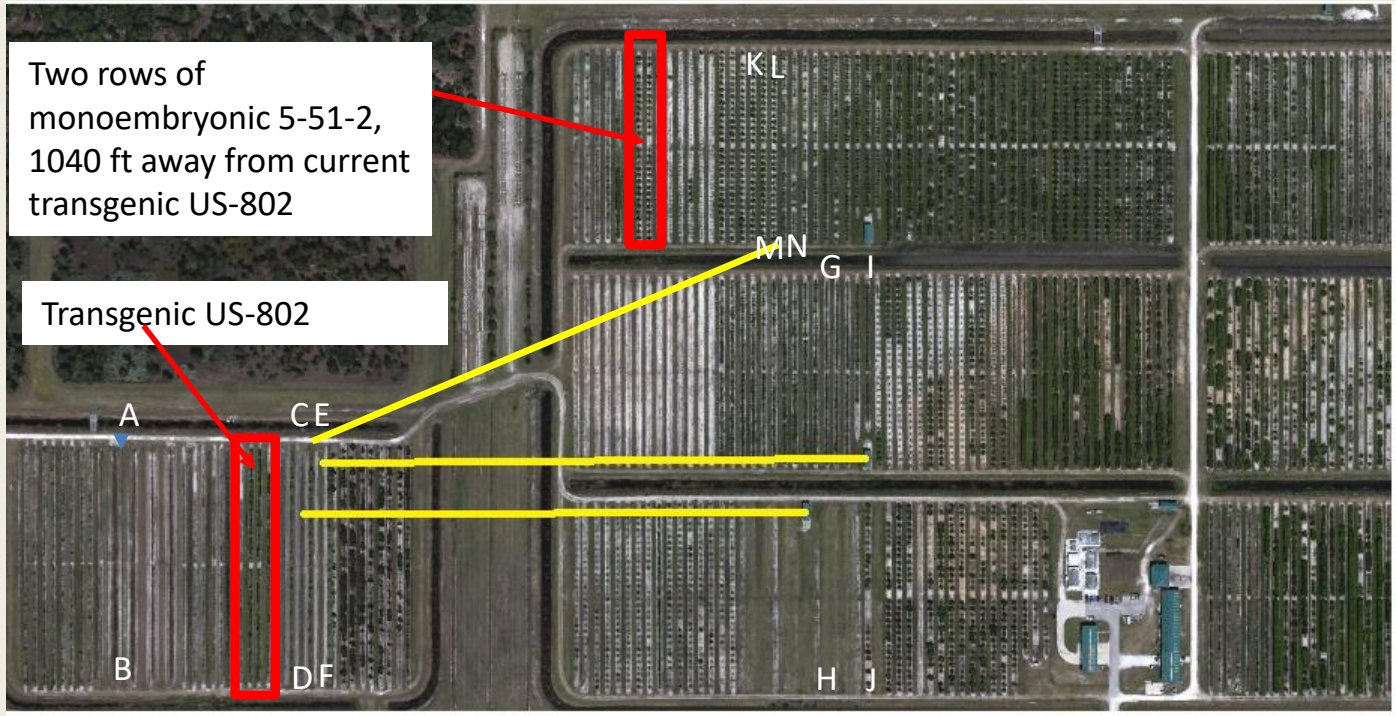
**One mile would mean no crosses without bagging anywhere and all rootstock seed would have to be screened for transgenes**

Longest line 3120 ft (1 km) from closest GE: row 43 block 5

Shorter line 2600 ft (1/2 mile) from closest GE: to row 20 block 5

Shortest line 2000 ft from closest GE: to row 4 from east block 2

# More moderate isolation contingent on pollen flow study




Picos farm with 1200 ft lines from irrigation to transgenic E edge

A: NW boundary, row 31 from East
B: SW boundary, row 31 from East
C: NW Boundary 1, row 13 from E (current UF)
D: SW Boundary 1, row 13 from E (current UF)
E: NW Boundary 2, row 11 from E transgenics
F: SW Boundary 2, row 11 from E transgenics
G: NE Boundary 1, block 2 row 32 from E, block 3 row 20 from E
H: E Boundary 1, block 2 row 32 from E, block 3 row 20 from E
I: NE Boundary 2, block 2 row 30 from E, block 3 row 18 from E
J: SE Boundary 2, block 2 row 30 from E, block 3 row 18 from E
K:NE Boundary 1, block 1 row 40 from E : L Boundary 2 row 38
M: SE Boundary 1, block 1 row 40 from E :N Boundary 2 row 38

# Planting of seed from 85 diverse citrus and relatives from NCGR gene bank

- Showed *Poncirus* among most-resistant to HLB and also psyllid colonization (Hall et al.)
- *Eremocitrus* and *Microcitrus*, also showed strong Las and psyllid resistance (Ramadugu et al)
- No resistance in *Citrus* but tolerance associated with citron in the pedigree (Stover et al.)

**Non-Citron Background**

Egami Buntan pummelo

C. maxima

Robinson

Pineapple Sweet Orange

Scarlet Emperor mandarin

Volkamer Lemon Hybrid

Diamante citron

S Coast Field Station Citron

Santa Barbara Rangpur

# Regressions: Tolerance and % Citron in Pedigree

Miles, Stover, Ramadugu, Keremane, Lee

| | % Citron | | |
|---|---|---|---|
| | $r^2$ | P-value | Slope |
| Canopy density | 0.32 | $1.0E^{-06}$ | + |
| Overall health | 0.30 | $3.0E^{-06}$ | + |
| Canopy volume | 0.42 | $5.0E^{-09}$ | + |
| Trunk diameter | 0.57 | $2.0E^{-13}$ | + |
| % leaves mottled | 0.20 | 0.002 | + |

- Initiated breeding of acid fruit adapted to Florida
- Beginning to introgress citron into standard market types
- Hortsci. 52:31-39

# UF Gmitter-led project on Citranges in 8th year- just identified 4 QTL associated with HLB-tolerance and manuscript accepted

Stover included USDA selections with Poncirus in pedigree, some with decent fruit quality and collecting data for potential juice usage in Baldwin-NIFA

# UCR Ramadugu-led project on HLB-resistance in Poncirus and hybrids from NCGR-CD in 6th and final year

# Stover/Smith (Queensland) project on inheritance of HLB-resistance from Microcitrus

# Malcolm Smith has most advanced program on breeding with Microcitrus- wide range of pedigree % and some with mandarin-like fruit

# UF Gmitter-led project on HLB-resistance from Citrus latipes. Only Citrus reported to be resistant

So far:

- Regularly remind the researcher community that we are accepting plantings with a good chance of providing the industry with HLB-resistant planting stock
- Have never turned down a request
- Plantings at full 10 acres specified in proposal – half transgenics now, but proportion will increase this year and likely into the future
- Where appropriate, some plantings have been used for additional projects such as ACP colonization, canker susceptibility, freeze tolerance

## Refereed publications from this project:


Huang, M., M.L. Roose, Q. Yu, D. Du, Y. Zhang, Z. Deng, M. Irey, E. Stover, and F.G. Gmitter Jr. 2018. Construction of high-density genetic maps and detection of QTLs associated with Huanglongbing infection in citrus. J. Exp. Biol. (in press).

Hall, D.G. C, Ramadugu, M.G. Hentz, F.G. Gmitter, Jr., and E. Stover. 2018. Survey of *Poncirus trifoliata* hybrids for resistance to colonization by asian citrus psyllid. Florida Ent. (in press).

Hall, D.G. M.G. Hentz, and E. Stover. 2017. Field survey of Asian citrus psyllid (Hemiptera: Liviidae) infestations associated with six cultivars of *Poncirus trifoliata*. Florida Ent. Soc. URL: http://www.bioone.org/doi/full/10.1653/024.100.0328

Dutt, M., G. Barthe, M. Irey, and J. Grosser. 2015 Transgenic citrus expressing an Arabidopsis NPR1 gene exhibit enhanced resistance against huanglongbing (HLB; citrus greening). *PloS one*, *10*(9), e0137134.

Miles*, G., E. Stover, M. Keremane, C. Ramadugu, and R.F. Lee. 2016. Tolerance to huanglongbing in a Florida field planting of diverse citrus relatives. HortScience 52:31-39.

Ramadugu, C., M. Keremane, S. Halbert, Y-P. Duan, M.L. Roose, E. Stover, R. Lee. 2016. Long term field evaluation reveals HLB resistance in *Citrus* relatives. Plant Dis.100:1858-1869.

Inch*, S., Stover, E., Driggers, R., and Lee, R.F. 2014. Freeze responses of Citrus and Citrus–related genotypes in a Florida field planting. Hortscience 49:1010-1016.

Stover, E.W., M.L. Richardson, R. Driggers, D.G. Hall, Y.P. Duan and R.F. Lee. 2013. Incidence and severity of asiatic citrus canker on *Citrus* and *Citrus*–related germplasm in a Florida field planting. Hortscience 49:4-9.

Richardson, M.L., C.J. Westbrook, D.G. Hall, E.W. Stover, Y.P. Duan and R.F. Lee. 2011. Abundance of citrus leafminer larvae on *Citrus* and *Citrus*–related germplasm. HortScience 46:1260-1264.

Westbrook, C.J., D.G. Hall, E.Stover, Y.P. Duan and R.F. Lee. 2011. Colonization of *Citrus* and *Citrus*-related germplasm by *Diaphorina citri* (Hemiptera: Psyllidae). HortScience 46:1-9.