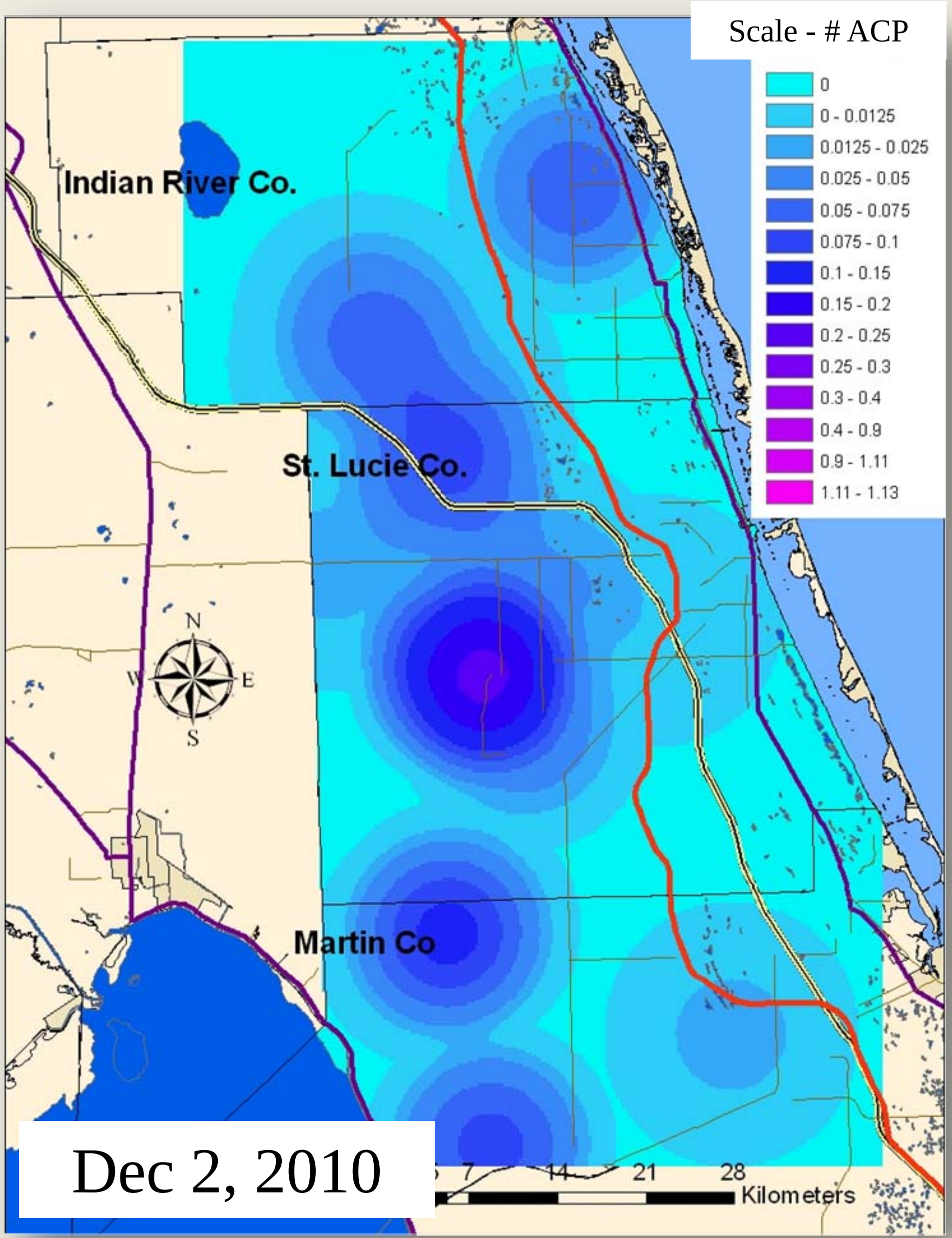
Current Research Activities on Asian Citrus Psyllid at USDA-ARS in Fort Pierce

David Hall
Research Leader, Subtropical Insects Research Unit

Area-wide management of Asian citrus psyllid

Growers in Florida are adopting area-wide management strategies for ACP.

Indian River Citrus Pilot Project



Of interest is the influence of ACP host plants in urban areas on populations of the ACP in commercial citrus.

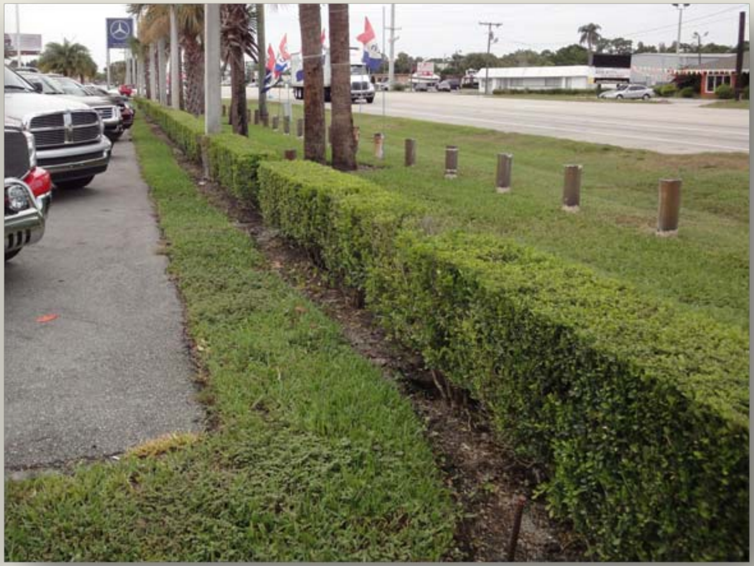
Orange jasmine (*Murraya paniculata*) is a member of the plant family Rutaceae and is a favored ACP host plant.

Orange jasmine is widely grown as an ornamental landscape plant in Florida.

Photo: Forest and Kim Starr

Biological control of Asian citrus psyllid (*Diaphorina citri*) in Florida by the parasitoid *Tamarixia radiata* in urban plantings of orange jasmine

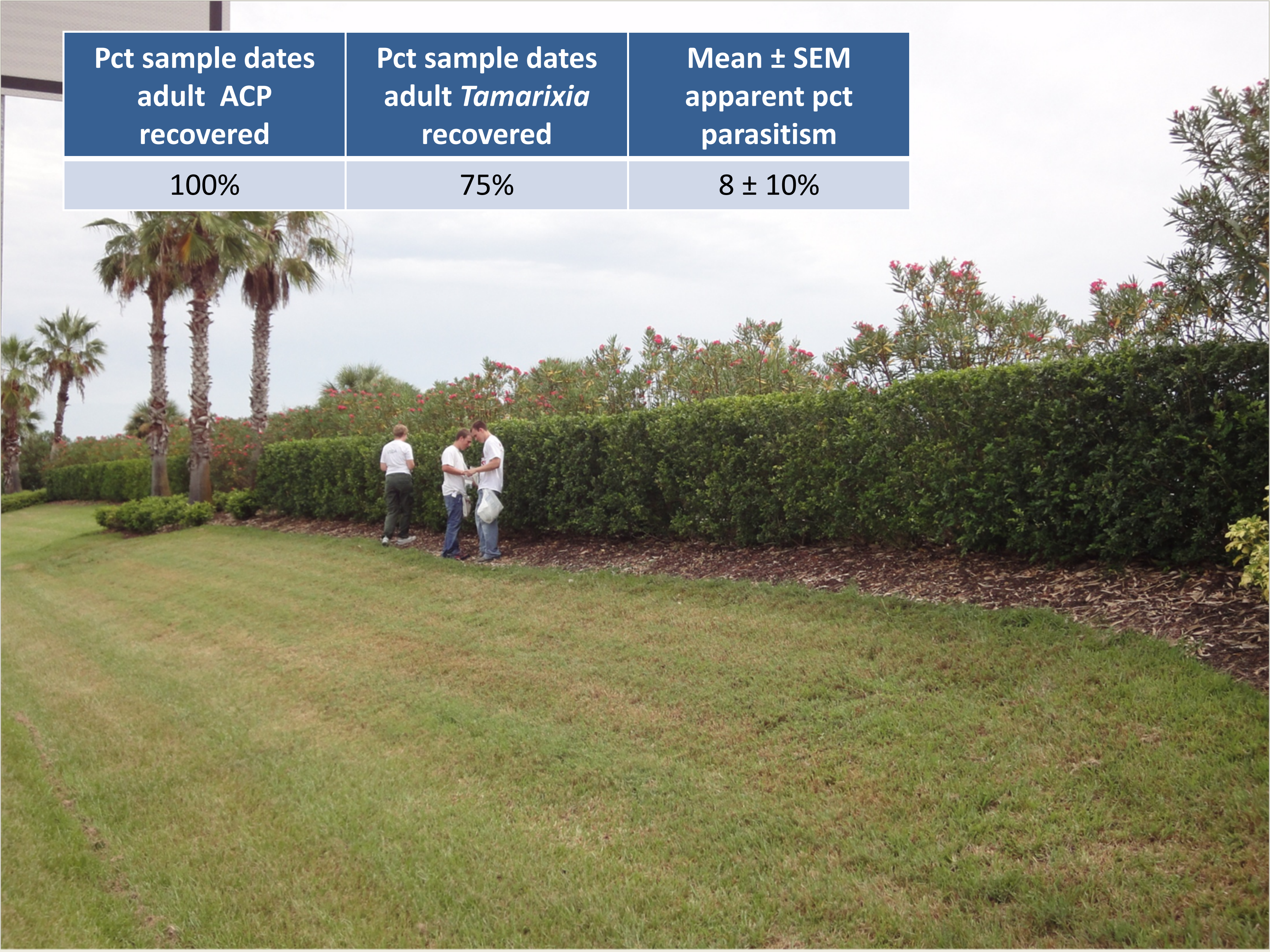
David G. Hall, Abigail Walter and YongPing Duan



Source of funding: ARS

Pct sample dates adult ACP recovered	Pct sample dates adult *Tamarixia* recovered	Mean ± SEM apparent pct parasitism
100%	100%	70 ± 12%

Pct sample dates adult ACP recovered	Pct sample dates adult *Tamarixia* recovered	Mean ± SEM apparent pct parasitism
100%	75%	8 ± 10%



Area-wide management of Asian citrus psyllid

Results to date:

- The Asian citrus psyllid is common in urban plantings of orange jasmine in St Lucie County.
- *Tamarixia radiata* commonly attacks the psyllid in urban plantings of orange jasmine in St Lucie County.
- Overall, percent parasitism has averaged 42%, some variability.
- The parasitoid persists in orange jasmine under regular management practices.
- *T. radiata* in urban plantings of jasmine is contributing to area-wide suppression of the psyllid .



Seasonal flight activity by the Asian citrus psyllid

D G Hall and M. G. Hentz

Of interest is the seasonal occurrence of psyllid flight activity distant from citrus.

We used sticky traps deployed at various distances from citrus to make inferences about psyllid flight activity.

Source of funding: ARS

Area-wide management of Asian citrus psyllid

Results indicated that flight activity by both male and female psyllids away from citrus can occur at any time of the year with consistent dispersal activity during the spring.

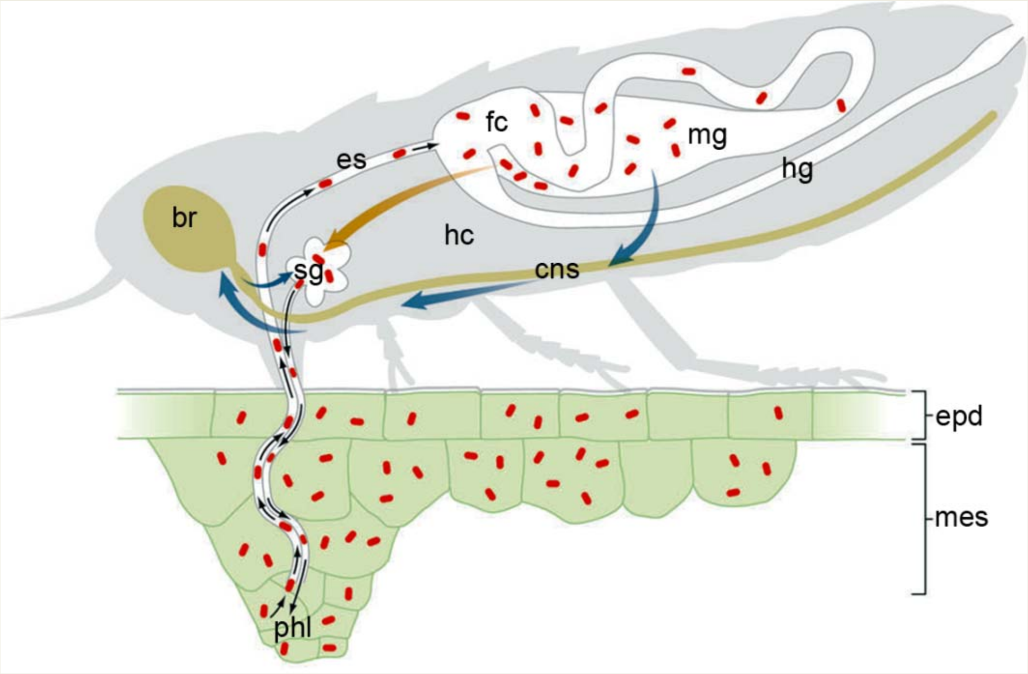
Flights 500 ft from citrus documented.

Citrus is continually subject to infestation by immigrating adults and that there is no time during the year that a citrus grower could be assured immigration would not occur.

Photo: Forest and Kim Starr

Distribution of *Candidatus* Liberibacter asiaticus in its psyllid vector *Diaphorina citri*

El-Desouky Ammar, Bob Shatters and David Hall

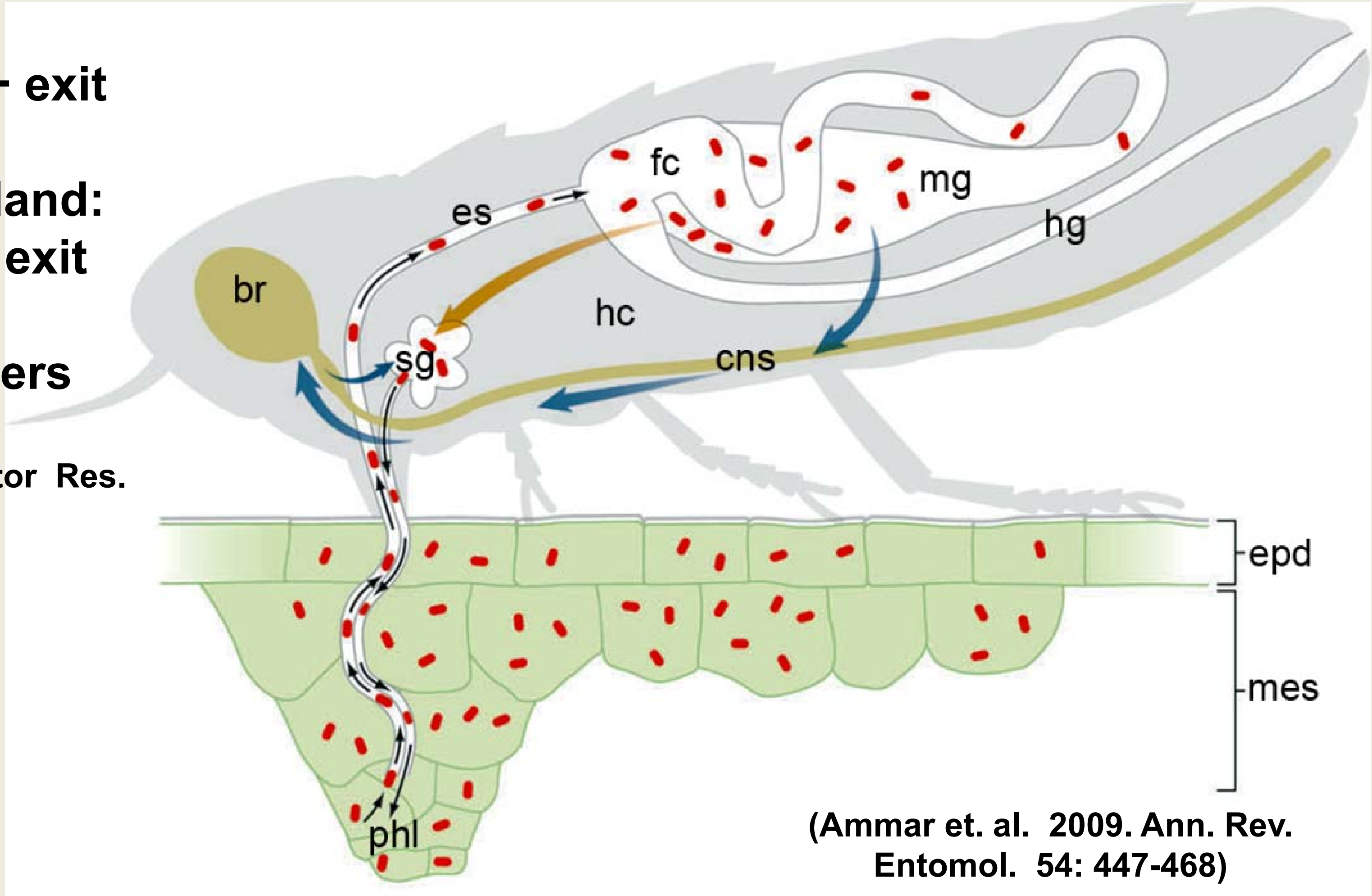


Source of funding: CDRF

Transmission barriers known for persistent pathogens in hemipteran vectors

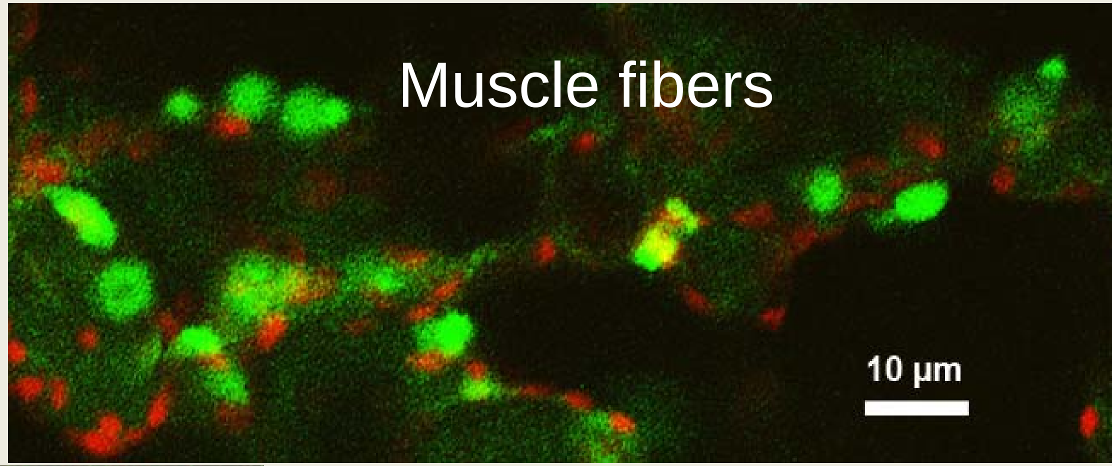
- **Midgut: infection + exit**
- **Salivary gland: infection+ exit**
- **Other barriers**

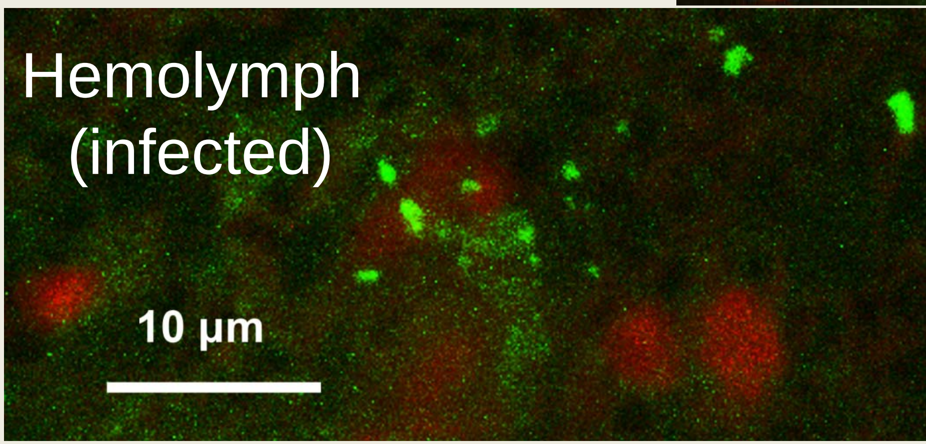
(Ammar 1994. Adv. in Dis. Vector Res. 10:289-332)

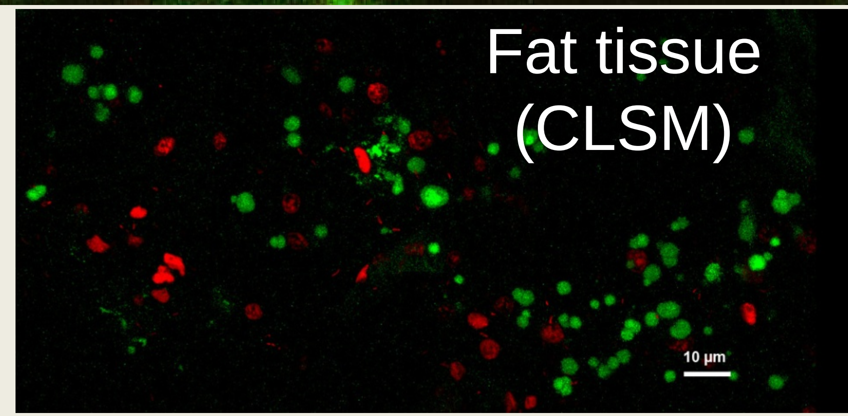


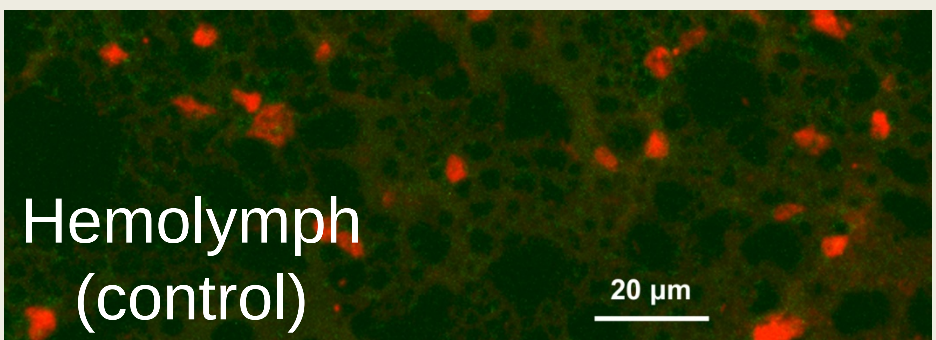
(Ammar et. al. 2009. Ann. Rev. Entomol. 54: 447-468)

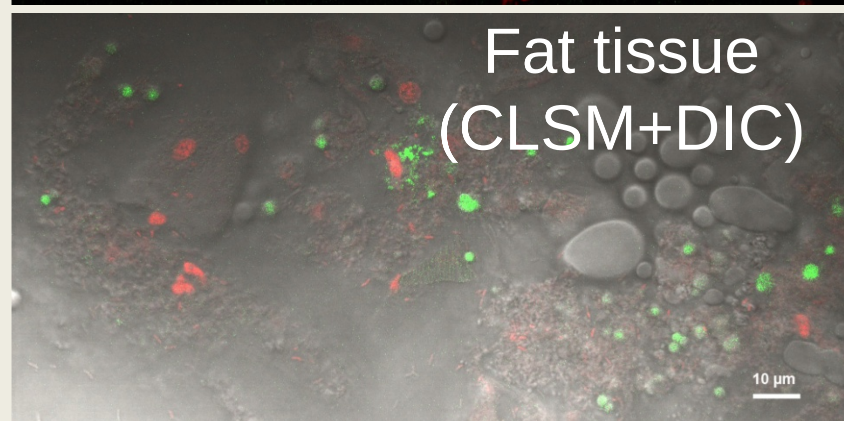
FISH-CLSM of Las in hemolymph smears, muscle and fat tissues







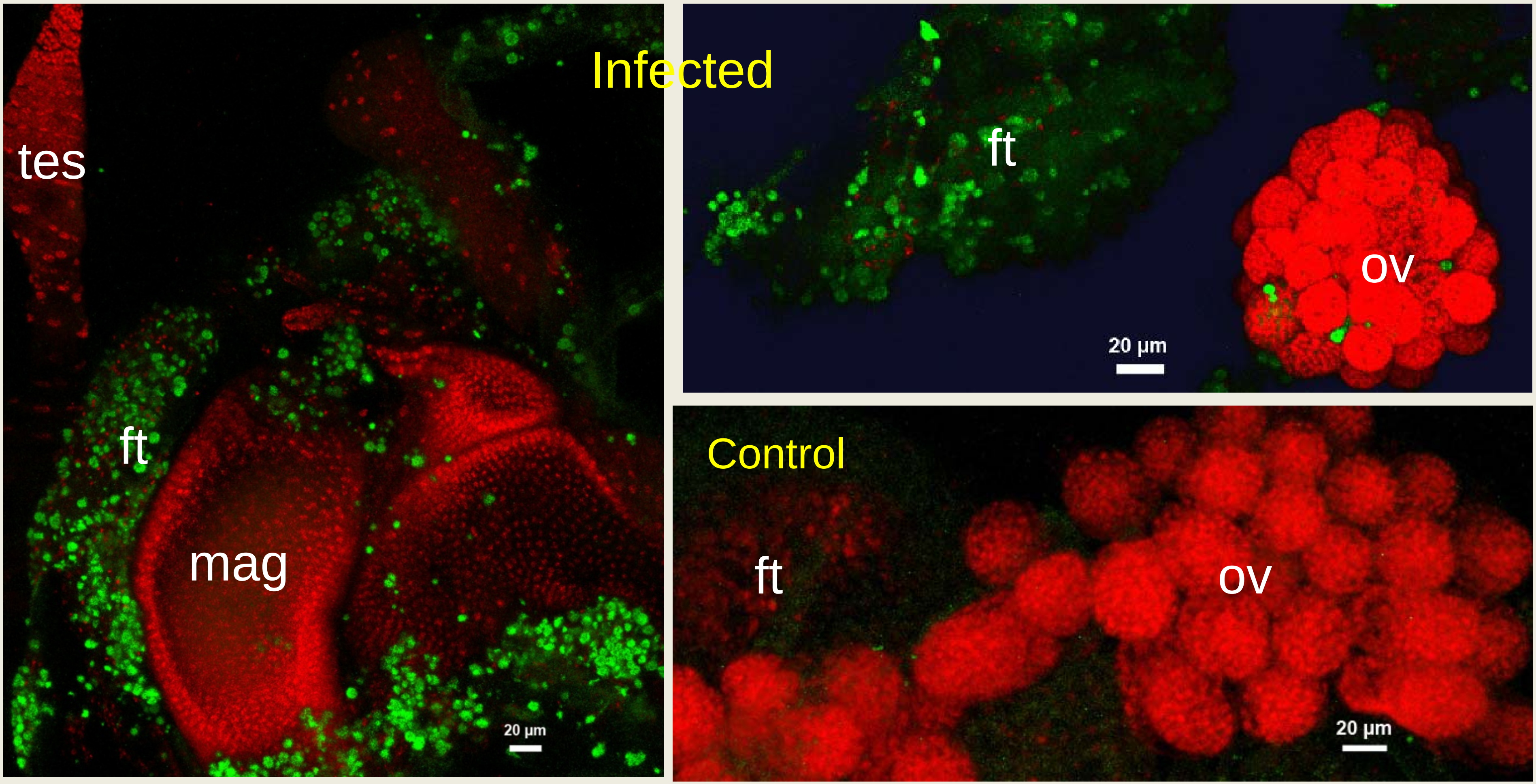




FISH-CLSM of Las in male and female reproductive system

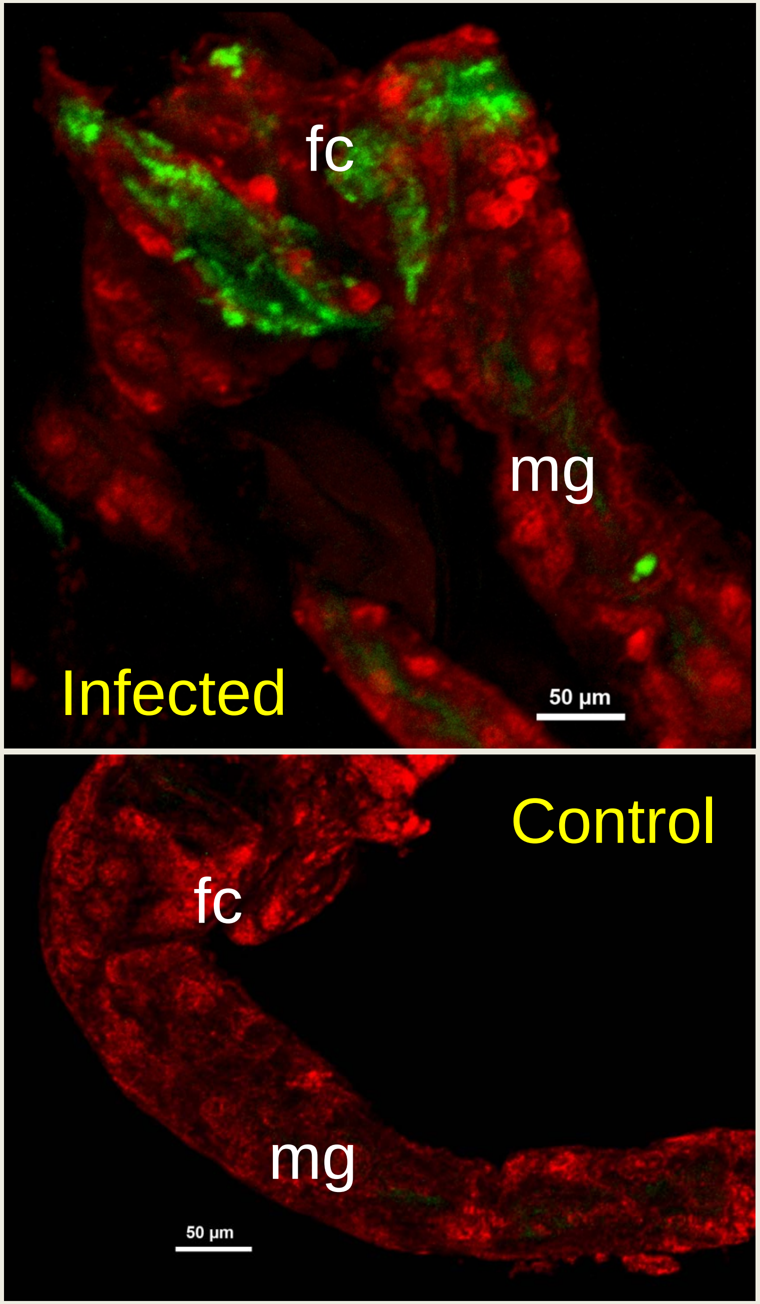
Male

Female

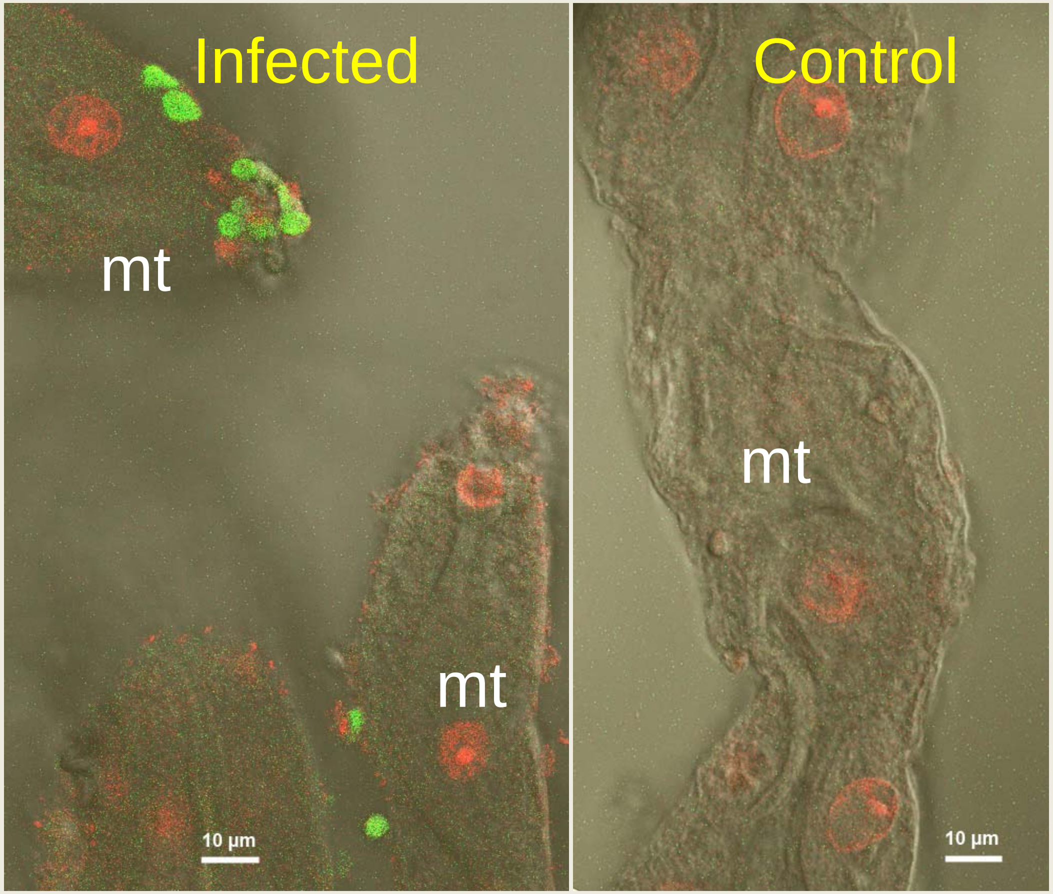


FISH on whole mounts of dissected ACP organs

CLSM
Midgut &
Filter chamber



CLSM and DIC:
Malpighian tubules



FISH-CLSM of Las in the principal salivary glands of ACP

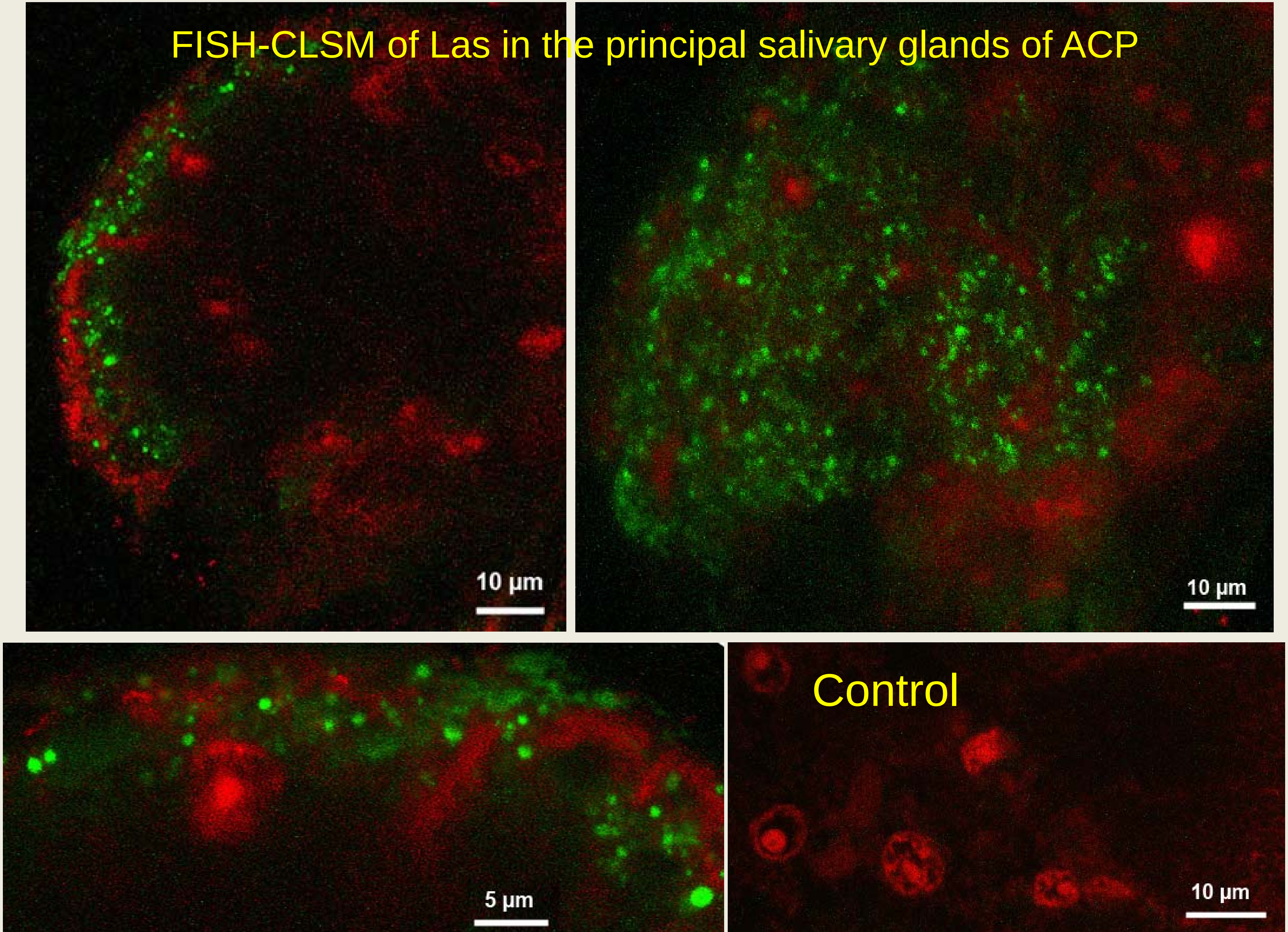


Table 1. Percentage of Las-positive organs of *D. citri* using Q-PCR on dissected adults collected from HLB-infected citrus trees in Fort Pierce, FL.

Psyllid source	Alimentary canal	Salivary glands	Other body parts
Field-infected	74%	45%	81%

Just because a psyllid is infected does not mean it can transmit the bacterium .

• Results suggest that the salivary gland constitutes a major barrier to Las infection and/or transmission.

• Las may replicate and/or accumulate in the salivary glands and alimentary canal of the psyllid.

Phloem infusion of chemicals into trees for HLB/Psyllid control

Bob Shatters

Development of a phloem infusion method that allows low volume treatment of citrus for applying:

1) antimicrobial compounds that will control/kill the HLB bacterium,
2) psyllid control/deterrent compounds.

The ultimate goal of this research is to develop a cost-effective and sustainable grove treatment strategy that will prevent HLB symptom development and reduce the amount of psyllid feeding on treated plants.

Source of funding: SCBG

Molecular Characterization of Psyllid Feeding to Identify Interdiction Point to Block Feeding Process

Bob Shatters and Kent Morgan

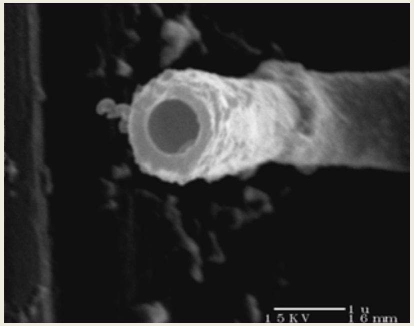
Psyllids secrete a salivary liquid that solidifies into a tube (salivary sheath, SS) when feeding on plants.

- Developed method for isolating SSs.
- Identified protein components of the SS including structural proteins.
- Currently studying assembly mechanism of these SSs in an attempt to find a way to block the assembly process.

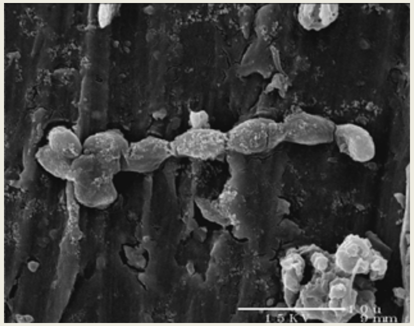
SDS-PAGE Gel of Salivary Sheath Proteins

Microscopy of Isolated Psyllid Salivary Sheaths (SSs)

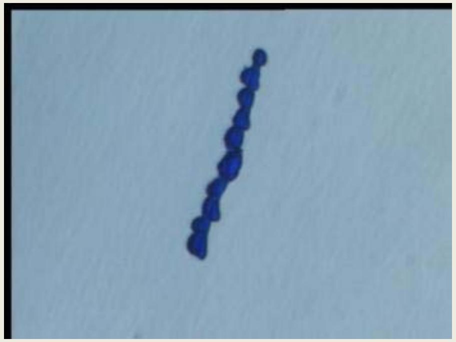
SEM of SS Showing Central Canal



SEM of SS Showing Globular Construction



Light Microscopy of Coomassie Blue stained SS



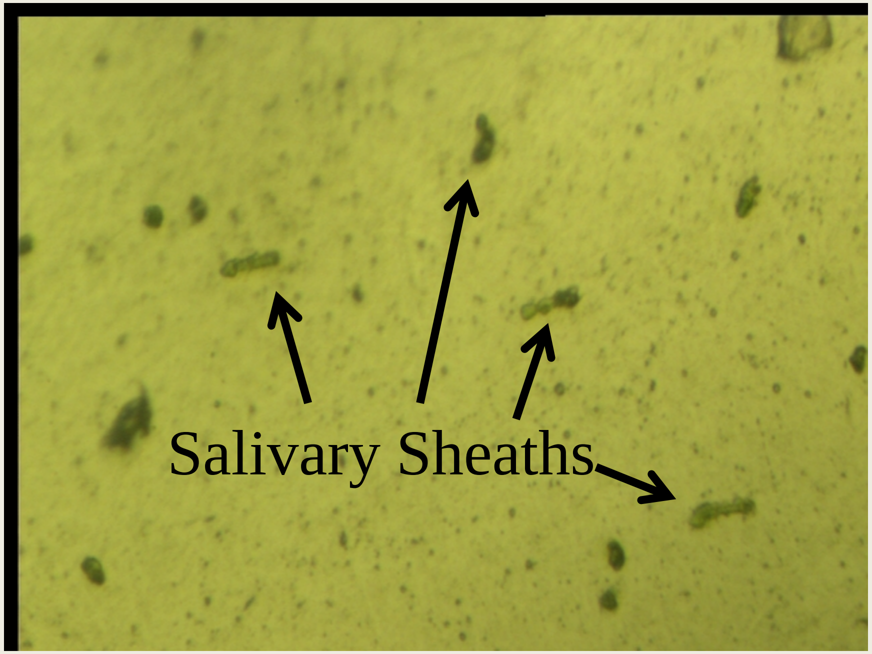
Source of funding: CDRF

Artificial Diet System For Harvesting Salivary Sheaths to determine their constituents.

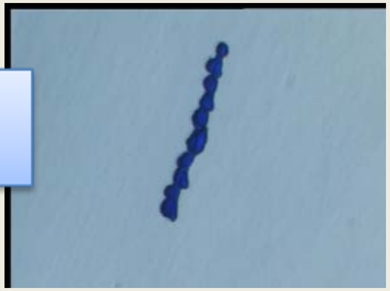
- Diet is sandwiched between two parafilm layers
- Diet Contains:
  - 30% Sucrose
  - Food Coloring Dye
  - Agar (Gelling agent)

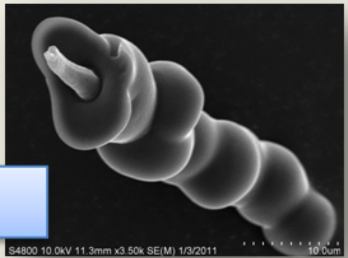
Observations on feeding activity and salivary sheaths

- Feeding readily occurred
- Salivary sheaths were formed through the membrane and into the diet medium.



Light Microscopy of Coomassie Blue stained SS



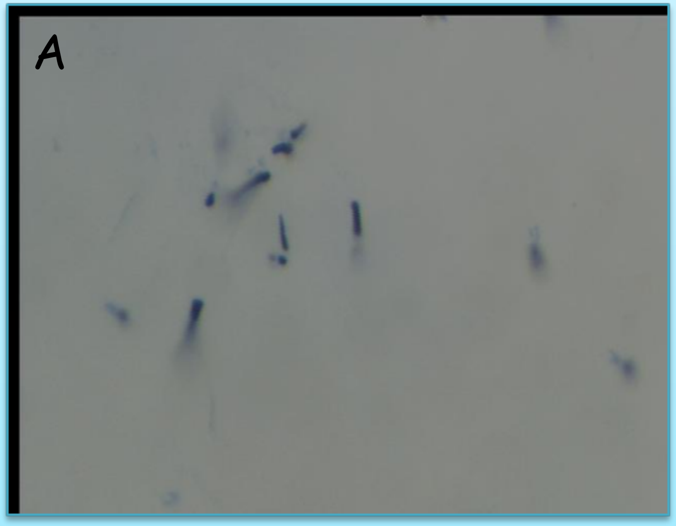


SEM of SS

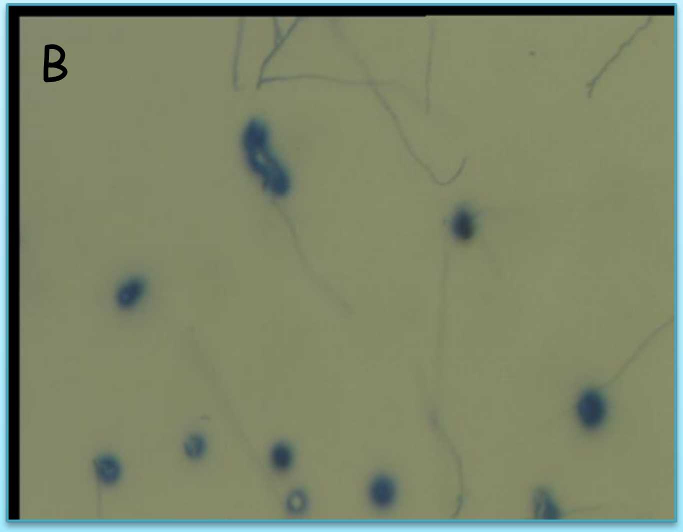
USE OF ARTIFICIAL DIET TO IDENTIFY INHIBITORS OF SALIVARY SHEATH FORMATION

BLOCKING SALIVARY SHEATH FORMATION

Normal diet



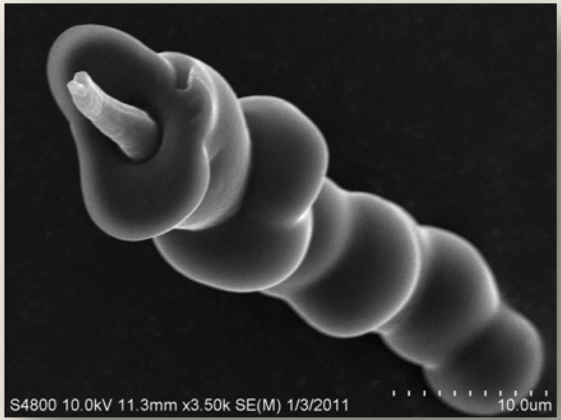
Addition of EDTA-divalent cation chelating agent

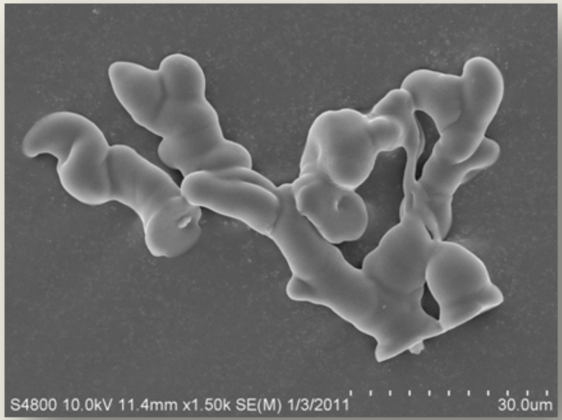


Block the psyllid’s ability to feed = block psyllid transmission of the HLB bacterium.

Research confirms that specific chemicals can block the psyllid feeding process.

Genetically engineered plants might be developed that produce these specific chemicals, or these chemicals might be applied directly to a plant using an infusion method.





Antennal responses of Asian citrus psyllid to host plant volatiles recorded using a gas chromatograph coupled to an electroantennograph detector system.

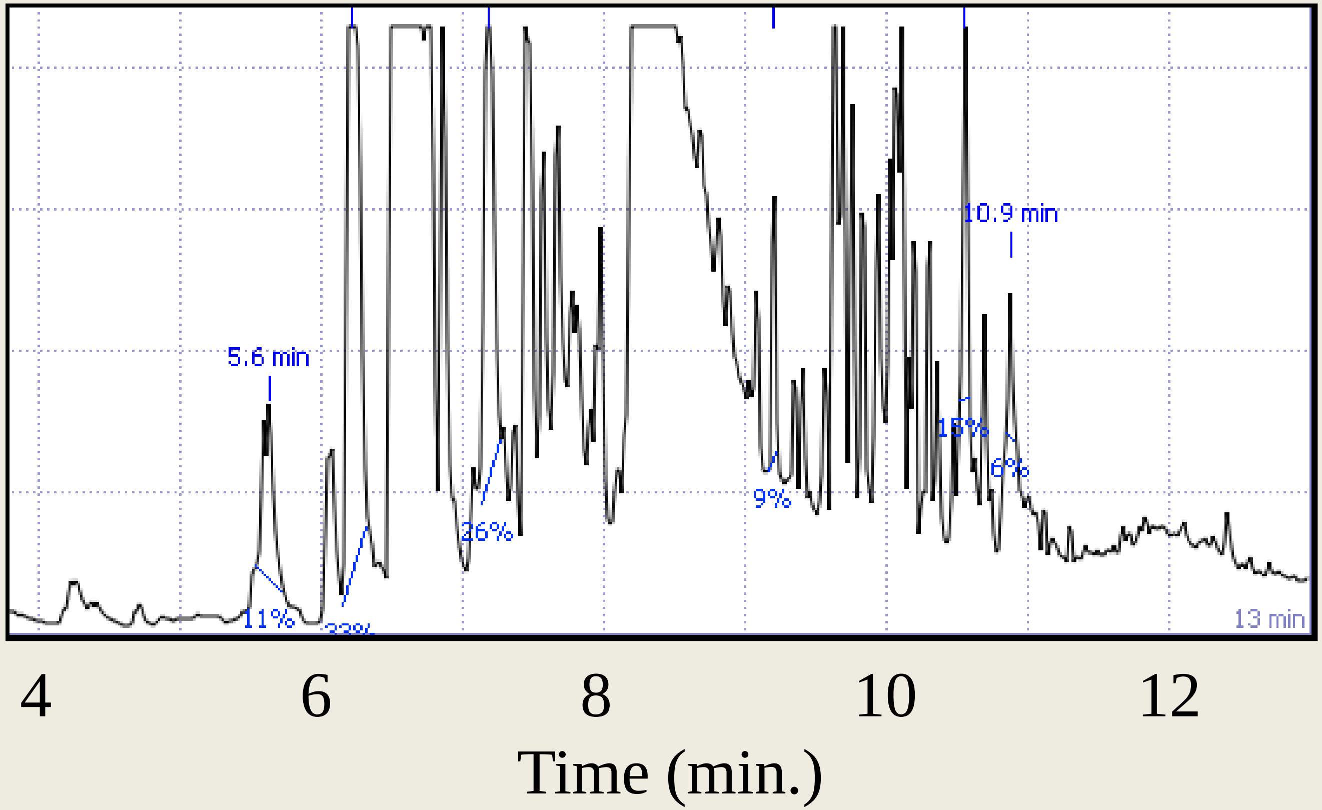
Paul Robbins, Rocco Alessandro and Steve Lapointe

The objective of this project is to find attractants and repellents for the psyllid.

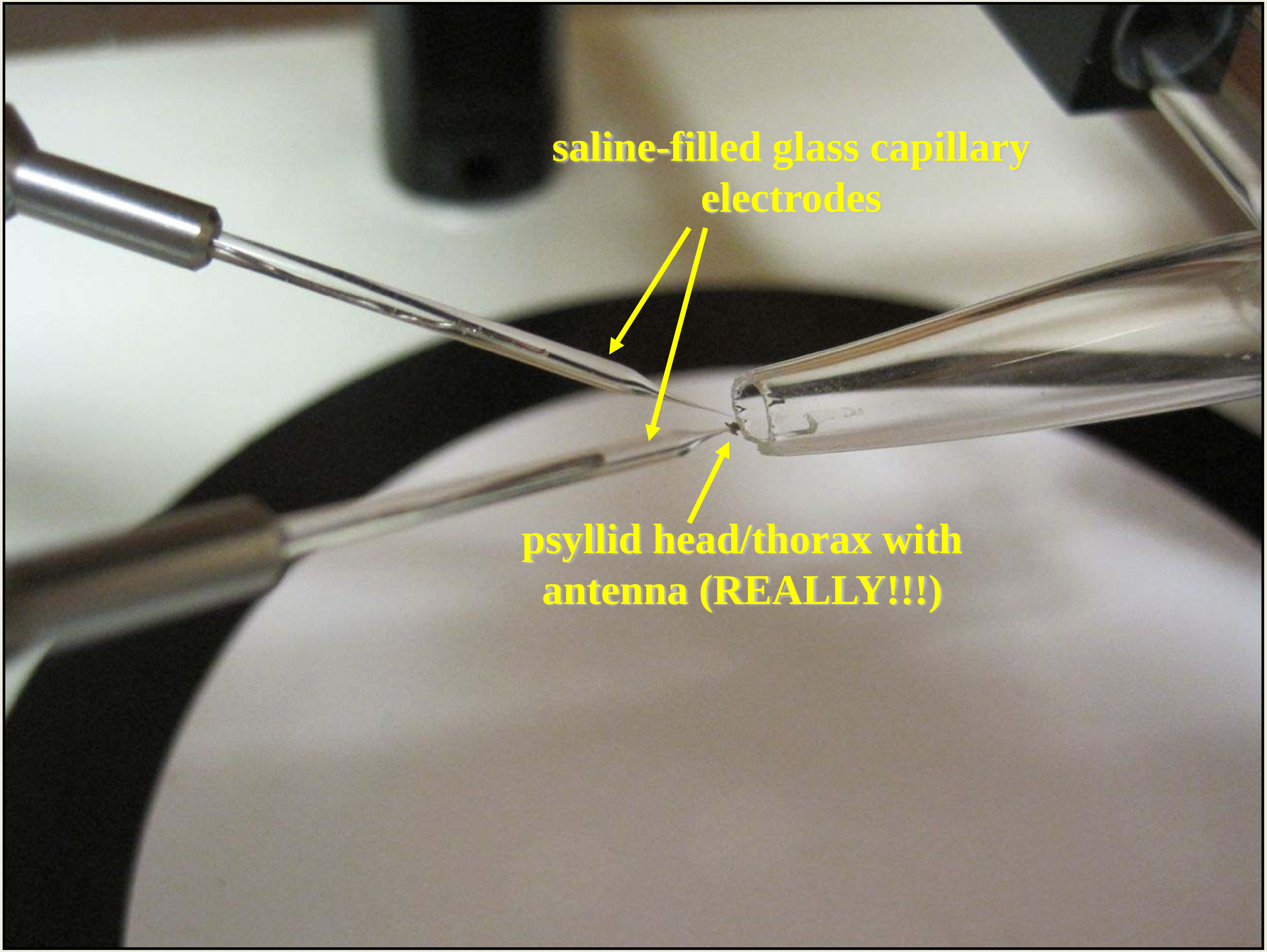
Antennal responses provide clues as to what volatiles might be candidate attractants or repellents.

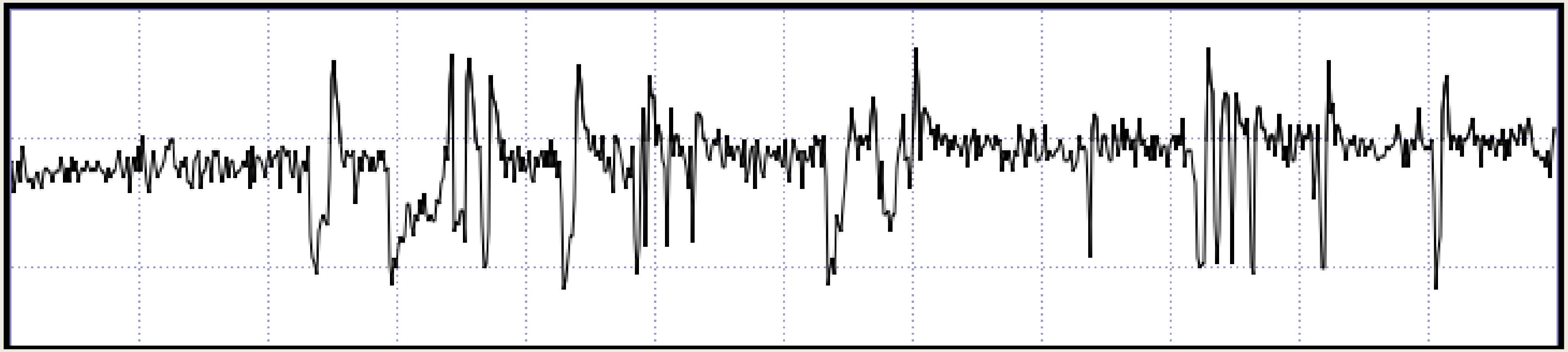
Source of funding: CDRF


Agilent Technologies
7890A GC System
12:24:24 Wed 5 Jan 2011
Last runtime 28.000
Next runtime 28.000
time = 0:00.0 1/t = 0.000<
Actual
Setpoint

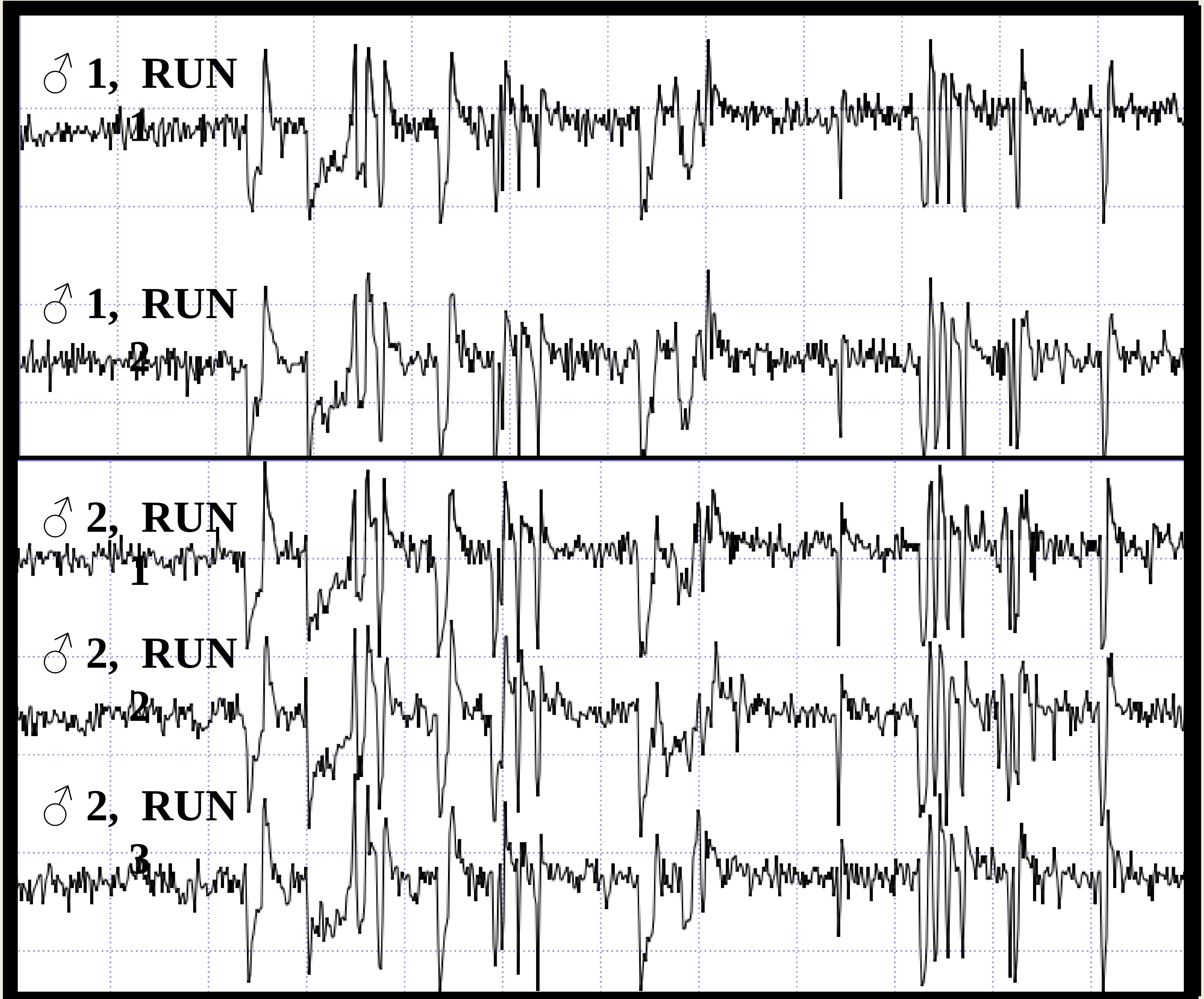


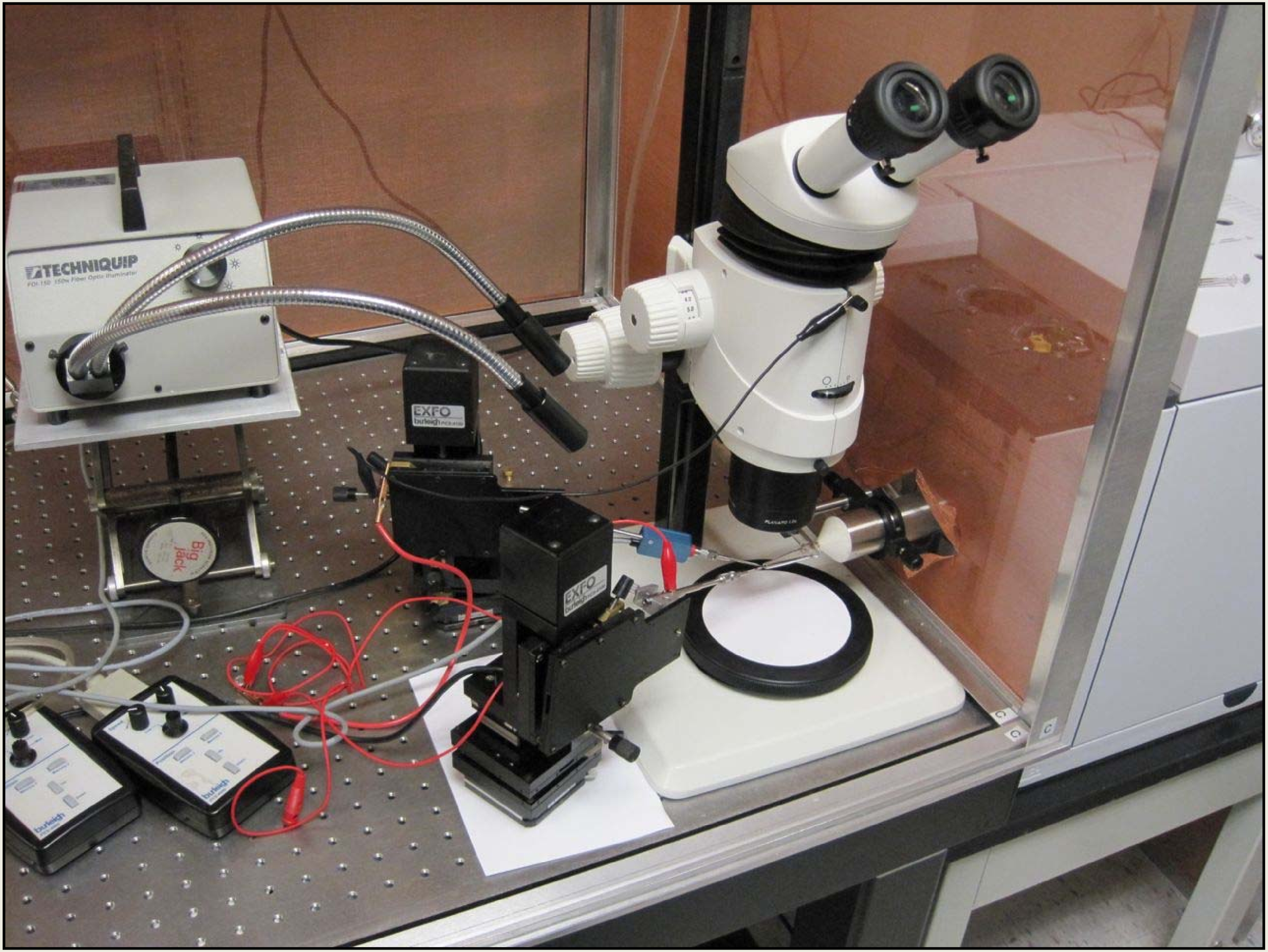
Gas chromatogram showing an array of peaks associated with volatiles in a sample – used to identify specific volatiles.

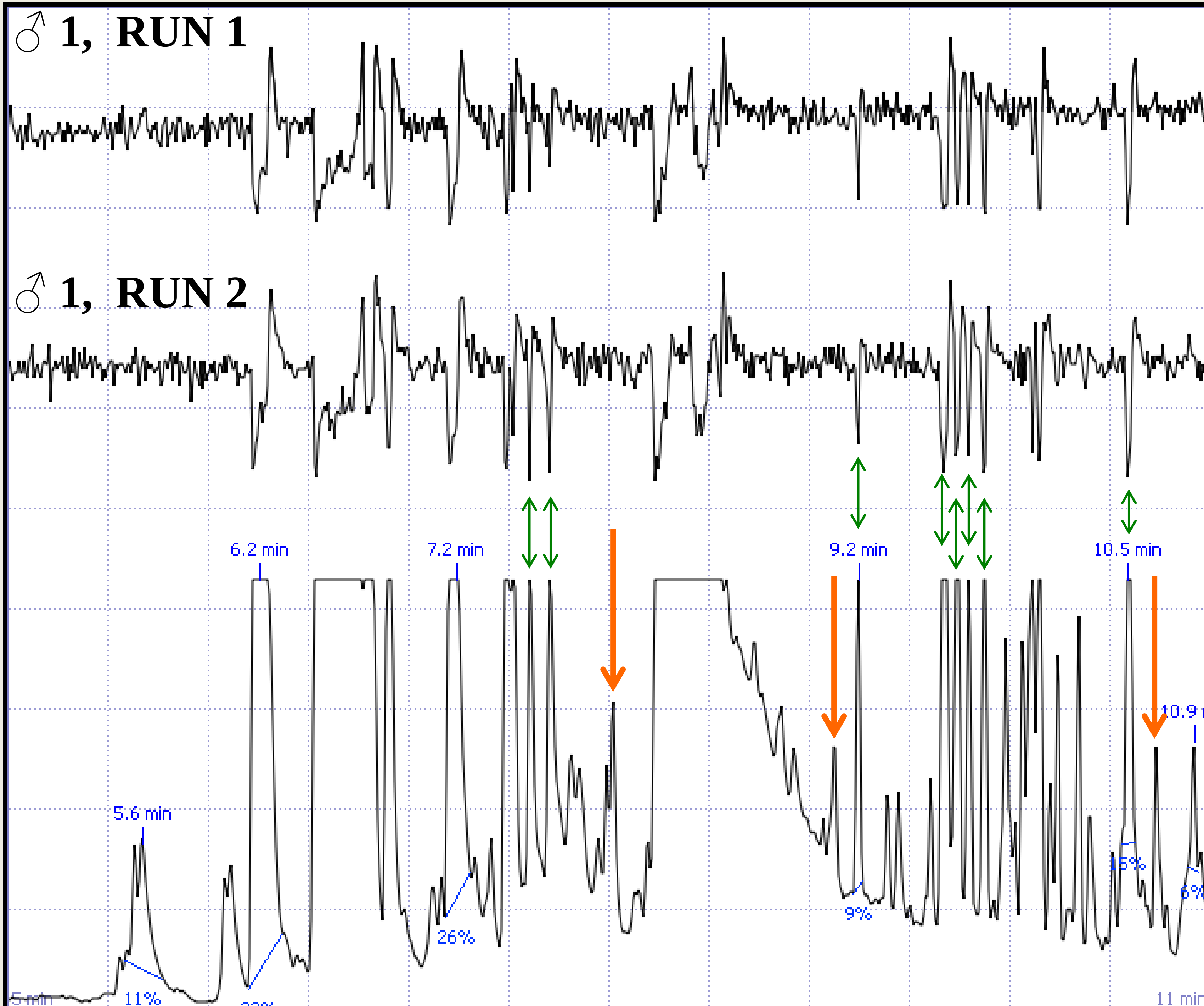

saline-filled glass capillary electrodes
psyllid head/thorax with antenna (REALLY!!!)



Antennogram showing an array of responses to volatiles in a sample.


♂ 1, RUN 1
♂ 1, RUN 2
♂ 2, RUN 1
♂ 2, RUN 2
♂ 2, RUN 3


TECHNIQUIP
EXFO
burleigh
Big Jack
EXFO
burleigh
burleigh
burleigh


♂ 1, RUN 1
♂ 1, RUN 2
6.2 min
7.2 min
9.2 min
10.5 min
5.6 min
26%
9%
15%
6%
11%
5 min
11 min

- Difficult research, notably because the insect antennae are so small.
- Continue to refine the technique to achieve results on a more consistent basis.
- Identify which host plant volatiles elicit antennal responses for use in behavioral bioassays.
- Identify compounds from females that elicit antennal responses in males.
- Both host plant volatiles and sex attractants could be useful in programs to manage the Asian citrus psyllid.

Identification of resistance to Asian citrus psyllid in citrus and related germplasm

D. Hall, C. Westbrook, Y.-P. Duan, E. Stover, R. Lee, M. Richardson

- Extensive survey of a planting of citrus and relatives
  - Approximately 90 genotypes
  - Sampled monthly (April – November 2010)
  - Presence and density of ACP life-stages

Source of funding: CDRF

Very susceptible to infestations of the Asian citrus psyllid

Scientific name	Common name
C. reticulata	Tien Chieh mandarin
Bergera koenigii	Curry tree
Murraya paniculata	Orange Jessamine
C. maxima	Mato Buntan pummelo
C. medica	Diamante citron
C. macrophylla	Alemow
xCitroncirus sp. (C. paradisi ‘Duncan'x P. trifoliata)	Swingle citrumelo
C. taiwanica	Nansho Daidai sour orange

<u>In free choice situations</u>, resistant to infestations of the Asian citrus psyllid

<u>Scientific name</u>	<u>Common name</u>
Zanthoxylum ailanthoides	Japanese prickly-ash
Casimiroa edulis	White Sapote
Poncirus trifoliata	Simmons trifoliate
Glycosmis pentaphylla	Orangeberry/Gin berry
Clausena harmandiana	*Clausena harmandiana*
Severinia buxifolia	Chinese box orange (brachytic form)
Microcitrus australasica	Australian finger lime var. Sanguinea

An RNA interference Strategy to suppress Psyllids

Wayne B. Hunter
In collaboration with University of Texas and Beeologics, LLC

A decade has passed since the discovery of RNA interference (RNAi).

Double-stranded RNA (dsRNA) are used to silence complementary messenger RNA sequences.

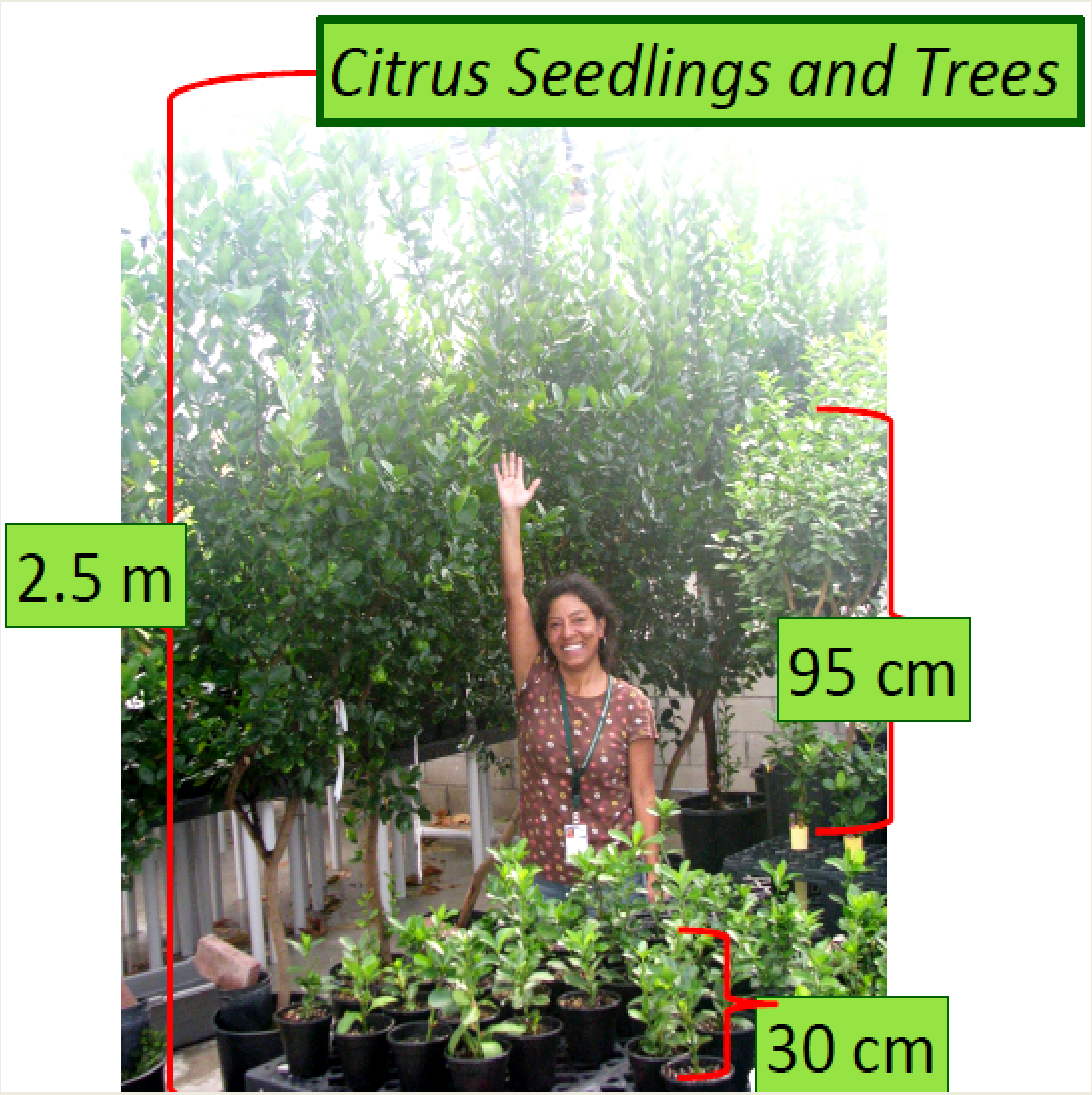
This interferes with the production of normal gene products.

Hunter is studying RNAi strategies for interfering with gene products associated with the psyllid midgut (for example, digestion, energy, membrane development, chitin synthesis).

In laboratory experiments, RNAi treatments increased ACP mortality within 5 days of initial ingestion.

Source of funding: ARS

Getting dsRNA into plants: Plant Absorption (Drench or Injection)



-Demonstrated that citrus trees can absorb dsRNA through roots and injection,

-Ingested by psyllids during feeding.

-Thus supporting a RNAi strategy for psyllid population suppression by host-delivered RNAi.

Sequencing of the Asian citrus psyllid genome

Wayne Hunter, Bob Shatters, David Hall

Projected benefits of sequencing the genome:

Identify enzymatic and metabolic pathways in search of weak links.

Identify gene targets for RNA interference.

Determine why the bacterium is pathogenic in the psyllid – identify methods of disrupting pathogenesis.

Bioinformatics team:
Justin Reese, Reese Consulting, LLC;
Nan, Leng, Illumina, Inc.
Working group 2011.

Source of funding: ARS

So far: Draft version 0.6 working towards a full Draft V.1.0

- Currently assembling draft genome sequences
- First draft completed this year.
- Estimate over 20,000 active genes will be identified.
- First run: 2,000 proteins align to genome with >50% identity of known Pea aphid proteins.
- Total contigs using program Velvet: 158,717 contigs (two or more sequences assembled), top 50 contig average length: 39,665 bases long.
- Sequence lengths: Max: 259,603 bases;   Min: 120 bases
- Total number of base pairs: 379,323,837   which is close to our original genome size estimate of ~360-400 million bases.
- Genome coverage estimated between 76%-80%
- Average contig size overall: 2,008 bp
- 37.89% GC (nearly identical to genome of Pea Aphid)
- Bioinformatics to follow
- All information available to the scientific community

Thanks to:

Florida Citrus Research and Development Foundation

Florida Department of Agriculture, Specialty Crops Block Grant

Subtropical Insect Research Unit

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