Biology and Worldwide Situation of *Diaphorina citri* Kuwayama, 1908 (Hemiptera: Liviidae)

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Outline

- Nomenclature
- Geographic origin and current distribution
- Origin of *D. citri* present in the Americas
- Description, Life cycle and Biology
- Host plants
- Factors affecting *D. citri* biology (preference and development) and population growth
- Behavior and spatio-temporal distribution
- CLas acquisition and transmission by *D. citri*

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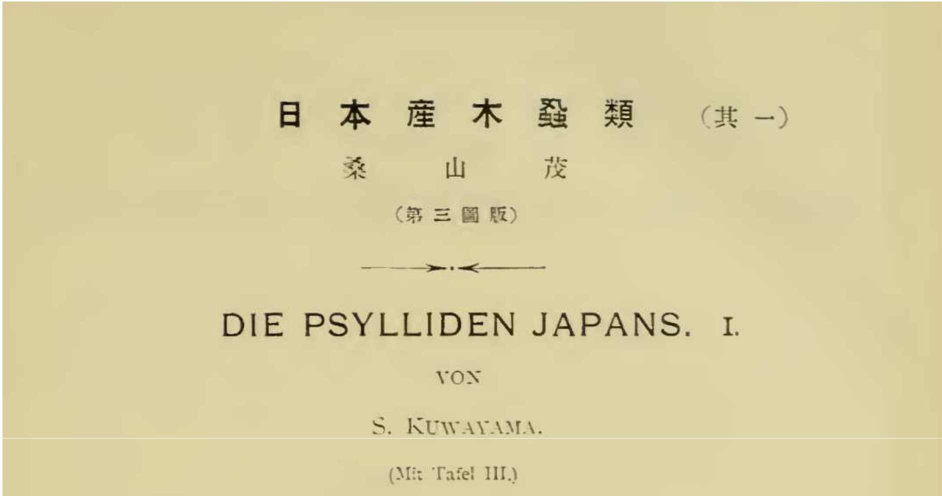
Diaphorina citri Kuwayama, 1908



日本產木蝨類　（其一）

桑山茂

（第三圖版）

DIE PSYLLIDEN JAPANS. I.

VON

S. KUWAYAMA.

(Mit Tafel III.)

Kuwayama Sh. 1908 - Die Psylliden Japans. I. *Transactions of the Sapporo Natural History Society* 2: 149-189 [160].

Common Name: **Asian citrus psyllid (ACP)**

Diaphorina citri Kuwayama, 1908

- ❑ **Liviidae Löw, 1879**
  - ❑ **Euphyllurinae Crawford, 1914**
    - ■ ***Diaphorina* Löw, 1880**

Löw F. 1880 - Mittheilungen über Psylloden. *Verhandlungen der Zoologischbotanischen Gesellschaft in Wien* 29: 549-598 [567]

Synonyms

- ❖ ***Diaphora* Löw, 1879**
- ❖ ***Gonanoplicus* Enderlein, 1910**
- ❖ ***Pennavena* Capener, 1968**
- ❖ ***Eudiaphorina* Loginova, 1975**
- ❖ ***Brachypsylla* Froggatt, 1901**

Mittheilungen über Psylloden.

Von

Dr. Franz Löw in Wien.

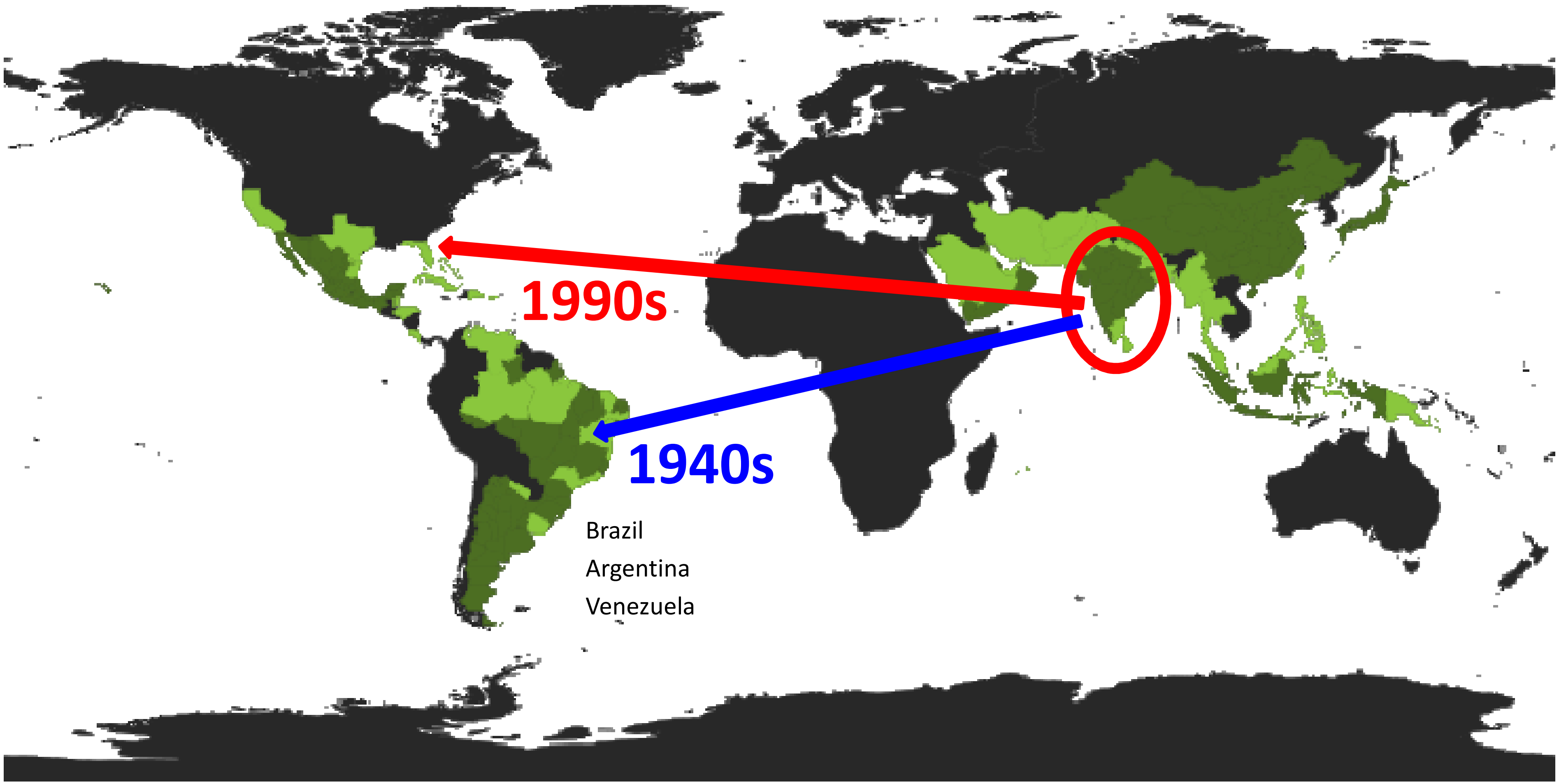
(Mit Tafel XV.)

(Vorgelegt in der Versammlung am 5. November 1879.)

Geographic origin of *D. citri*

- South-western Asia (Halbert & Manjunath 2004, Beattie et al. 2009)
- ACP has evolved in India (Beattie et al. 2009)
- In Asia: China, India, Myanmar, Taiwan, Phillippine Islands, Malaysia, Indonesia, Sri Lanka, Pakistan, Thailand, Nepal, Ryukyu Islands (Japan), Afghanistan, Saudi Arabia, Reunion, and Mauritius
- Americas: Most of South, North and Central American countries
- In the USA: FL (1998), TX (2001), CA (2008), but also present in AZ, LA, AL, GA, MS and SC

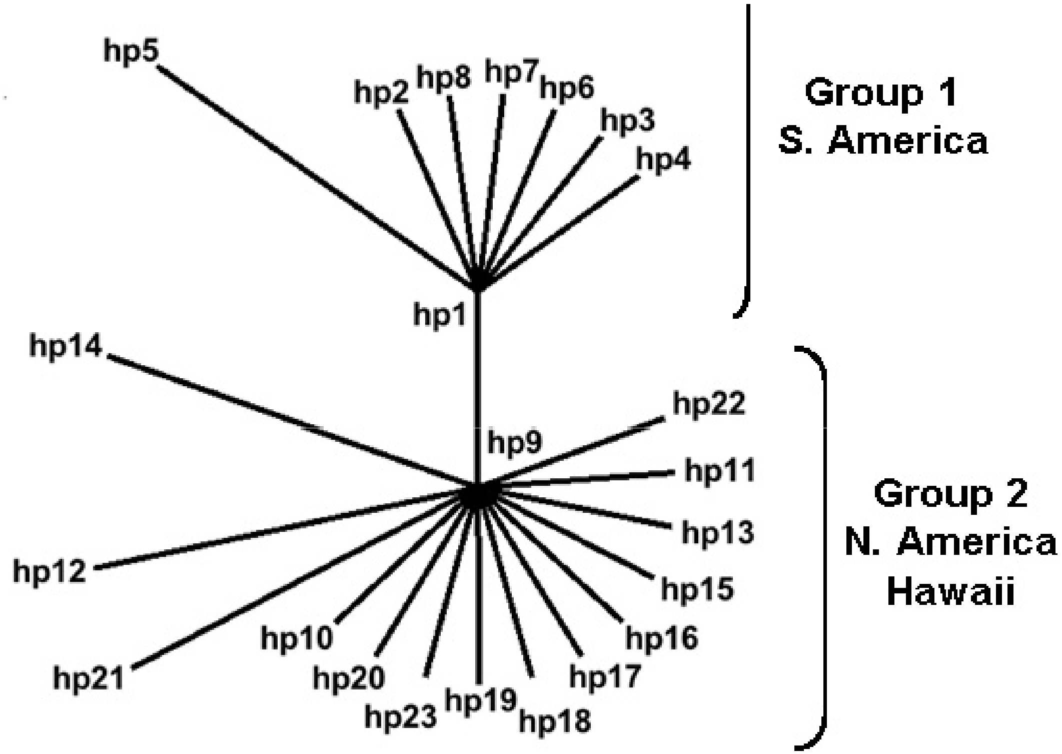
Geographic origin: Southwestern Asia, "India"



Ouvrard, D. (2013) Psyl'list - The World Psylloidea Database. [http://www.hemiptera databases.com/psyllist](http://www.hemipteradatabases.com/psyllist) - searched on 15 August 2013

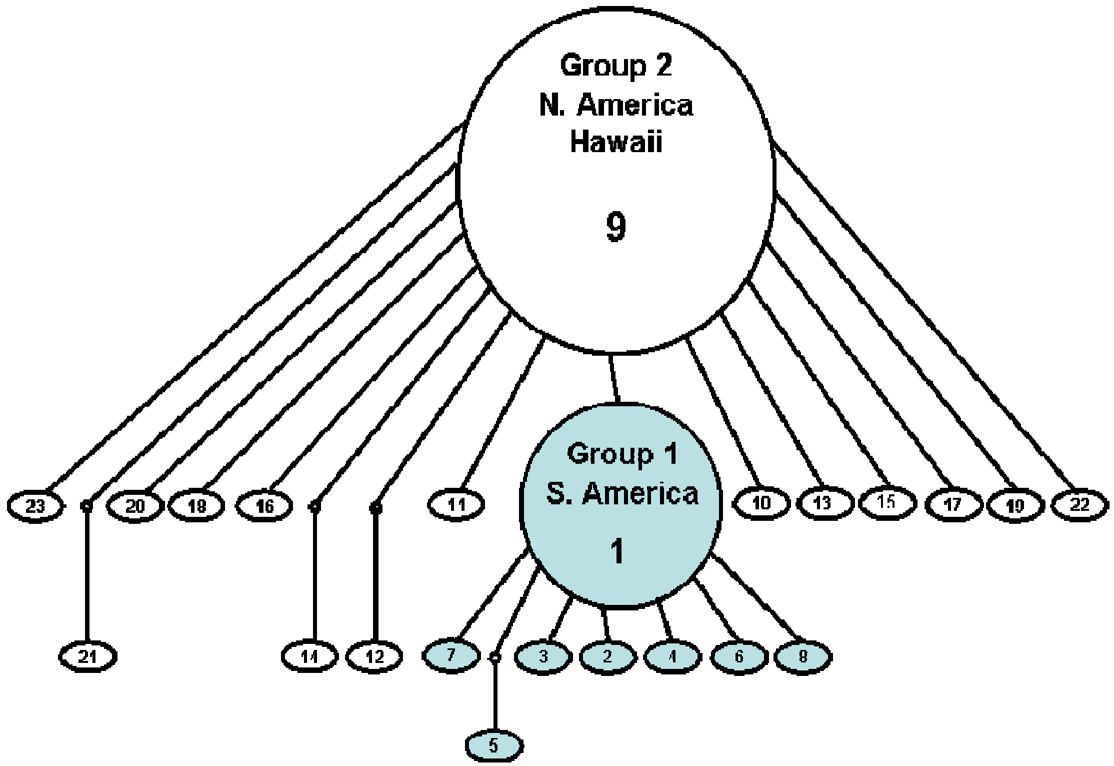
Network of evolutionary relationships among COI haplotypes of *D. citri* in the Americas and Hawaii

- 23 haplotypes identified that fell into two groups
- hp1 present in highest frequency in group1
- hp9 present in highest frequency in group 2
- No hp shared between the 2 groups
- (Φ_{CT}=0.733; *P*<0.001)



De Leon et al. 2011, Annals of Entomological Society of America 104: 1392-1398.

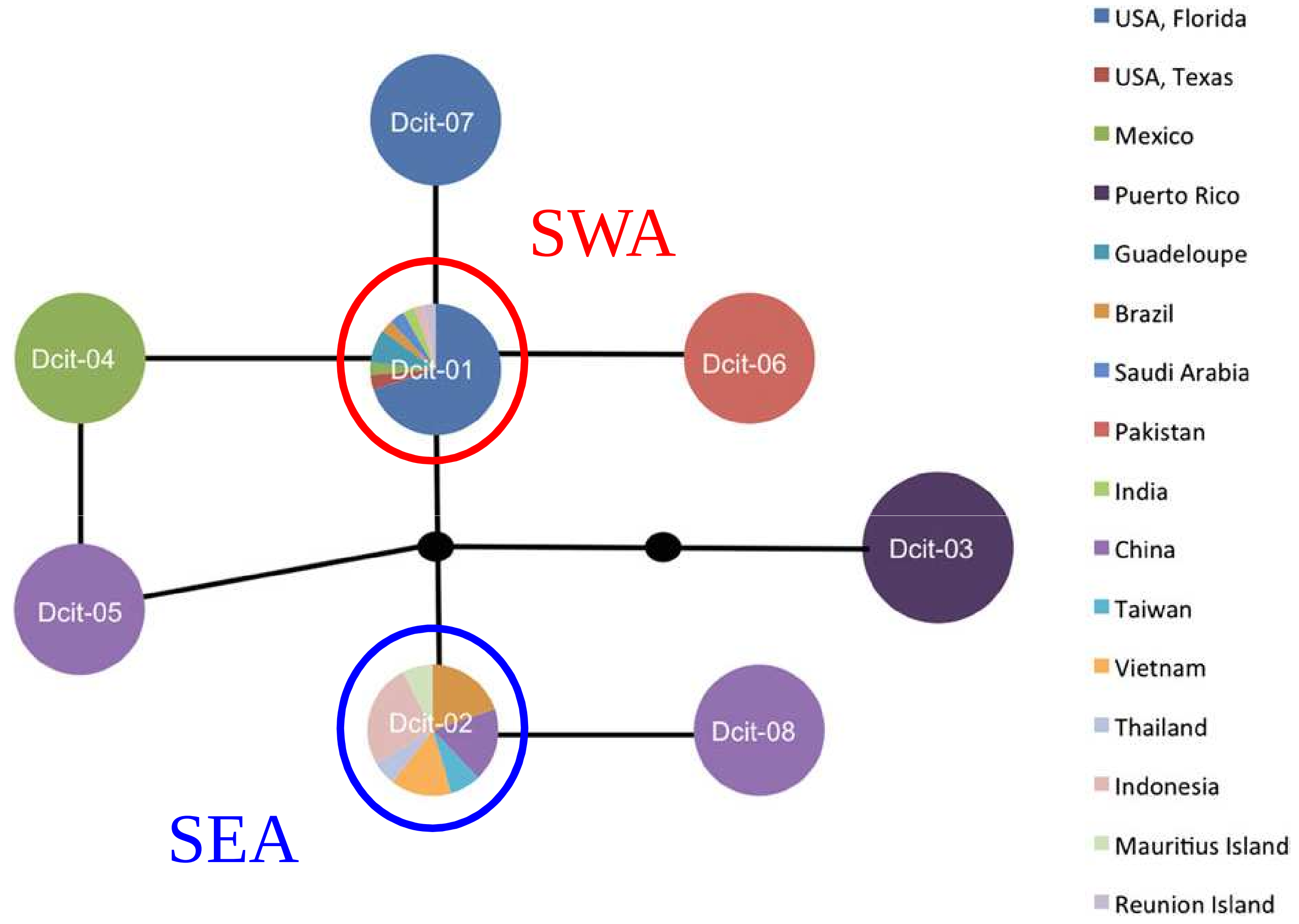
Network of genealogical relationships among COI haplotypes of *D. citri* in the Americas and Hawaii



- ParsimonySplits and Statistical Parsimony discriminated 2 groups
- hp1 & hp9 considered ancestral haplotypes
- In each group all other hp derived from them

Two founding events of *D. citri* in the Americas

Phylogenetic relationships between worldwide population of ACP



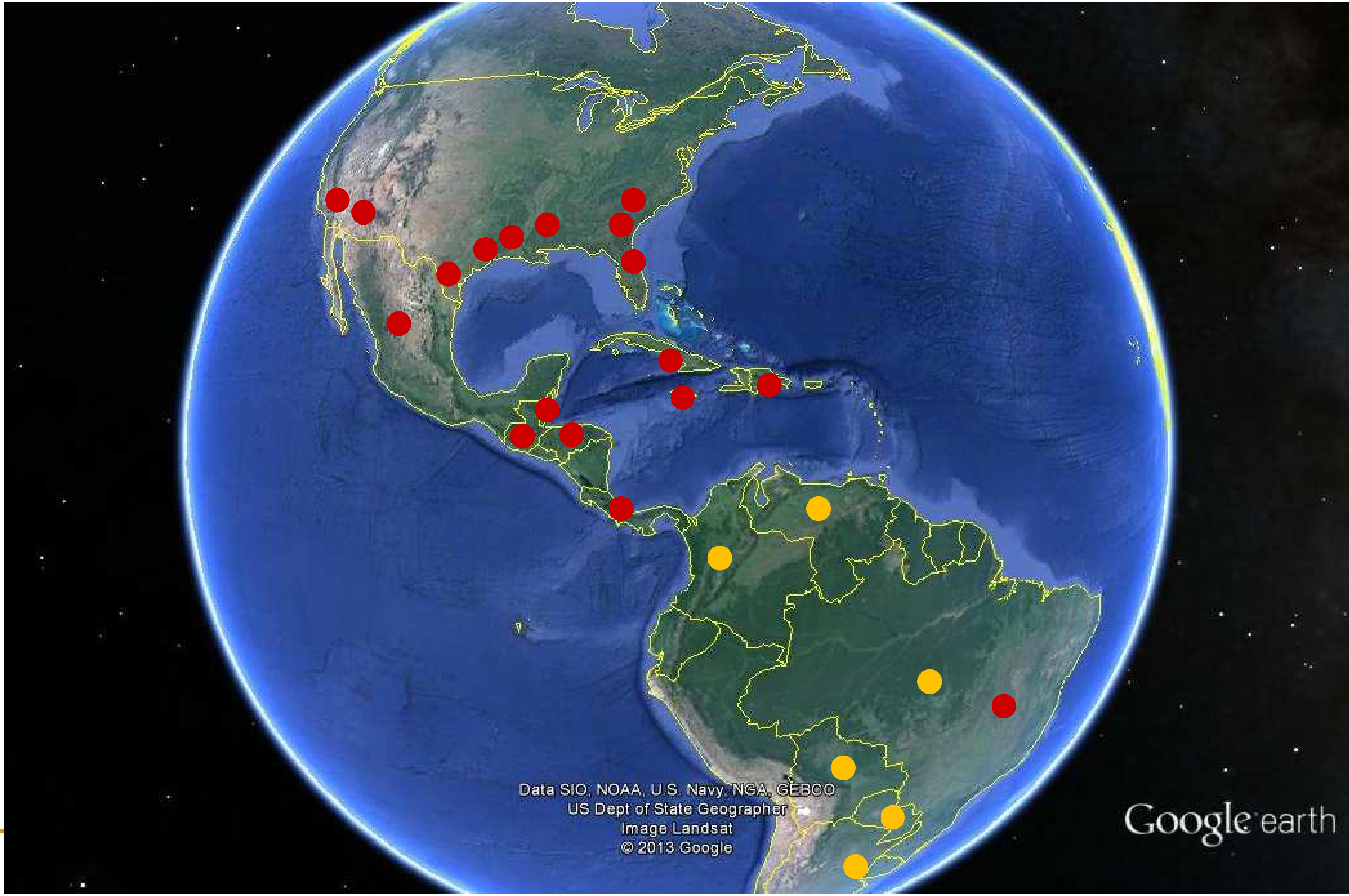
Haplotype network generated with statistical parsimony analysis of *D. citri* mtCOIDNAsequence

(Boykin et al. 2012, Bulletin of Entomological Research 102: 573-582

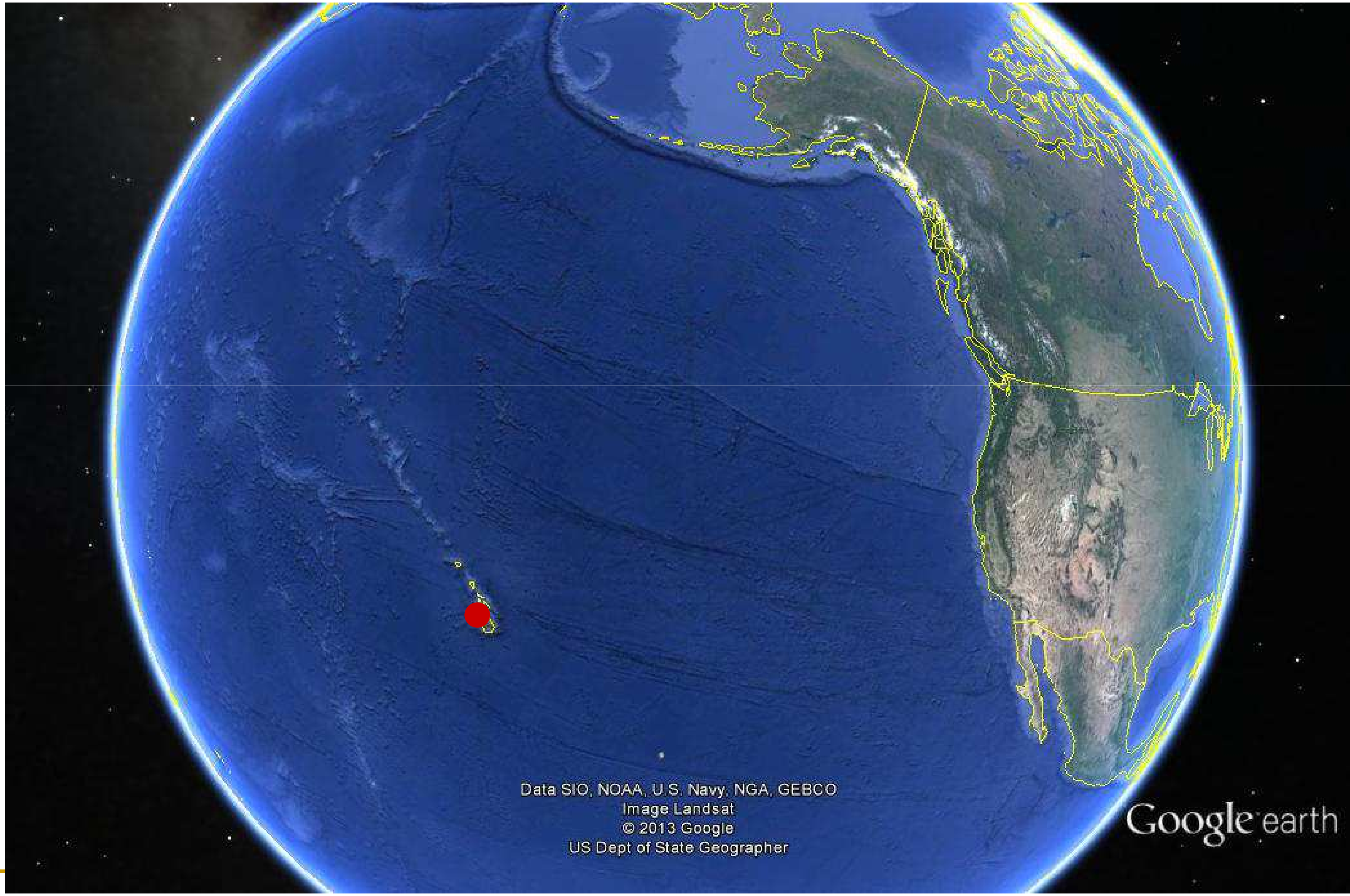
Two founding events of *D. citri* in the Americas and Hawaii

Major Group	Countries	Haplotype group
South America	Argentina, Brazil, Paraguay, Uruguay	Southeastern Asia
North & Central America	Belize, Costa Rica, Hawaii, Mexico, and USA (California, Florida and Texas)	Southwestern Asia
Both haplotype groups invaded separate locations of Brazil. **Haplotype unique to the Caribbean (Puerto Rico & Guadeloupe) was also found** (Boykin et al. 2012)		

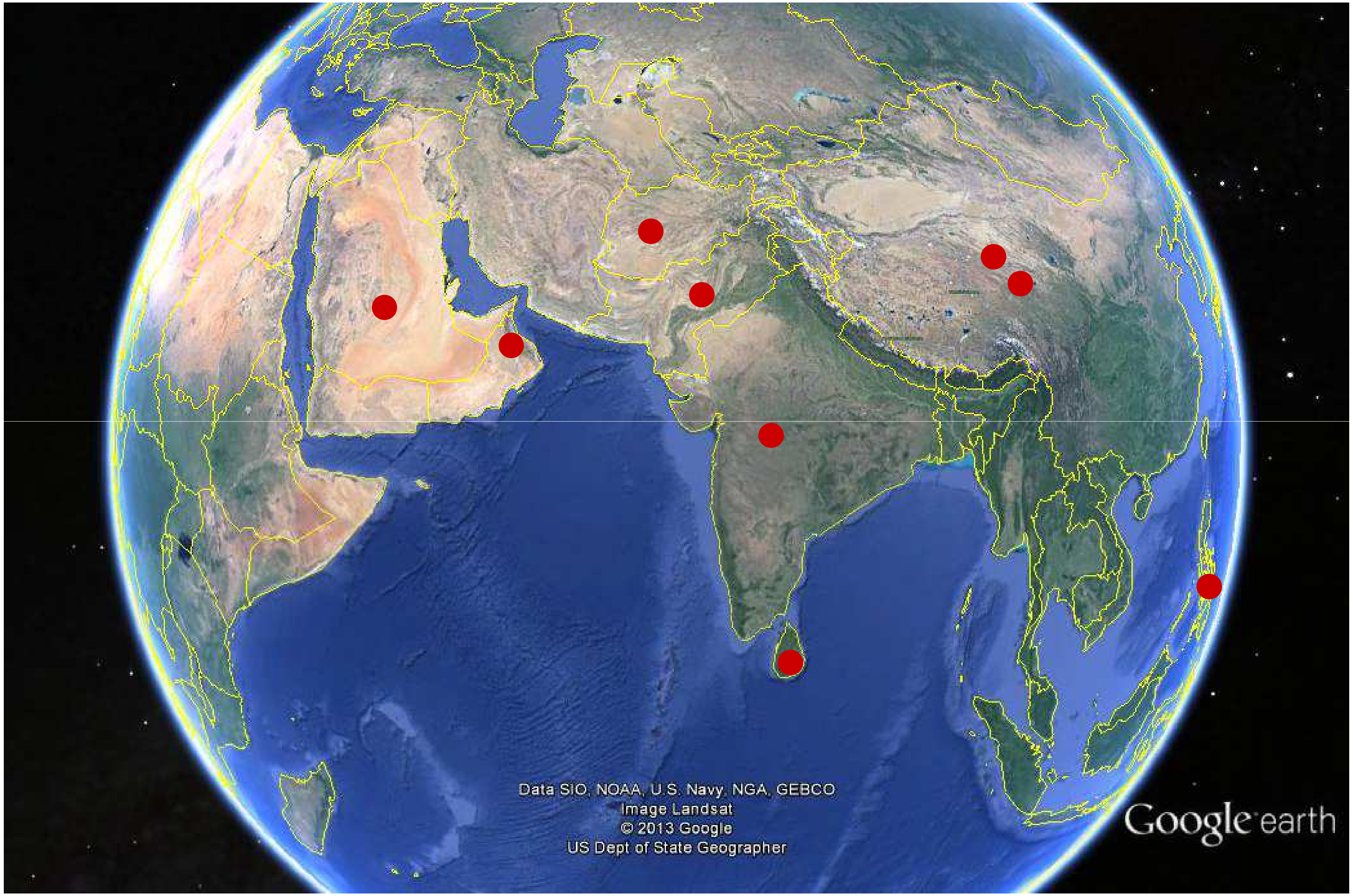
World distribution of *D. citri* in the Americas



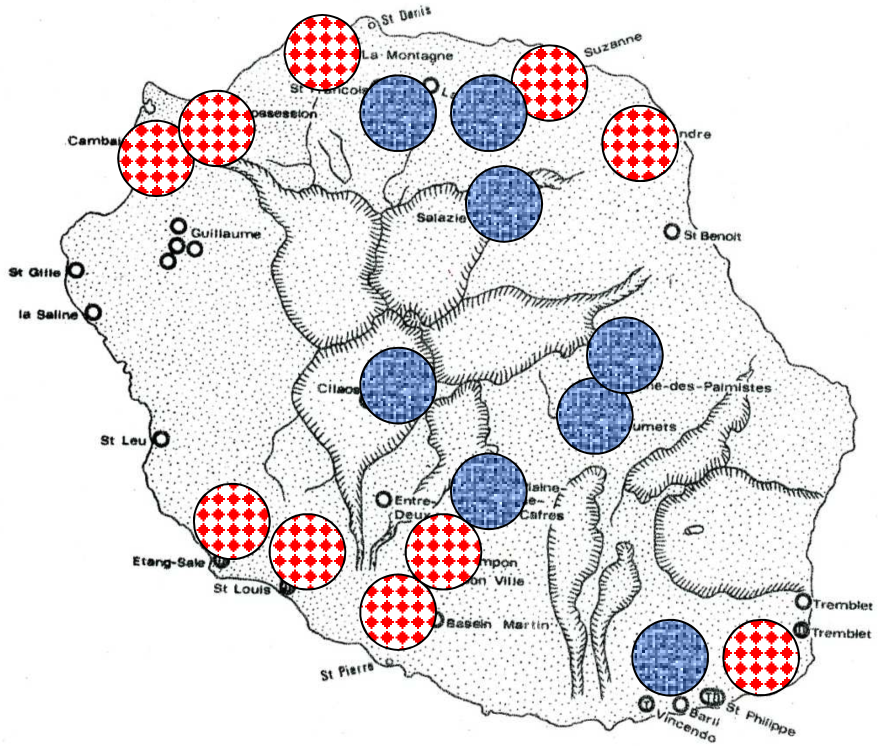
D. citri in Hawaii



D. citri in Asia



Distribution of (1) Heat tolerant Asian psyllid, *Diaphorina citri*, and (2) Heat sensitive African psyllid, *Trioza erytreae*, in Reunion island



Diaphorina citri : below ~500m

Trioza erytreae : above ~500m

Description, life cycle and biology of ACP

Diaphorina citri-Adult

- Small (2.7-3.3 mm long) with mottled brown wings
- 3 abdominal colors (**gray/brown**, **blue/green**, **orange/yellow)**
- Gravid females are mostly orange/yellow but significant of colors remain largely unknown
- Sex ratio is ca. 1:1
- Adult feeding position is 30-45° from plant surface

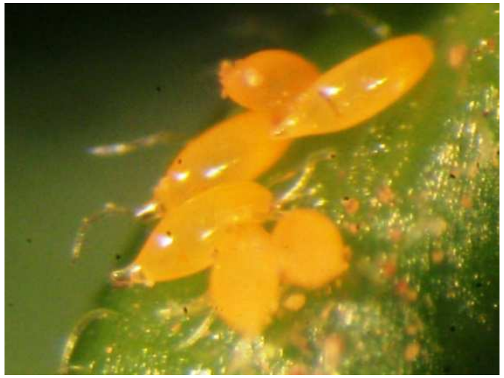
Diaphorina citri-Adult

Feeding sites of adults	Trees flushing	No new flush
Young shoots	**49.5** A	-
Midrib/veins of leaf	**24.0** B	**38.5** a
Leaf margin	**13.0** C	**43.0** a
Twig	**13.5** C	**18.5** b
Chi-square	*8.32 **	*25.44****

Diaphorina citri-Adult

- Adult locate their mates using vibrational sounds, and females emitted pheromones
- Adults mate multiple times
- ACP is a diurnal insect: Mating, oviposition, movement restricted to daytime (light is important)
- Female lay eggs throughout life time only if young shoots are present (500-1,000 eggs)
- Reproductive maturity reached in 2-3d after emergence
- Egg laying begin 1-2d after mating
- Adult live 2-3 months, but longevity is host plant, temperature and RH dependent

ACP Eggs

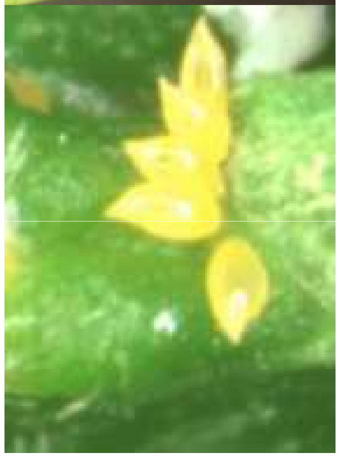


- Almond-shaped eggs
- Bright-yellow in color when freshly deposited but turn orange when mature with 2 visible red eye spots
- Laid on the tips of new flushes or in crevices of unopened leaves
- Fixed to the site by a small stalk driven into the tissue called pedicel (moisture driven)

Psyllid Nymphs

- Eggs hatch into small nymphs that go thru 5 instars (gradual metamorphosis)
- Flat, yellow-orange (0.01-0.07 inch)
- 2 red eye-spots that can be seen from 2nd instar
- Wing pad from the 3rd instar
- Settle and start feeding after hatching, but 2nd instar move to young stems
- Produce honeydew and waxy tubules that direct the honeydew
- Nymphal development takes 10 to 21 days; 16 d at 25°C

D. citri development


- Oviposition and development are temperature and humidity dependent
- Egg laying occurs between 16-42°C, w/ peaks at 25-30°C
- Very few eggs laid when RH < 40%
- Developmental time from egg to adult ranged from 14.1d at 28°C to 49.3 at 15°C with optimum temp at 24-28°C
- Generation time is 20-22d at 25°C
- ACP overwinters as adult in citrus
- Adults and nymphs can survive at -8 to -6°C for several hrs. Mild to moderate freeze events are non-lethal to ACP, but may lower spring pop.

D. citri feeding

- ACP adults and nymphs have piercing-sucking mouthparts, feed by inserting stylet into phloem tissue leaving their salivary sheaths and stylet tracks behind
- Adults found on young stems and leaves of all ages while nymphs only found on young leaves and stems (shoots)
- Nymphs produce tube-like waste covered by waxy white materials
- Adult females also produce waxy excretory substance but males produce clear substance

D. Citri life cycle

2.6-8.2 d

11-39 d

13.3-47.5 d

D. citri host plants

- ACP is an **oligophagous** with host plants restricted to the Rutaceae family, sub-family Aurantioideae (Citrus and relatives)
- A Moraceae (*Ficus carica* L.) has been reported as hosts by Thomas & De Leon (2011), but we did not confirm these findings
- Within its host plant family, ACP exhibit strong host plant preference which affects its oviposition, development, longevity, reproduction and size (morphometric analysis)
- Larger adults recorded on preferred hosts (e.g. Lemon)

D. citri host plants

- Most species in the genus *Citrus* are common hosts but there is huge variation in the preference/performance of ACP on these hosts
- Plants in other genera are also recorded as host plants of ACP: *Bergera koenigii*, *Murraya exotica* & *M. paniculata*, *Microcitrus sp.*, *Alatantia* sp., *Eremocitrus glauca*, *Fortunella* spp., *Merrillia caloxylon*, *Zanthoxylum fagara*, *Poncirus trifoliata*, *Choisya ternata*, *C. arizonica*, and *Helietta baretta*
- *Other Rutacae are just egg laying sites in no-choice situations but do not support nymphal development (Amyris madrensis, A. texana, Zanthoxylum clavahercules)*
- *Some Rutaceae are known non-host species (Esenbeckia berlandieri, Ptelea trifoliata, Casimiroa tetrameria, C. edulis, Sargentia greggii)*

D. citri host plants

- Reason of acceptance of some Rutaceae and non-acceptance of others as host plants is poorly understood
- What we do know is that on yellow chapote (*Casimiroa greggii*) the presence of hairs does not allow successful establishment of nymphs for feeding although adult survival and egg laying occur
- Elucidating this will help in developing resistance materials to ACP
- Among the known host plants, ACP exhibit strong host plant preference

D. citri preferred host plants

Bergera koenigii

(curry leaf plant)

Murraya

(orange jasmine)

Microcitrus

(finger lime)

Citrus limon

(lemon)

Citrus aurantifolia

(lime)

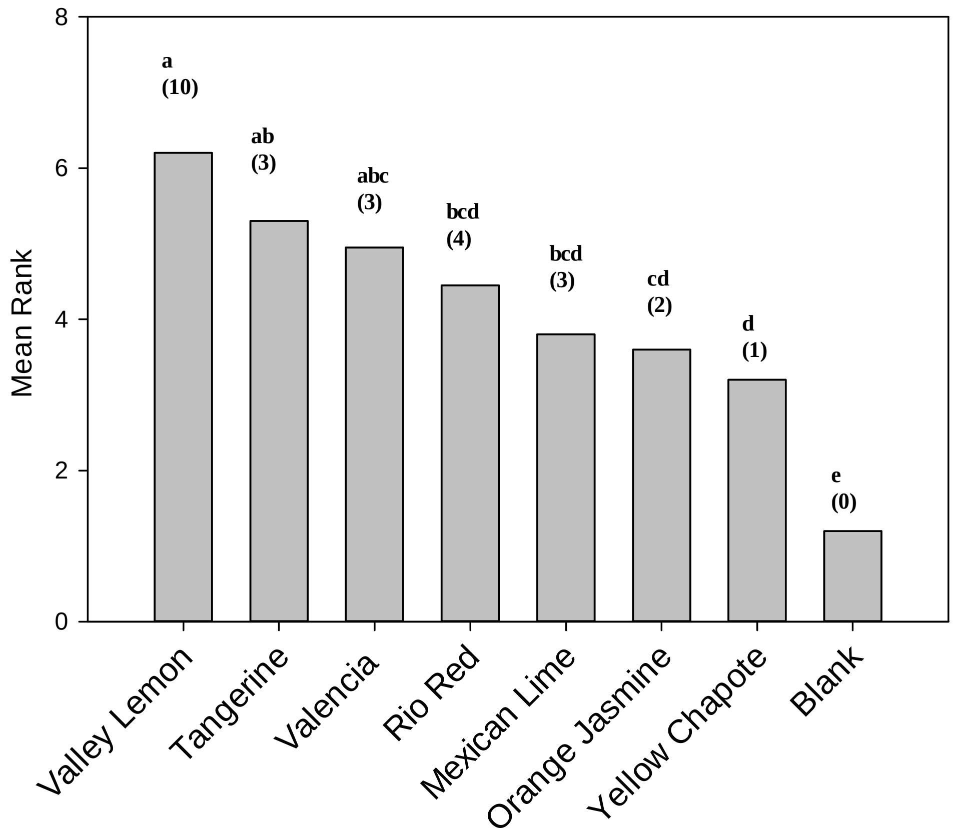
Citrus sinensis

(sweet orange)

D. citri host preference

- Psyllid choice arena (**PCA**) used in *D. citri* host preference study (8 host plants can be used simultaneously)

D. citri host preference

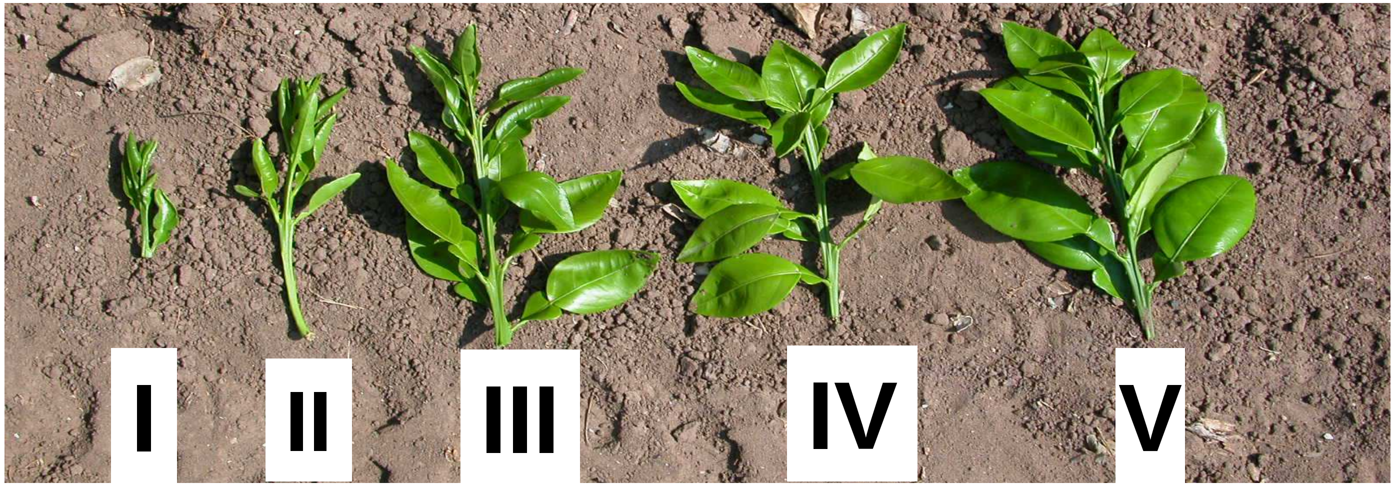


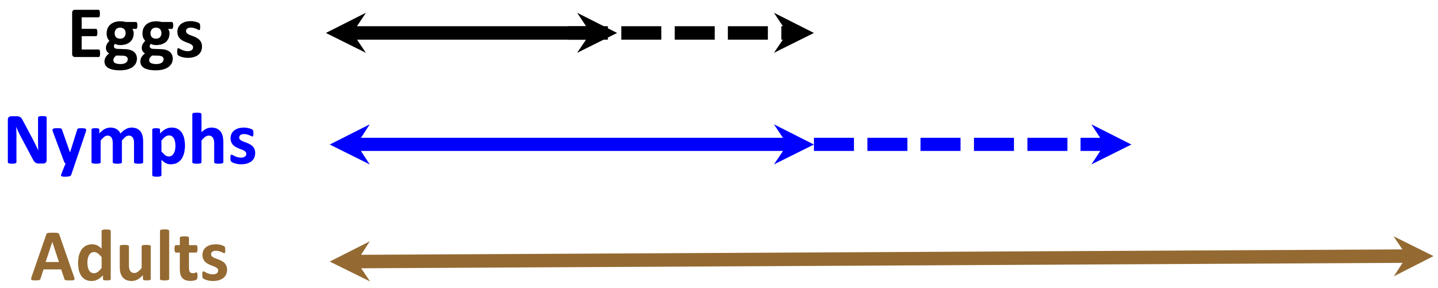
Values in parenthesis depict the number of first choices for each host plant

Factors governing D. citri host plant selection

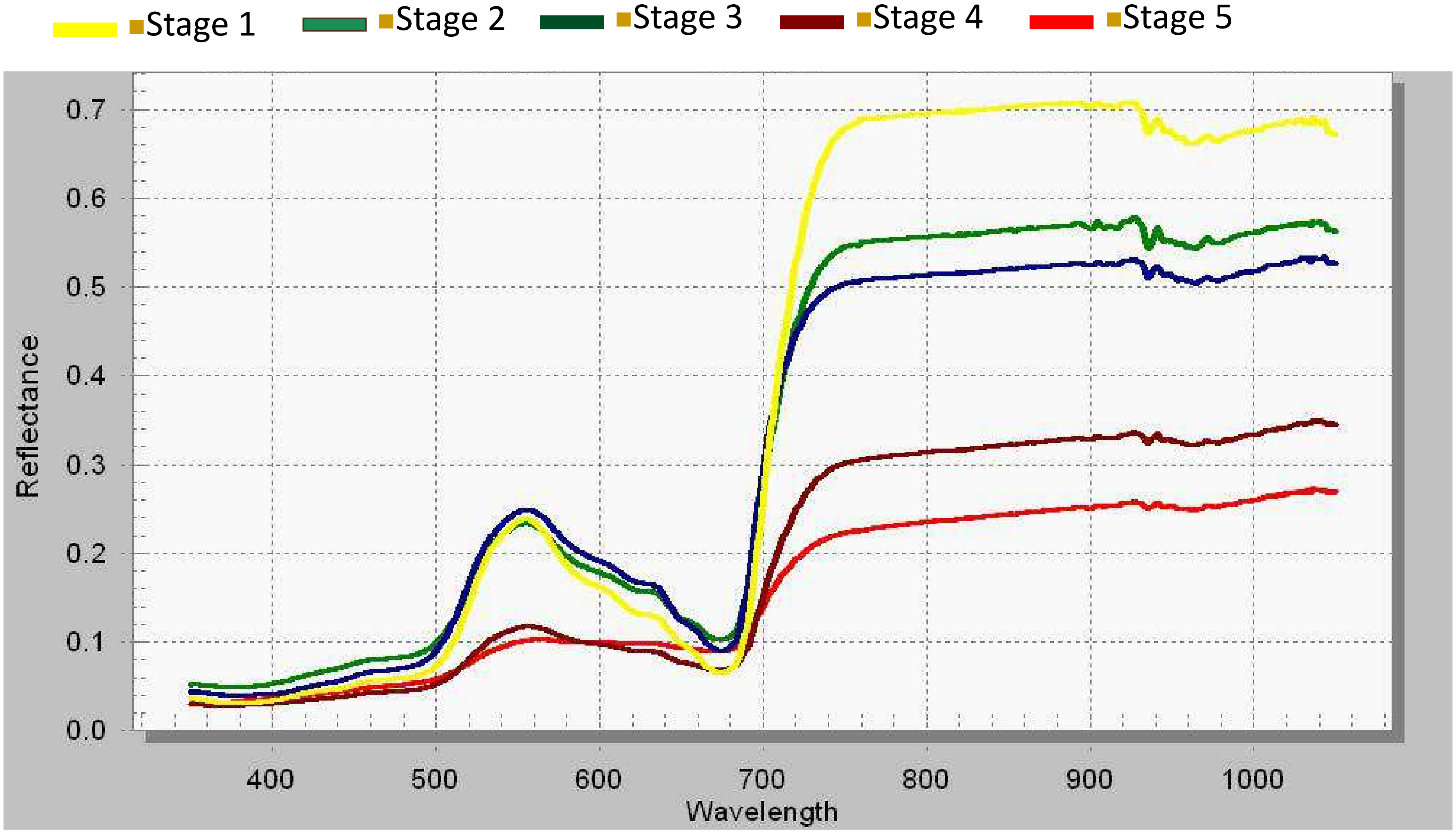
- Combination of visual, olfactory, tissue softness and nutritive value of flush shoots
- Visual characteristic (higher reflectance values in the yellow range)
- Olfactory cues (some sesquiterpenes involve in ACP adult attraction)
- Tissue softness allowing copious feeding and egg laying
- Nutrient content of flush shoot (high N and protein content)

Flush shoot growth stages and ACP phenology

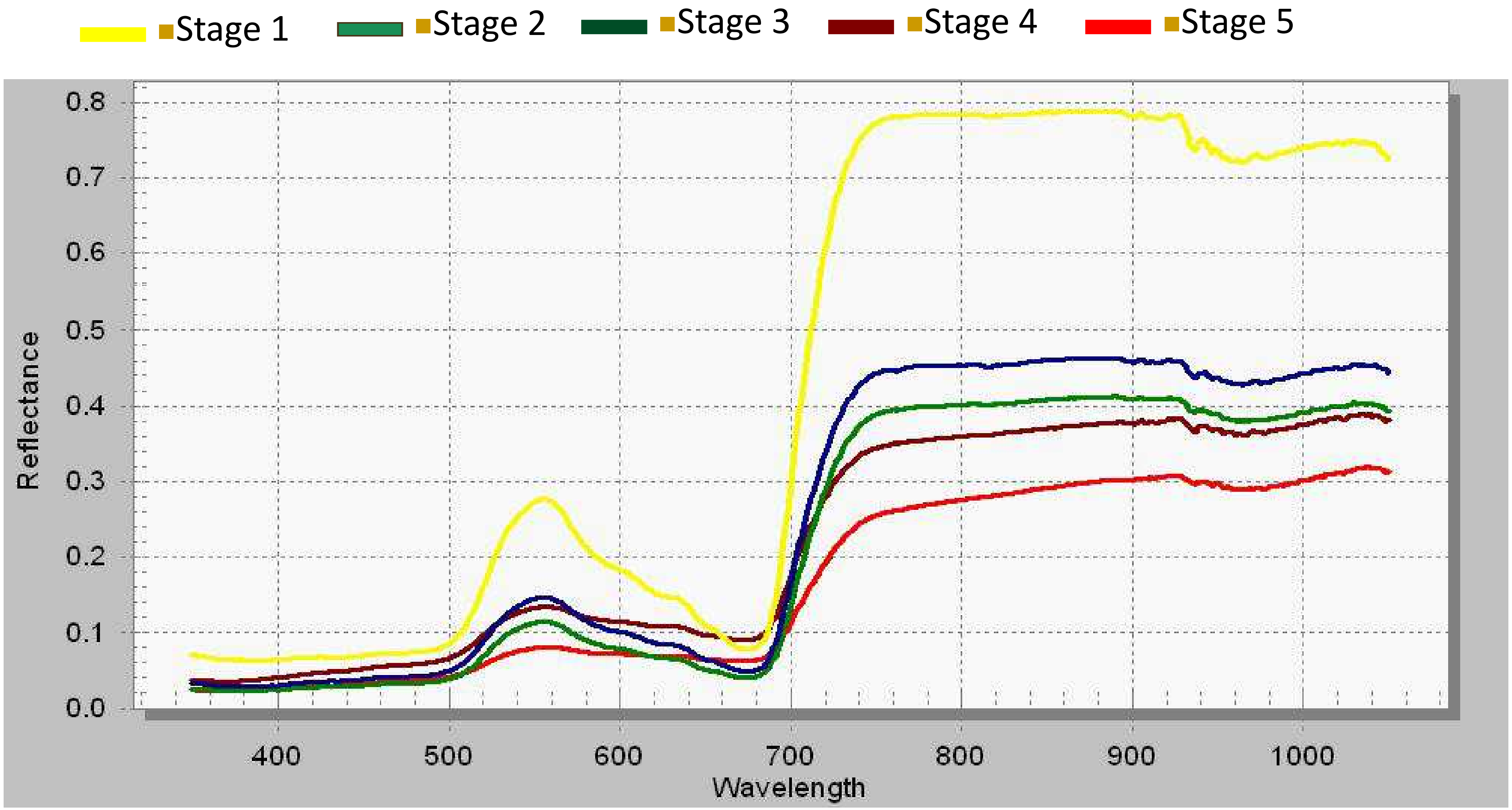




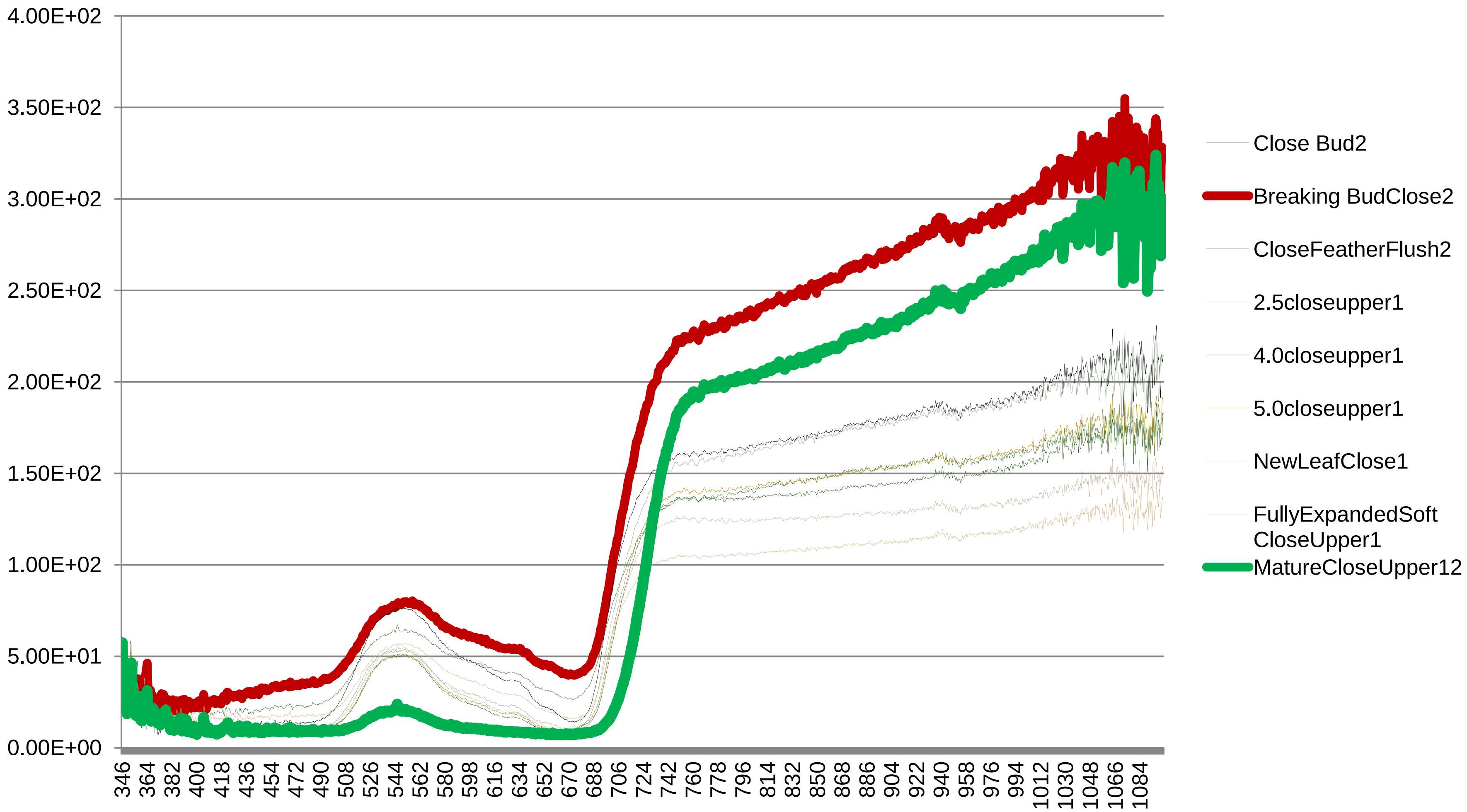
Spectral reflectance of citrus flush shoots (sweet orange)



Spectral reflectance of citrus flush shoots (Grapefruit)

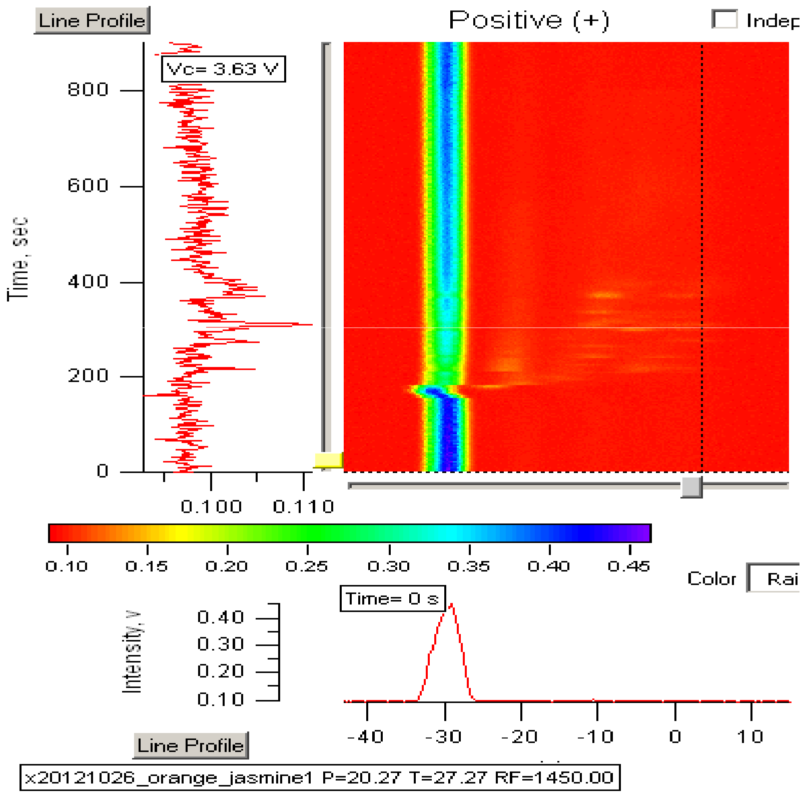


Factors governing D. citri host plant selection: Flush shoot reflectance

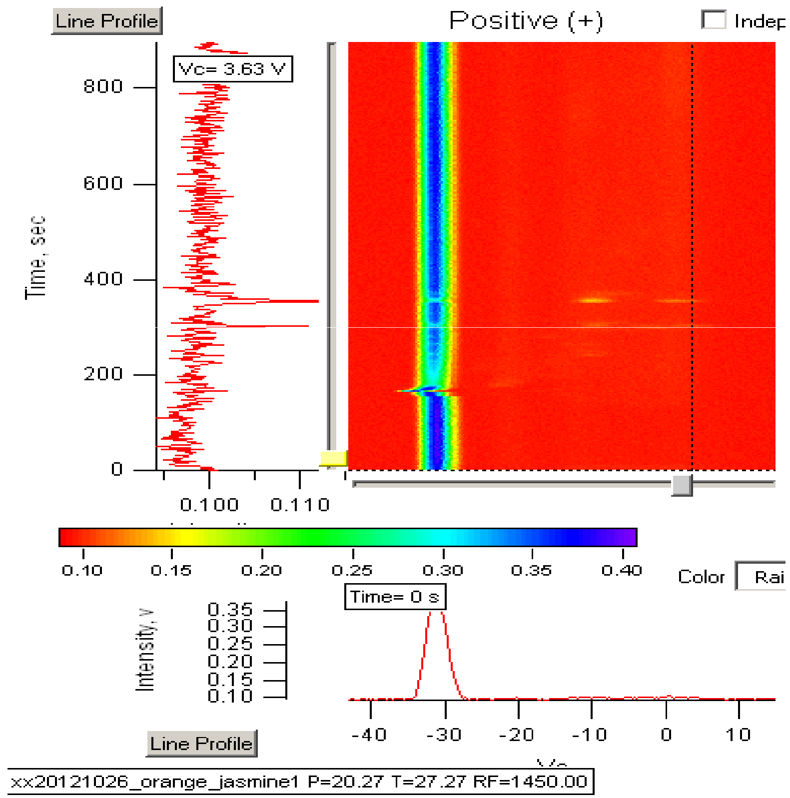


Volatile profile of flush shoot

Young flush shoot



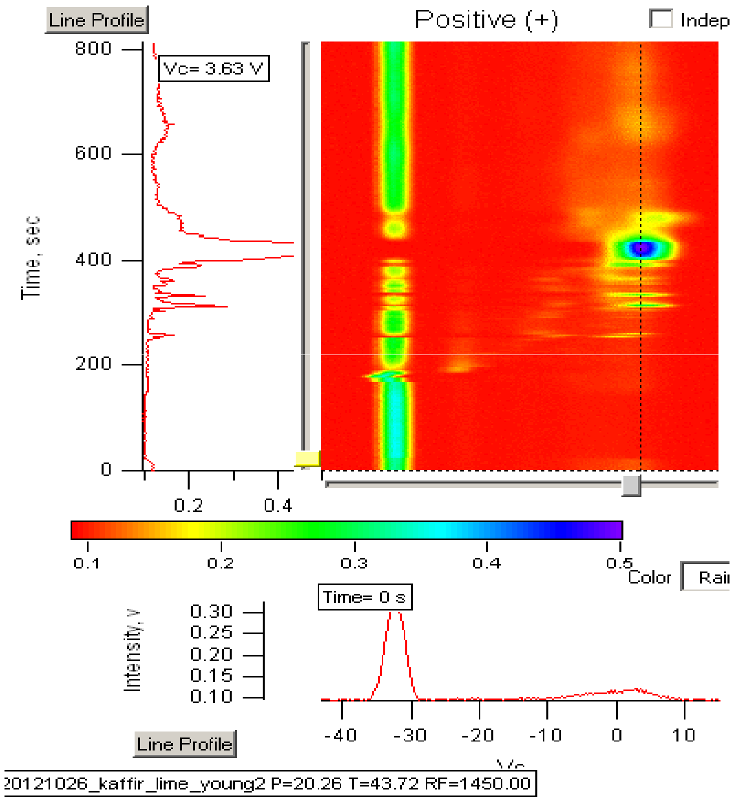
Mature flush shoot



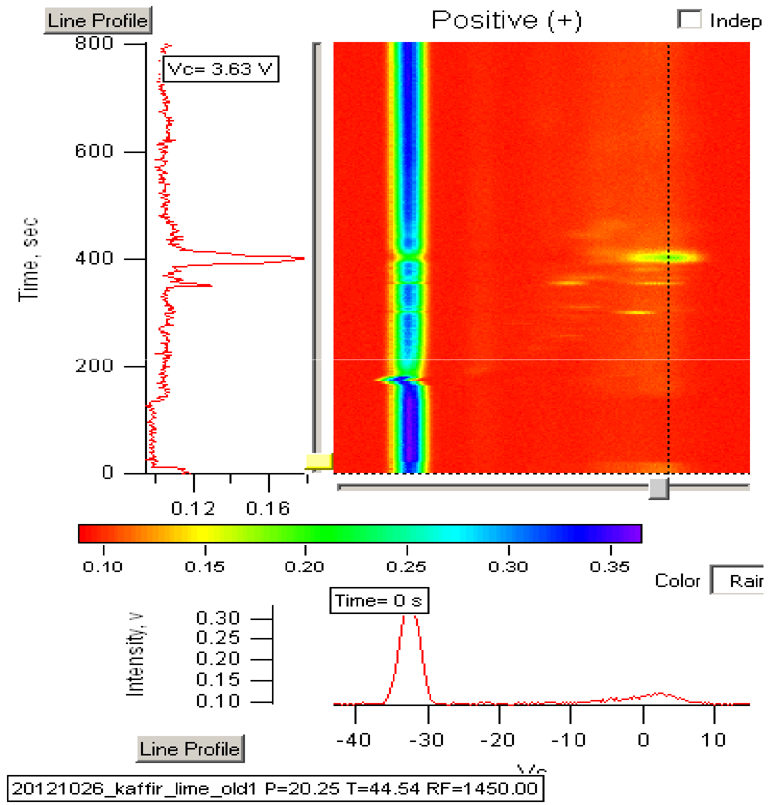
Orange jasmine

Volatile profile of flush shoot

- Young flush



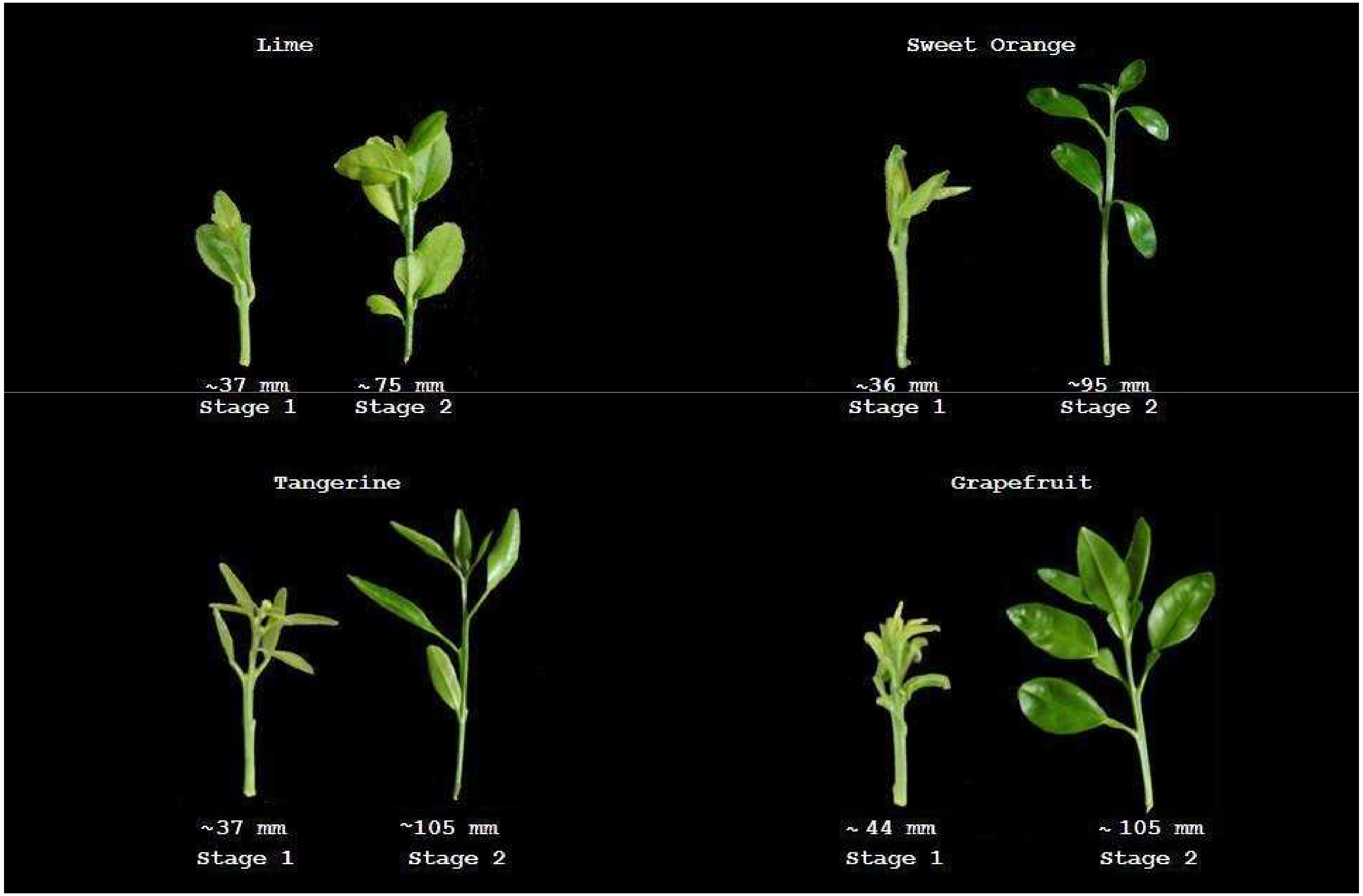
- Mature flush shoot



Kaffir lime

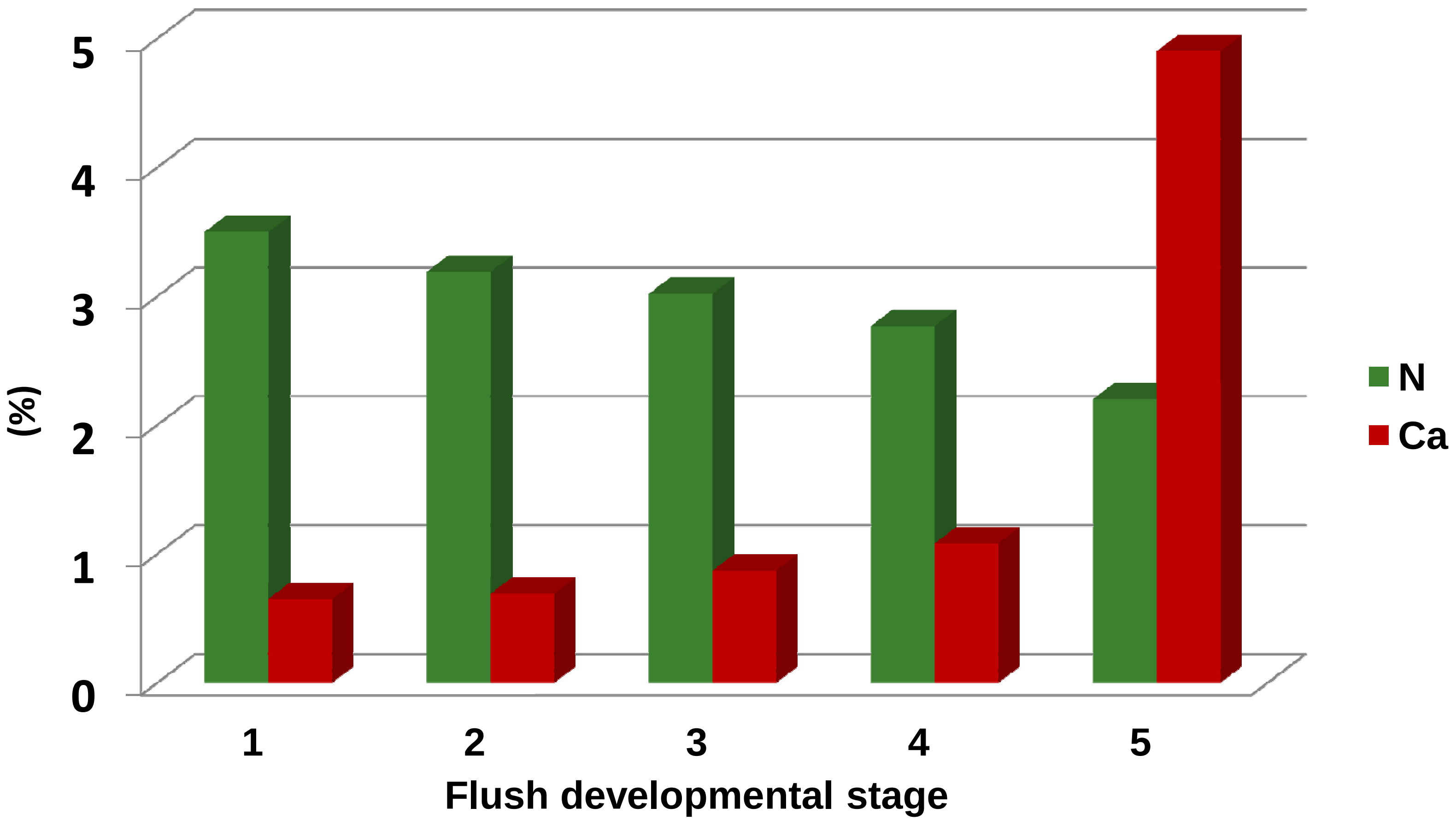
Factors governing D. citri host plant selection:

Flush shoot architecture



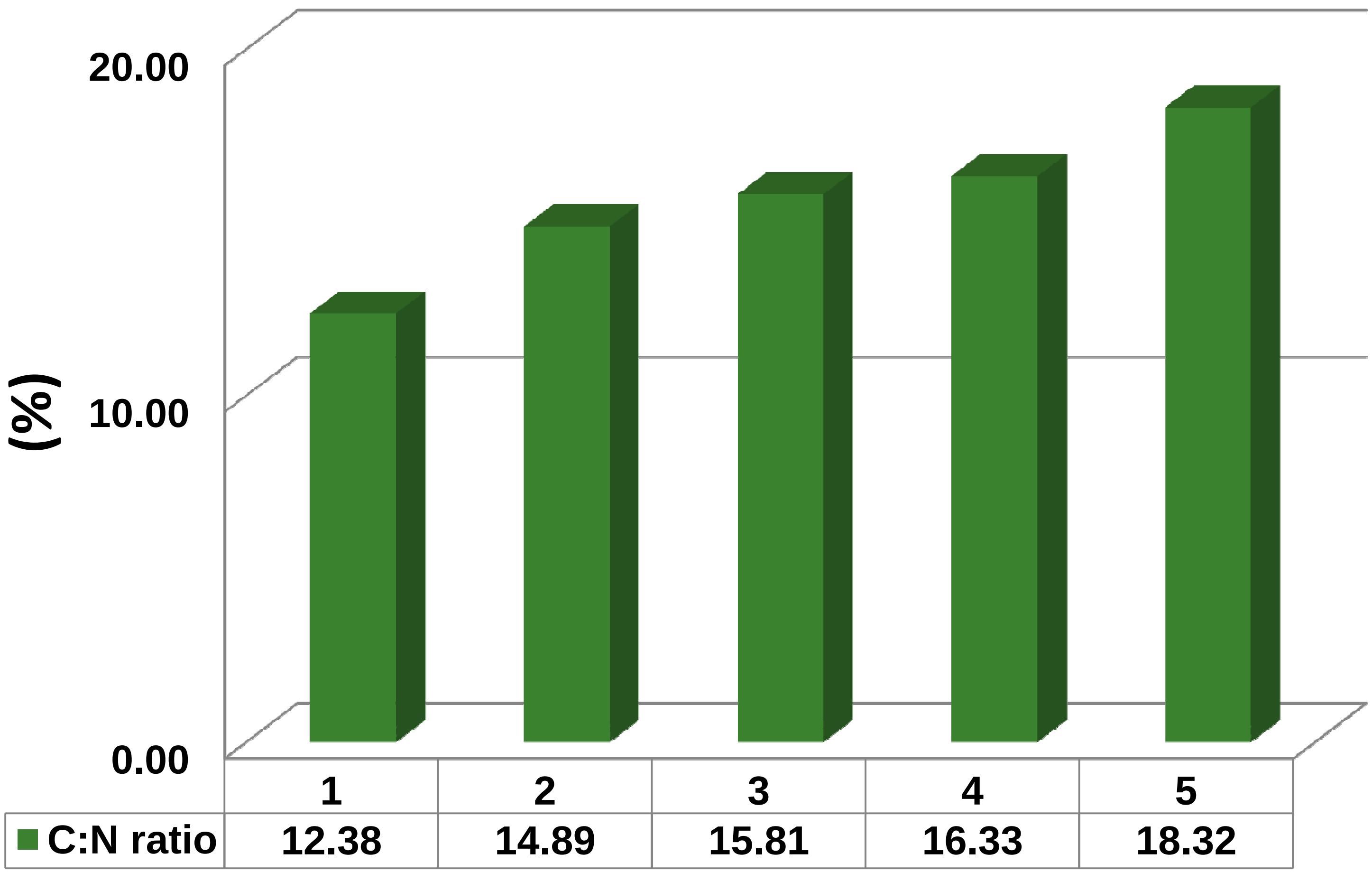
Factors governing D. citri host plant selection:

Flush nutrient content



Factors governing D. citri host plant selection:

Flush shoot C:N Ratio



Factors governing D. citri host plant selection:

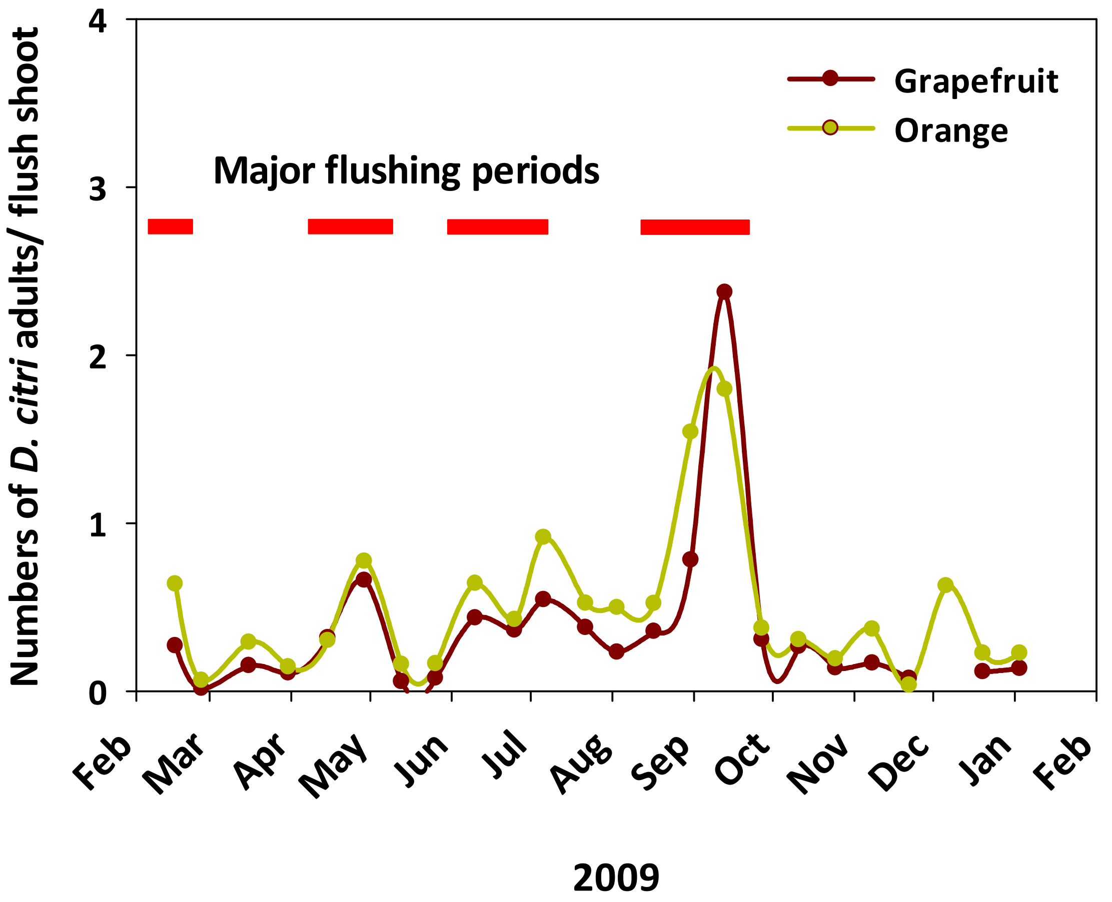
Tissue softness

- Eggs are deposited on soft tissue-As flush shoots mature their softness decreases. Good relationship between tissue softness and number of eggs laid
- Flush shoot availability, softness and speed of maturity could explain differential densities of ACP on ≠host plants (lemon shoots are soft, lime & lemon frequent flushing)

Factors governing D. citri populations

- In citrus groves, ACP population is intimately related to flush cycles because egg laying and nymphal development ONLY occur on young shoots
- Host species also determine ACP population
- Periods of peak ACP populations depend on the region: E.g. in Florida large ACP infestations occur from late Spring to mid-Summer, in contrast in Texas, ACP populations gradually increased reaching its peak in September (late Summer to Fall)

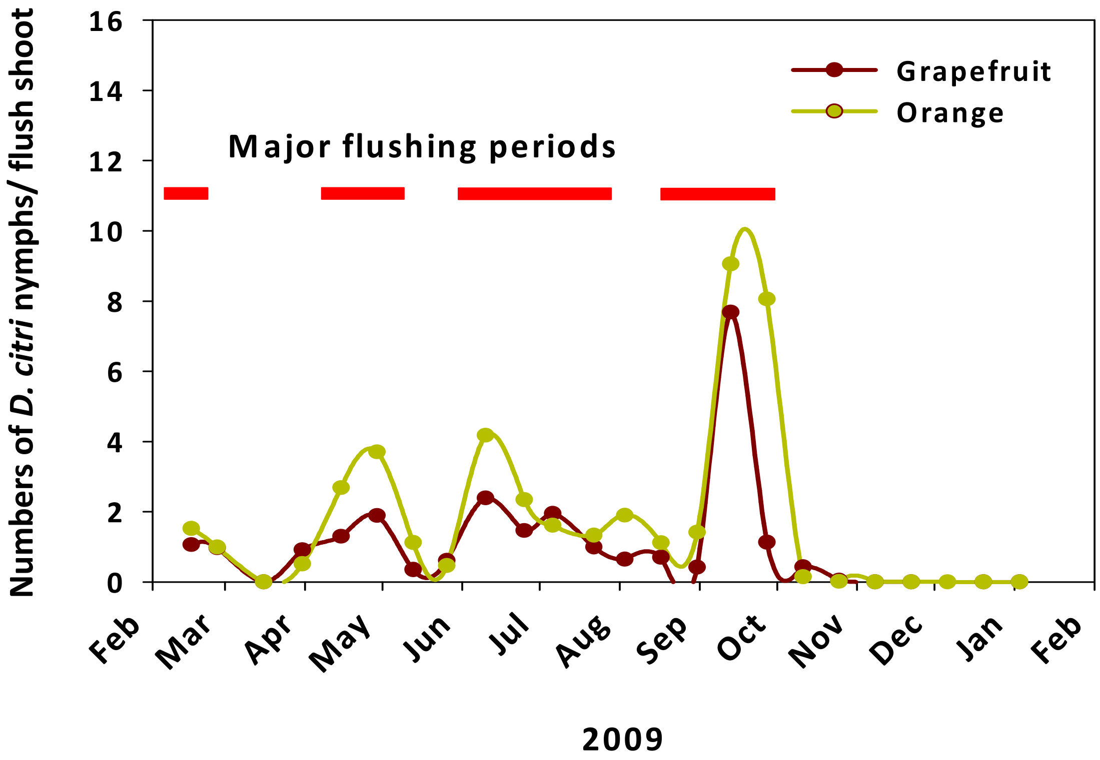
Factors governing D. citri populations



- Adult numbers increased with new flush shoots

Factors governing D. citri populations

Immatures only present when new flush are produced



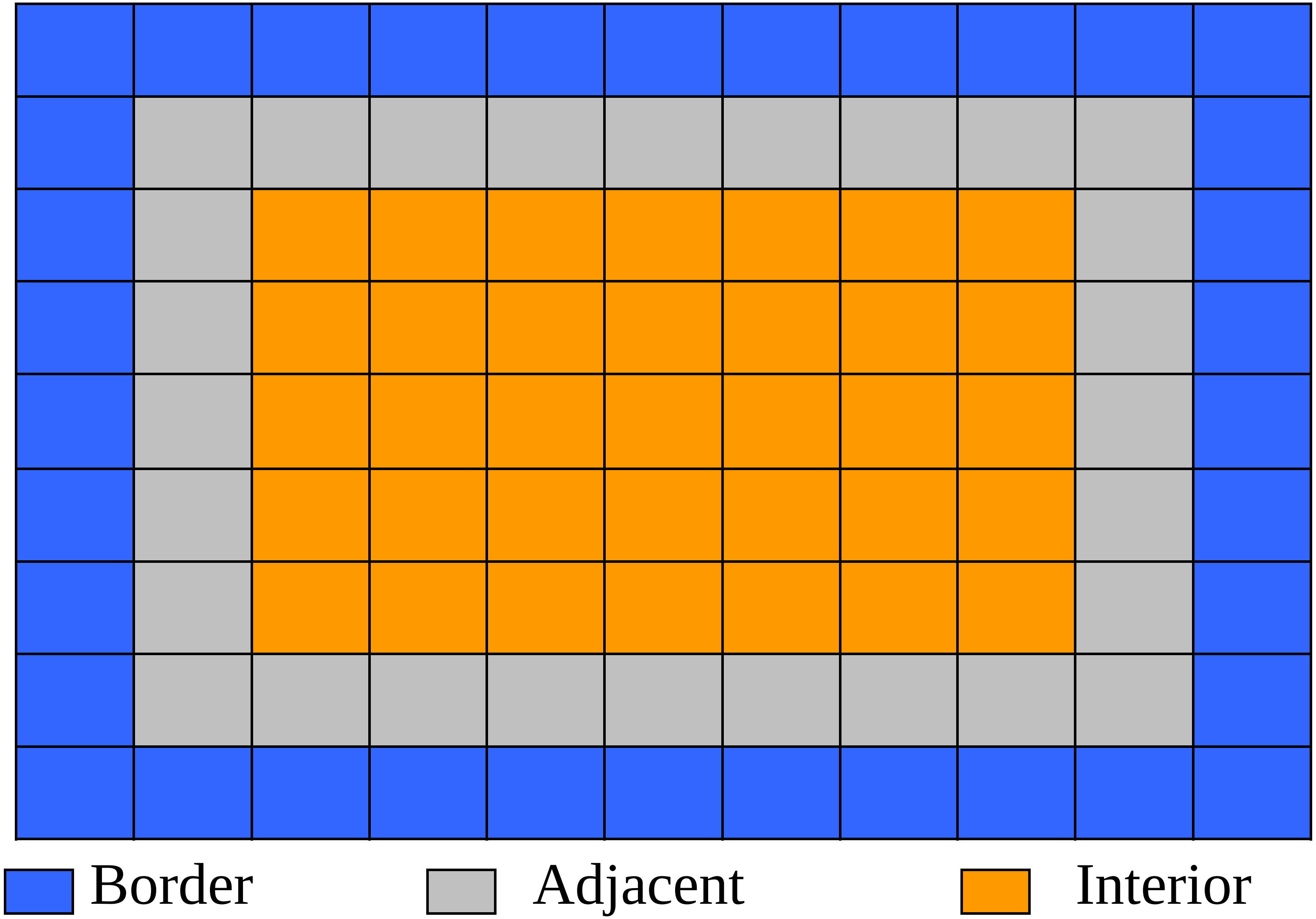
ACP flight behavior

- **ACP is a weak flyer**: Adults cannot fly very far or sustain long flights because of weak muscles relative to large size of wings
- Longest flight is 47-49 minutes in flight mill (Arakawa & Mivamolo 2007)
- Longest distance is 978m for females and 1.2 km for males (speed 1.4 km/hr)
- ACP active during daytime
- ACP can be transported by winds over long distance
- However due to its posture, ACP adults mostly face the direction where wind is coming from: explain higher densities on southeast of groves in FL & TX **(dominant wind SE)**

Field distribution

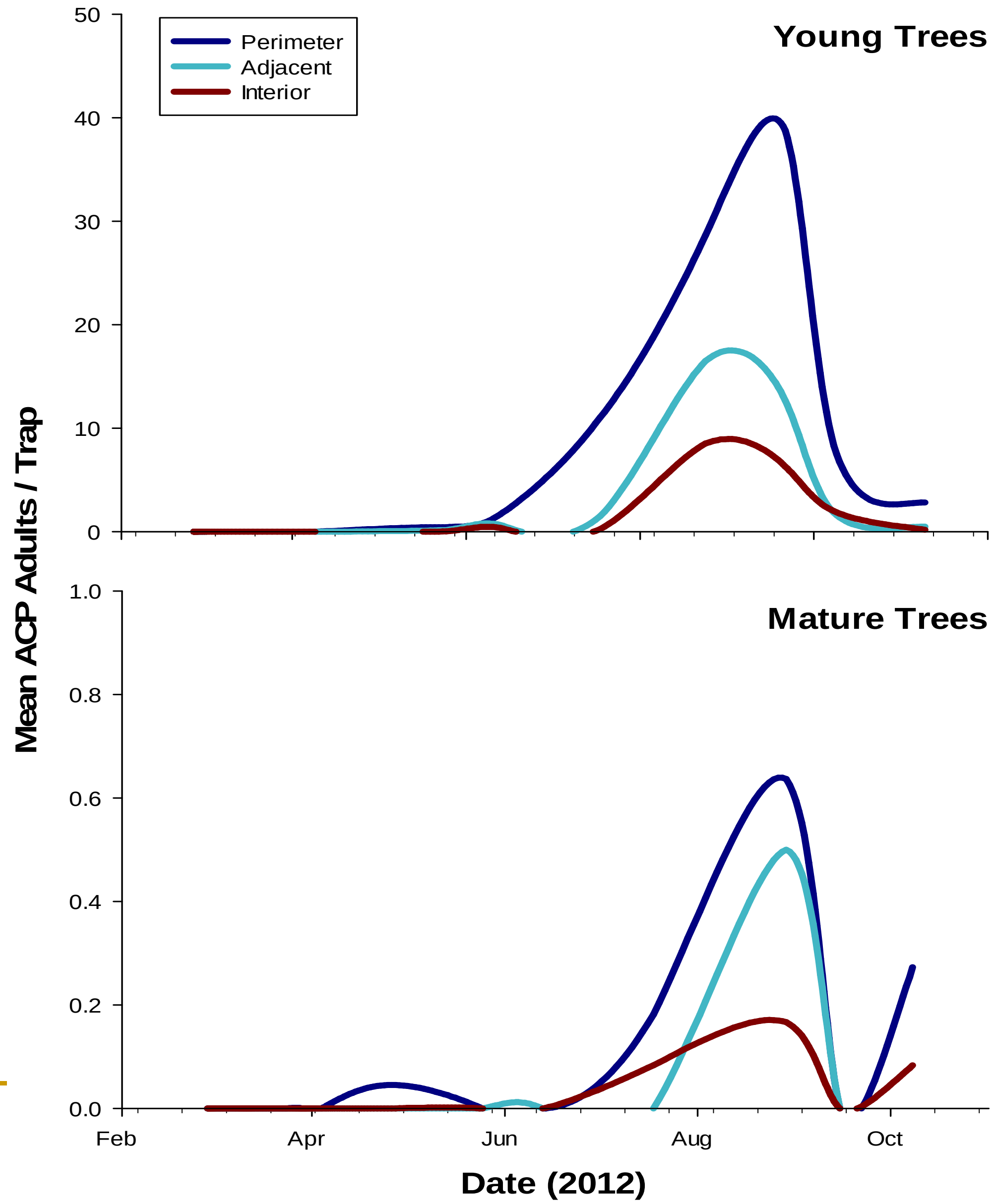
- ACP are frequent movers, move frequently between adjacent groves, thus explaining the border effects in ACP spatial distribution in the field
- Studies need to be conducted within each region to determine which side of the grove is the most infested

Field distribution studies of *D. citri*



Field distribution

- Strong edge effects in the distribution of ACP that could be used for developing control methods



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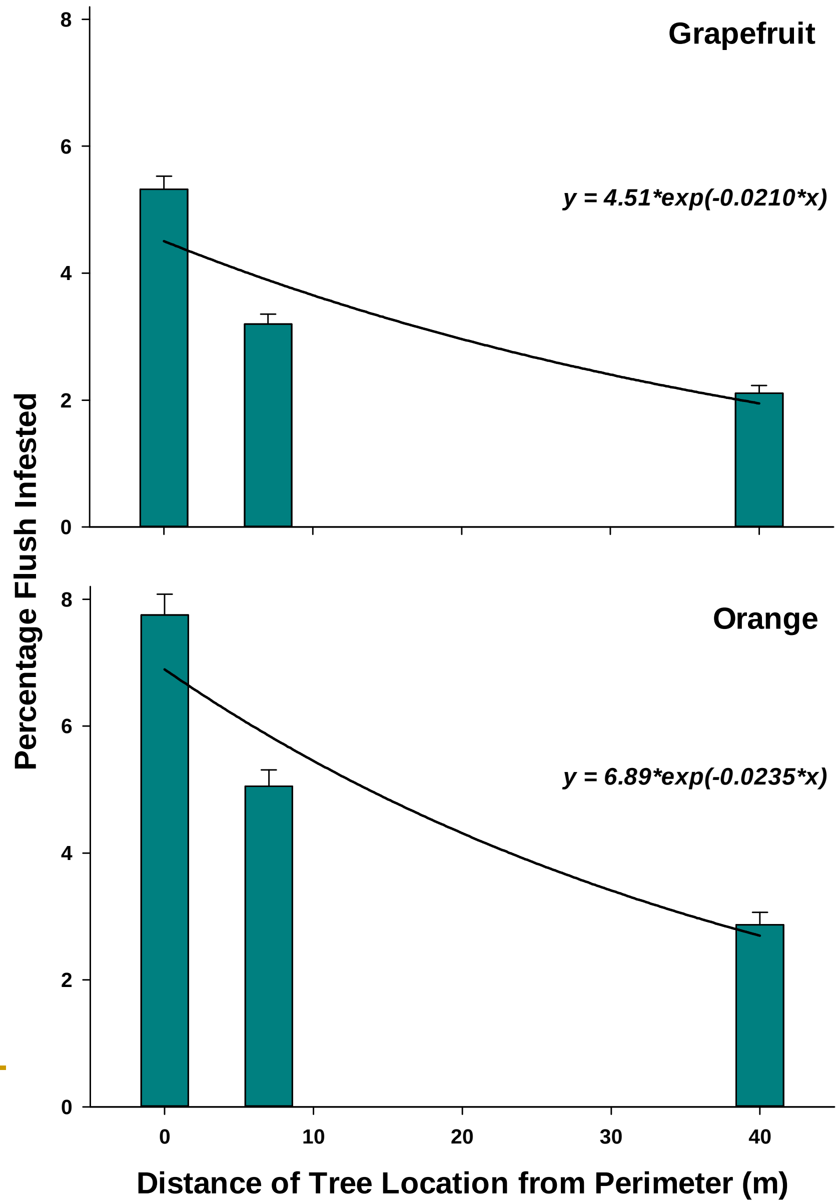
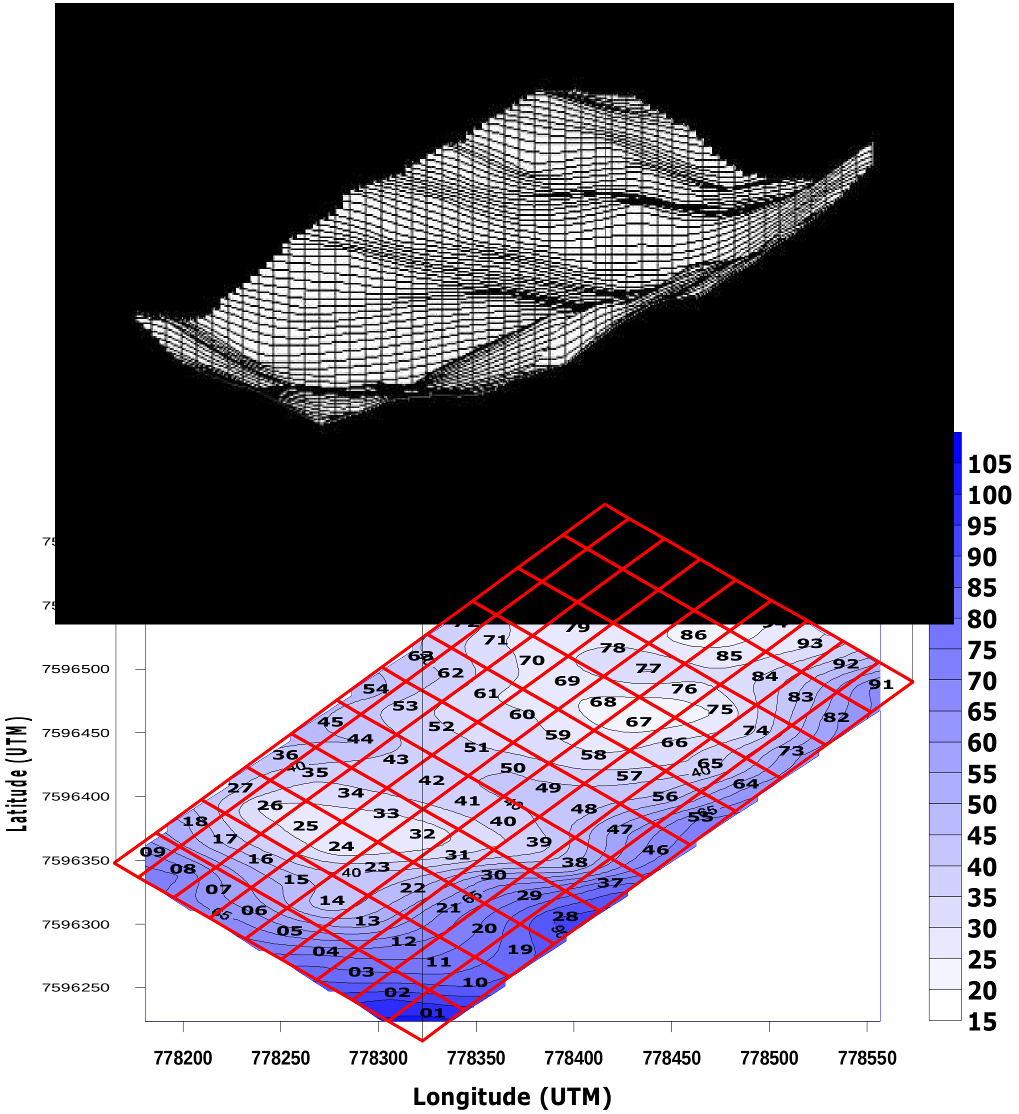


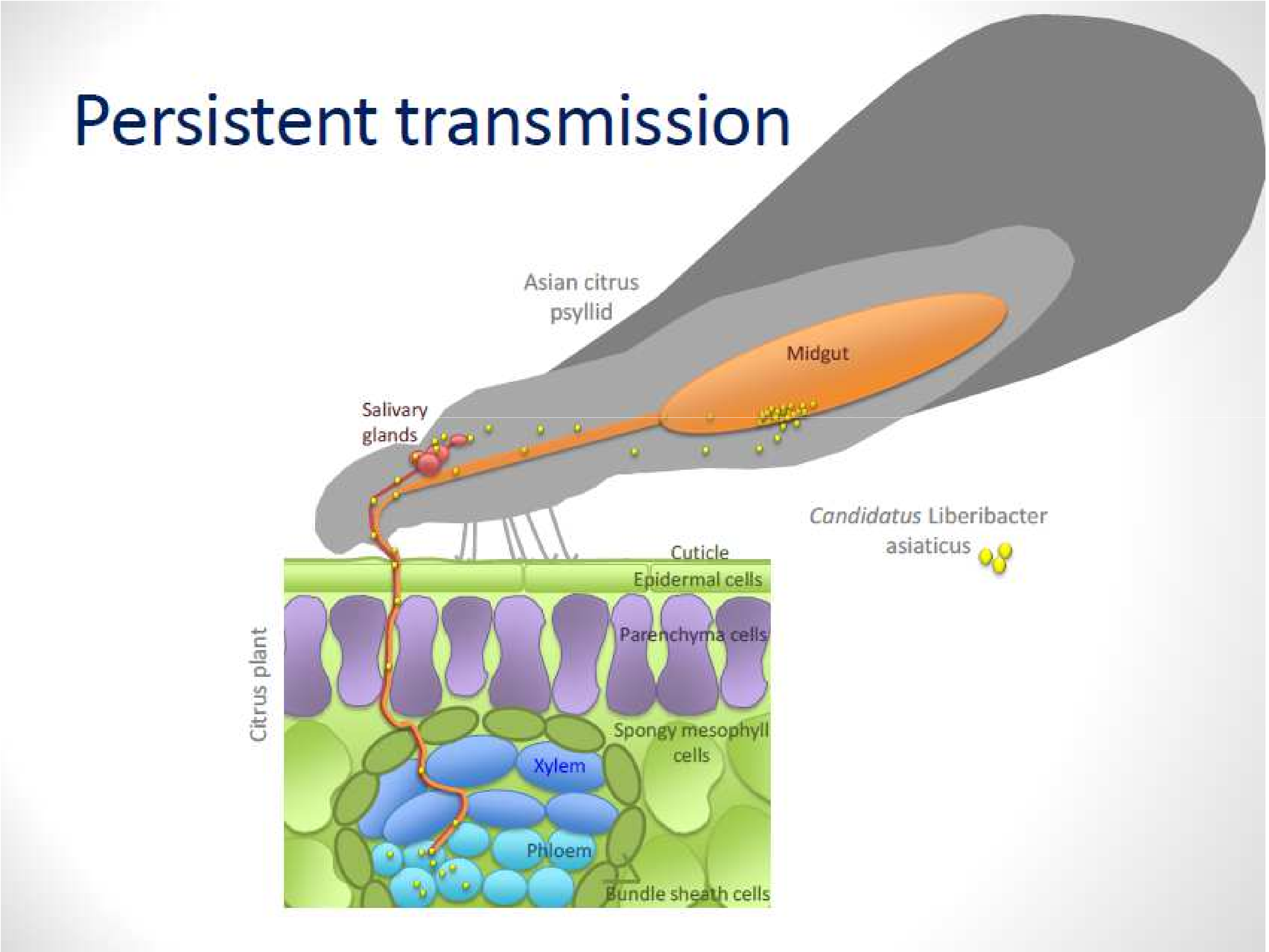
Field distribution



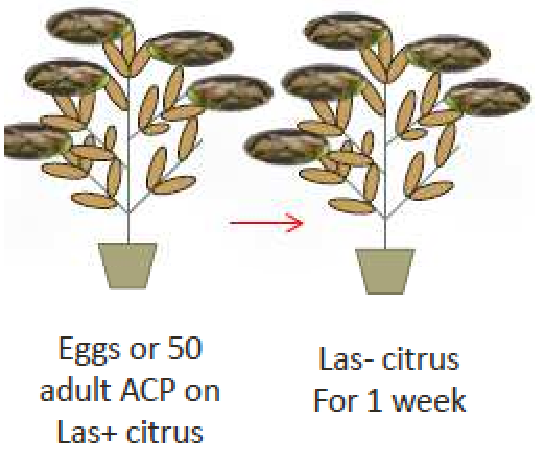
ACP Monitoring

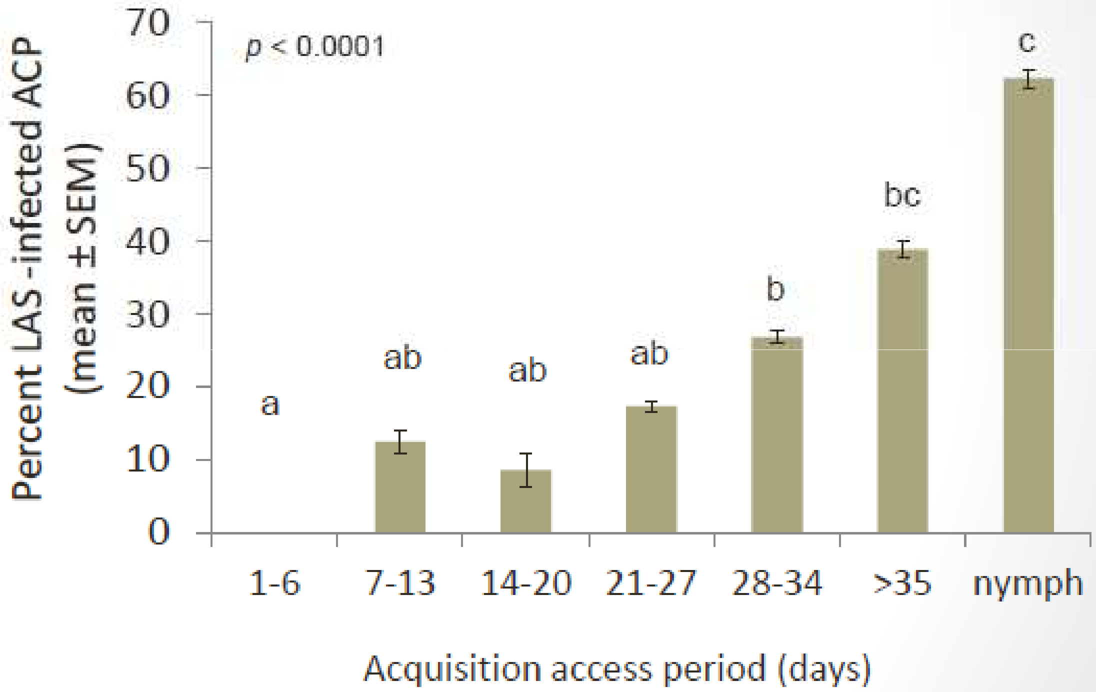
- **Monitoring of ACP**:
  - visual observations (for immatures)
  - tap sampling (beat sheets, bucket)
  - Sticky traps (yellow & lime-green)
  - Sweep nets & vacuum
- Choice of method depends on objectives of study

CLas acquisition and transmission by ACP



CLas acquisition and transmission by ACP



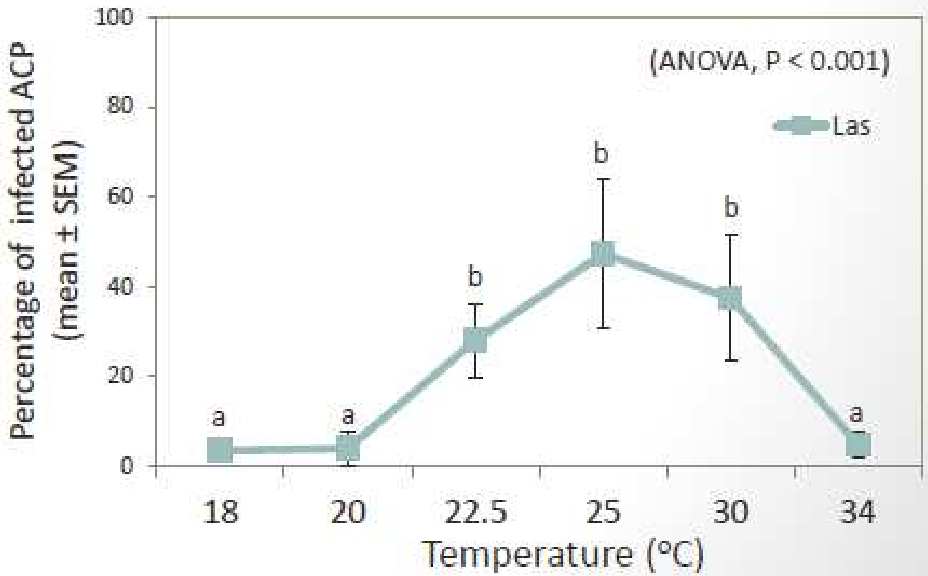


- Acquisition of Las by ACP greatest when reared on infected plant

CLas acquisition and transmission by ACP

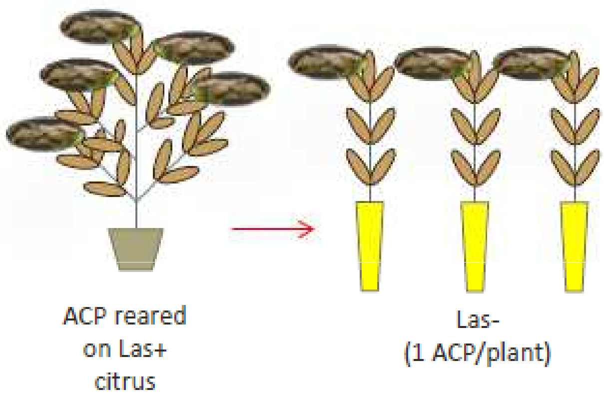
Does acquisition change with temperature?

- ACP reared on Las-infected plants and removed two weeks after adult emergence



CLas acquisition and transmission by ACP

Transmission: Inoculation

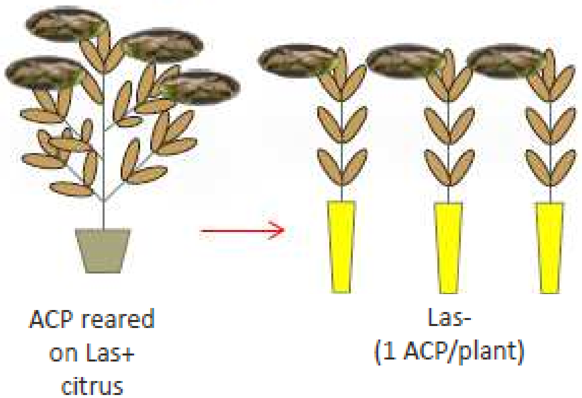


- ACP transferred to healthy citrus for 1-15 d inoculation access periods (IAPs)

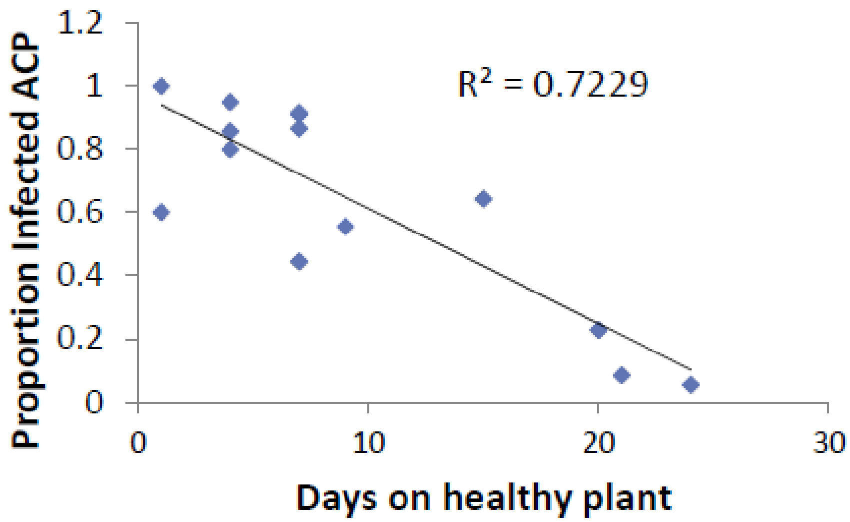
IAP (days)	% Positive psyllids (N)	Infected plants (%)
1	45.7 (35)	6.3 a
4	72.4 (58)	7.1 a
7	81.3 (48)	7.7 a
15	69.2 (39)	3.7 a

CLas acquisition and transmission by ACP

Do psyllids retain the pathogen throughout their lifetime?



- ACP reared from eggs on Las+ citrus
- Held for 1-25 d, then tested for Las with real-time PCR
- N= 10-40 ACP per day



Retention of Las by ACP decreases over time

CLas acquisition and transmission by ACP

Pathogen-vector interaction:

Transovarial Transmission

Developmental stage	*n*	% Infected offspring
Eggs	49	2.0
Nymphs	48	6.3
Adults	42	2.4

- Low rate of vertical transmission (parent-offspring)

Summary

- ACP from India, 2 founding events in America
- ACP in North America from southwestern Asia
- ACP exhibit strong host plant preference: **Lime, Lemon >> Sweet oranges >> Grapefruit**
- Population fluctuations depend on flush cycles
- Border effects on ACP distribution
- Frequent movement of ACP between groves
- Spatio-temporal behavior of ACP dictates AWM strategies for ACP
- Acquisition of CLas greatest at nymphal stage (management strategies should prevent nymphal development)

Acknowledgments

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Dr. Pelz-Stelinski, UF

Dr. M. Rogers

Dr. P. Yamamoto Univ of Sao Paolo

TX Citrus Growers

THANK YOU!

GRACIAS