BioControl Updates for Asian Citrus Psyllid in Southern California

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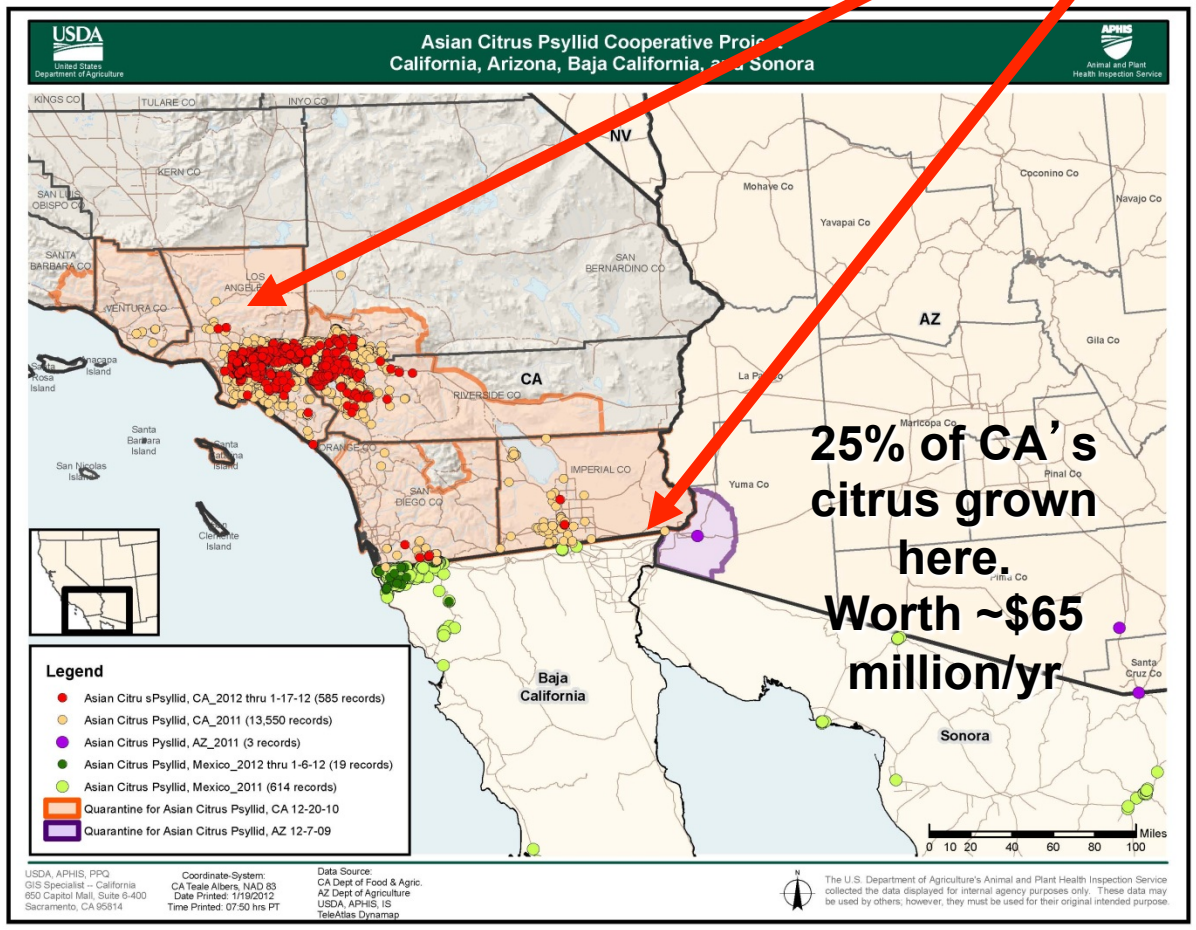
Distribution of ACP in California



25% of CA's citrus grown here. Worth ~$65 million/yr

Is HLB in California?

- At present there is ONE known HLB infestation in CA
  - Hacienda Heights in LA County
- It is highly likely that HLB is in other parts of SoCal
  - Plants smuggled into CA from Asia have been intercepted at airports
    - Some plants have been contaminated with ACP and infested with HLB
    - How many infected plants are in people's gardens waiting to ACP to arrive?

Management Options

- **Biological Control**
  - Use of natural enemies, in particular parasitoids of great interest for suppressing ACP populations
    - First work on ACP parasitoids conducted by Husain & Nath (1927) in the Punjab of Pakistan
      - Study sites: Sargodha, Lyallpur, and Gujranwala
      - Trees dry up, fruit is insipid, leaves fall to ground
    - Nine species of parasitoid associated with ACP nymphs
      - No adult or egg parasitoids recorded
      - Hyperparasitoids exist
      - 1 species named from this project, *Tamarixia radiata*

Tamarixia radiata (Waterston) (Hymenoptera: Eulophidae)

- First described from specimens collected from lemons in Lyallpur, Punjab, 2 Jan 1921
  - Solitary ecto-endoparasitoid
  - Arrhenotokous: 1.8♀ : 1♂
  - At 25°C egg-adult = 24 days
  - Attacks 3rd, 4th, & 5th instar ACP
  - Females live 12-24 days
  - Females lay 166-300 eggs
  - Kills ACP via host feeding too

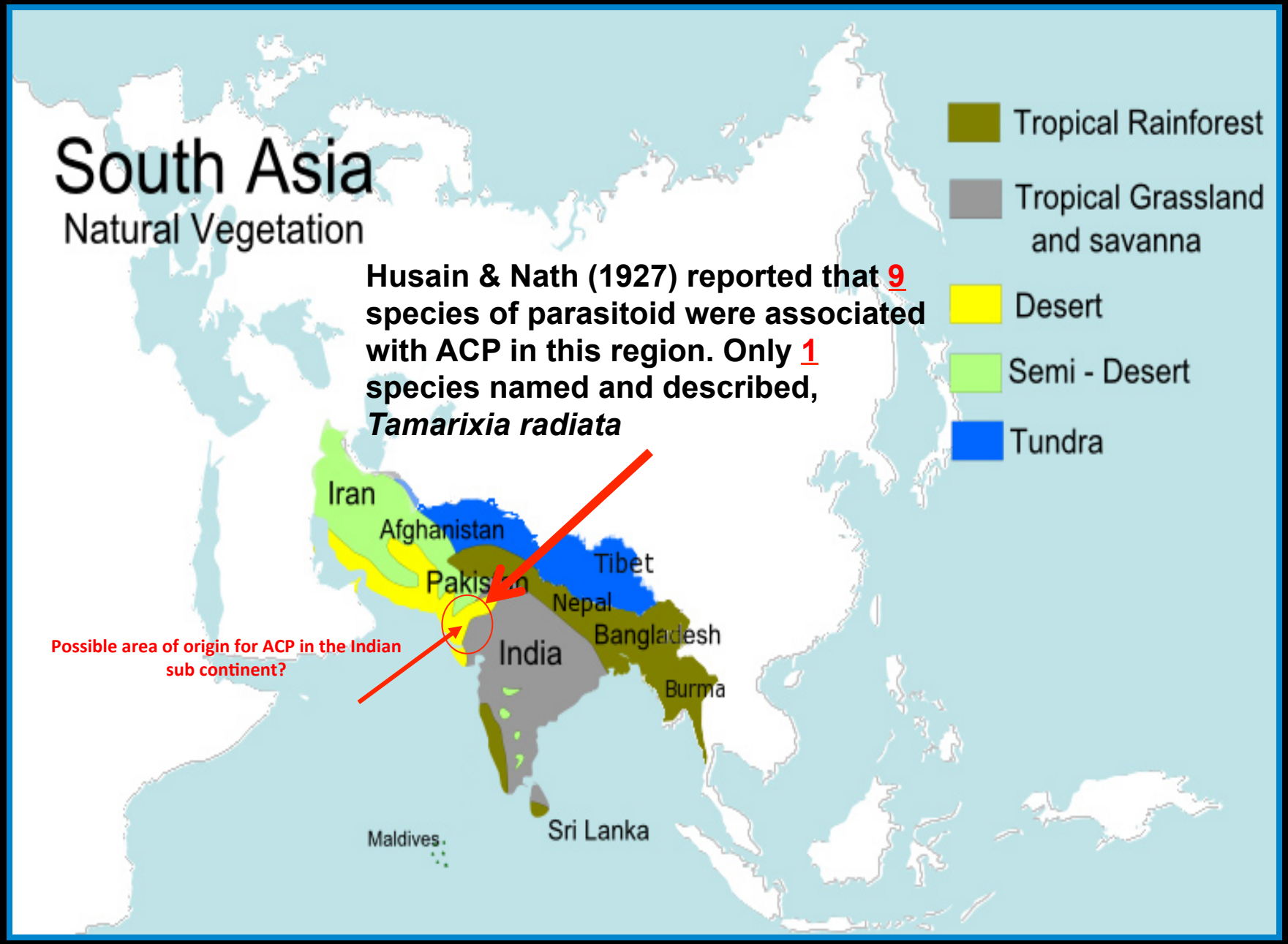
Adult Female and Male *Tamarixia*

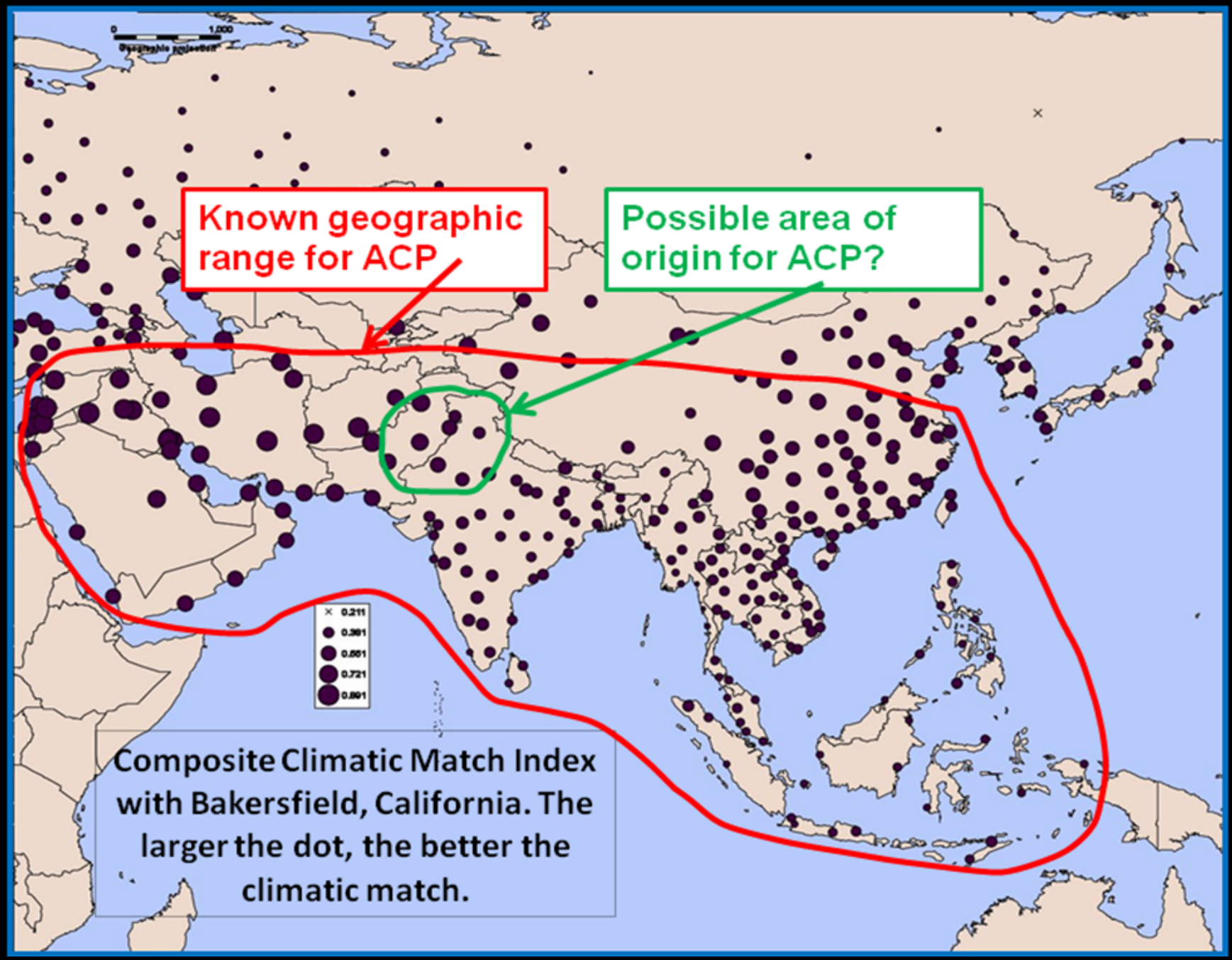
Female *Tamarixia* have clubbed antennae

Male *Tamarixia* have setose or plumose antennae

Developing a BioControl Program in CA with *Tamarixia*

- Parasitoids from Punjab of Pakistan are of most interest for establishment in CA because of the very good climatic match the major citrus growing regions of CA


South Asia
Natural Vegetation
Husain & Nath (1927) reported that 9 species of parasitoid were associated with ACP in this region. Only 1 species named and described, *Tamarixia radiata*
Tropical Rainforest
Tropical Grassland and savanna
Desert
Semi - Desert
Tundra
Iran
Afghanistan
Pakistan
Tibet
Nepal
Bangladesh
India
Burma
Sri Lanka
Maldives
Possible area of origin for ACP in the Indian sub continent?


Known geographic range for ACP
Possible area of origin for ACP?
Composite Climatic Match Index with Bakersfield, California. The larger the dot, the better the climatic match.

Summarized in Citrograph

http://www.citrusresearch.org/citrograph-sept-oct-2010

Foreign exploration for Asian citrus psyllid in Pakistan

The hunt for natural enemies and observations on 'Kinnow' mandarin

Mark S. Hoddle

Asian citrus psyllid (ACP), *Diaphornia citri*, is considered to be one of the world's most serious threats to economic citrus production because it vectors the bacteria, *Candidatus* Liberibacter asiaticus, that causes Huanglongbing (HLB) (also known as citrus greening), a disease that is lethal to most varieties of citrus.

In countries where this psyllid-bacteria combination have successfully invaded, citrus production has dropped markedly because trees go into decline -- losing vigor, leaves drop from trees and the canopy becomes thin, developing fruit tends to become small, misshapen, and juice quality may diminish. The most dramatic impacts in the last decade have been observed in Brazil and Florida, the world's two largest producers of orange juice. In Florida alone, about 10% of citrus acreage (~60,000 acres) has become unproductive because of HLB.

In September 2008, ACP was found in southern California, and it is thought that this insect entered California from Mexico where both the insect and HLB are present. Currently, HLB is not known to be found in California. However, it is thought the bacteria are likely present in citrus or curry plants that are growing in home gardens in California. The reason this possibility exists is because people may have smuggled plants from Asia into California to plant. Interceptions of smuggled plant material last year at Fresno international airport in California detected the presence of HLB in psyllids on Indian curry leaves. These intercepted psyllids and plant material were destroyed. However, it is possible that other illegal introductions may have escaped detection by border inspection and quarantine officials and this material has successfully entered California.

In an attempt to biologically manage ACP in California, entomologists at the University of California Riverside have been working at the University of Agriculture in Faisalabad in Pakistan (formerly known as the Punjab Agricultural College and Research Institute in Lyallpur) to better understand the impacts natural enemies have on controlling ACP in the Punjab region of Pakistan.

Healthy Asian citrus psyllid nymph. *Photo courtesy of Michael Rogers, University of Florida.*

Adult *Tamarixia radiata* parasitoid. *Photo courtesy of Marjorie Hoy, University of Florida.*

The reason this area was selected for investigation is because the Punjab region of Pakistan and India may be the area of origin where ACP and HLB evolved. The reason for this assumption is that the first study on ACP was published by two "Imperial Entomologists", Mohammad Husain and Dina Nath, who studied ACP attacking citrus primarily in the Punjab region of Pakistan. Their research entitled "The Citrus Psylla (*Diaphorina citri* Kuw.) Psyllidae: Homoptera" was published in 1927. Since this study, ACP has spread to many different parts of the world where citrus is grown.

From the point of view of a biocontrol specialist, one of the most intriguing statements made in Husain and Nath's study is on page 24 of their 27-page publication. Here they state that nine species of parasitoid wasps attack ACP in the Punjab, and several of these parasitoid species have their own parasitoids, or hyperparasitoids, attacking them. Hyperparasitoids are parasitoids attacking the parasitoids that are killing ACP. Consequently, hyperparasitoids may limit the impact some ACP parasitoids have on the pest because they are killing these ACP natural enemies.

This high diversity of parasitoid species associated with ACP as reported by Husain and Nath also suggests that the Punjab may be the home region for ACP. Biological control theory states that natural enemy diversity should be highest at the evolutionary center of origin of a pest, because at this location, the greatest amount of time has been available for the evolution of a diverse guild of natural enemies to exploit ACP as food.

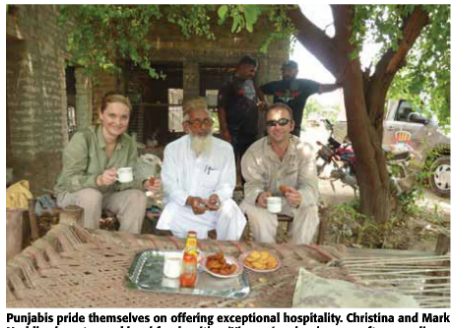
What is very curious about Husain and Nath's work is that today we know of only two species of parasitoids that attack ACP, not nine species. One of these parasitoids, *Tamarixia radiata*, was reared and described from ACP living on lemon leaves collected in the Punjab of Pakistan in 1921. The second parasitoid, *Diaphorencyrtus aligharensis*, was described in 1975 after it was reared from ACP collected from Alighar in the Punjab of India.

This lack of information on parasitoids associated with ACP in the Punjab of Pakistan raises several important and intriguing questions that need to be resolved: What are the other eight species of parasitoids attacking ACP in the Punjab of Pakistan? Is *D. aligharensis* from India one of these eight species or is it an additional species that was not reared by Husain and Nath in Pakistan? What is the collective impact of this parasitoid guild on ACP in Pakistan? Is it great enough to reduce the pest status of this insect in citrus? Could the performance of some of these ACP parasitoids be greatly improved if they were imported into California without their hyperparasitoids?

To get a better handle on the ACP/natural enemy situation in Pakistan, Mark and Christina Hoddle visited the University of Agriculture in Faisalabad in Pakistan in September 2010. This trip was supported by funds provided to the California Department of Food and Agriculture (CDFA) by the Citrus Health Response Program (CHRP).

The major citrus variety grown in the Punjab region of Pakistan and India is 'Kinnow', a type of mandarin. 'Kinnow' was developed in 1935 by H.B. Frost, a citrus breeder at the University of California, Riverside. 'Kinnow' is a hybrid that was produced by crossing the 'King' and 'Willow leaf' citrus varieties. 'Kinnow' was exported to the Punjab Agricultural College and Research Institute in Lyallpur in Pakistan in 1943-1944. 'Kinnow' has performed exceptionally well in the Punjab, it appears to be extremely well adapted to the climate of this region, and perhaps more importantly, this cultivar appears to be tolerant to agents that collectively cause "citrus decline" in the Punjab. Citrus decline is a term that describes a combination of maladies (e.g., HLB, tristeza virus, citrus canker, and Phytophthora) that collectively reduce the productivity and vigor of trees without one culprit being pinpointed as the major cause.

In the Punjab of Pakistan, citrus orchards cover 195,000 hectares, and in the 2008-2009 growing season 2,132,000 tons of citrus were harvested. The Punjab produces 95% of Pakistan's citrus, and 'Kinnow' makes up more than 75% of production in this region. 'Kinnow' accounts for 90% of Pakistan's citrus exports which primarily go to the United Arab Emirates. Further, Pakistan is amongst the world's top ten 'Kinnow' producing and exporting nations. There are 28 'Kinnow' processing plants in Pakistan located in Sargodha and Karachi which have the capacity to move 5-10 metric tons of fruit per hour. Discussions with faculty at the University of Agriculture in Faisalabad indicated that 'Kinnow' acreage in the Punjab is expanding; there are plans for developing more processing plants; seedless 'Kinnow' varieties are under consideration; and efforts are being made to develop disease-free planting materials for growers.



Punjabis pride themselves on offering exceptional hospitality. Christina and Mark Hoddle share tea and local foods with a 'Kinnow' orchard owner after sampling for Asian citrus psyllid.

Many farmers in the Punjab are not well trained in the appropriate and proper use of pesticides, and accidental poisonings are not uncommon. Most farmers are unable to afford safety equipment and clothing to protect themselves from pesticides. *Photo by Mark Hoddle.*

What was lacking from these discussions with faculty was immediate concern over managing ACP and HLB, and that research efforts were directed at much more pressing issues, in particular production of arable crops and protection of stored grains from arthropod pests.

This lack of research activity suggests that diseases afflicting citrus, in particular HLB mortality of 'Kinnow', a major source of vitamin C for many struggling people, is not an overly important issue in Pakistan. This fact may be supported indirectly by several lines of evidence.

The first and most obvious is the result of a natural field experiment that

Pakistan Collections

- March 10 to April 10 2011 spent at UAF Pakistan looking for ACP and its natural enemies
  - ~5,000 psyllids collected and reared
  - Malaise trap set to monitor ACP and natural enemy phenology
  - MS student recruited and trained to work on ACP
    - ACP & natural enemy phenology
    - Flush phenology of kinnow and sweet orange
    - % parasitism measures and rearing of ACP parasitoids
- June 4-13 2011 - ~400 parasitoids returned to UCR
- Oct. 23-28 2011 - > 1,000 parasitoids returned to CA
- June 16-23 2012 – > 1,000 parasitoids returned to CA


PEPSI




PETZ
PLUS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4-9-11

Releasing Pakistani Parasitoids

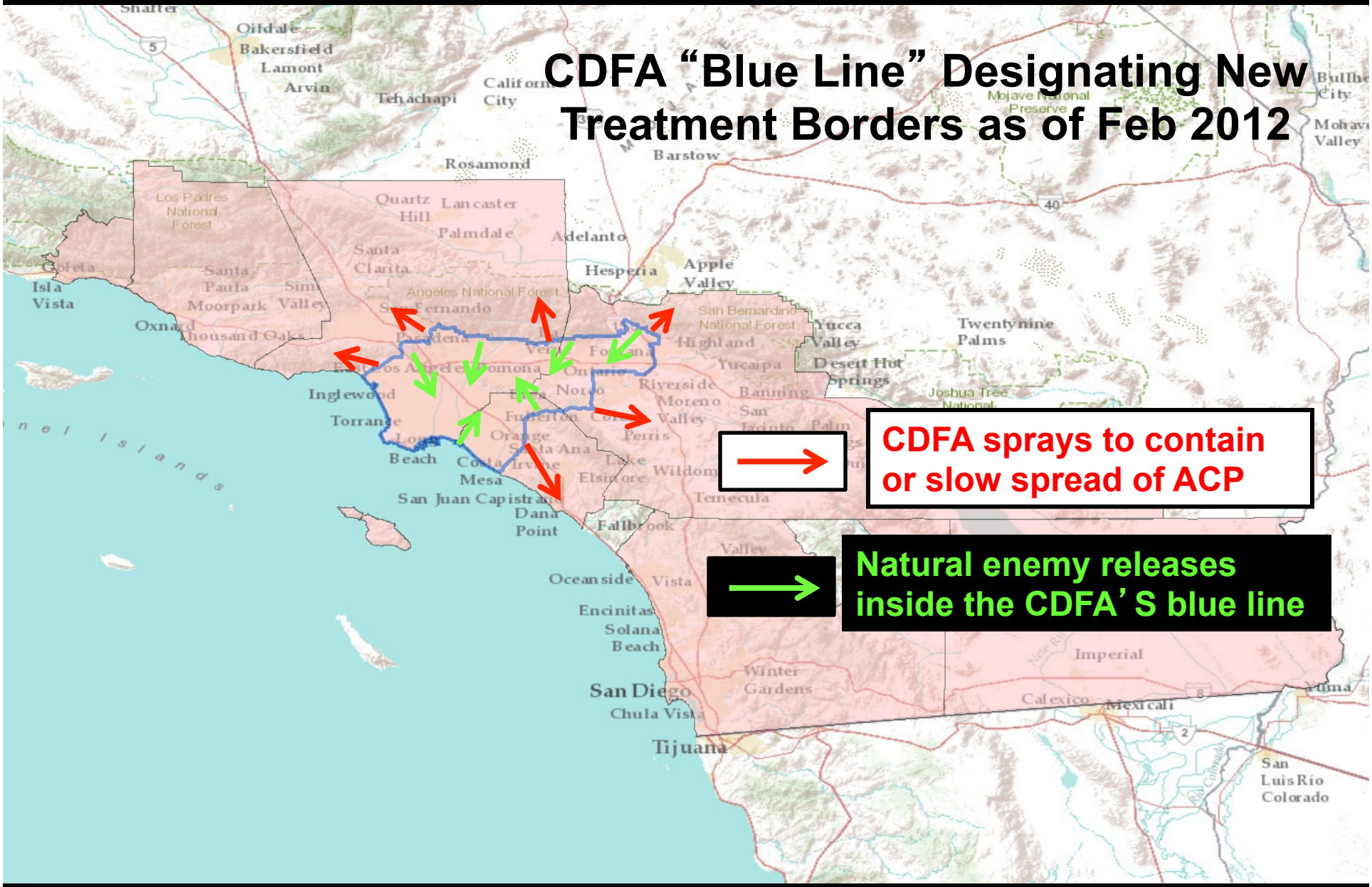
- Safety testing for *Tamarixia* is completed
  - Second parasitoid, *Diaphorencyrtus*, is in the queue for safety testing
- EAR was submitted to APHIS, 6:00pm, 15 Nov 2011
- USDA issued release permit Dec 7 2011
- *Tamarixia* released at UC Riverside, Dec 20 2011
  - >17,000 *Tamarixia* released by Sept. 2012
  - Establishment likely in 20% of release sites
    - Parasitism found ~ 100 yds from some release sites

The Cost of Spraying

- ACP infestations are high in LA County
- 2000 Census Data (on Wikipedia) indicates there are 3,270,909 housing units in LA
  - 806 houses per square mile
- Crude surveys suggest that 63% of residences have 1^{+} citrus in LA
  - 2,044,318 residences with citrus
- CDFA treated 67,863 residences by Oct 2011
  - 3% of properties with citrus treated in LA
    - Cost $10,722,657 or $158/residence (CPDPC 2011/12 Budget)
      - Resistance development documented in Florida (Tiwari et al. 2011 Pest Management Science 67: 1258-1268)
      - 35x resistance to Imidacloprid; cross resistance to thiamethoxam before it was used; Resistance building to chlorpyrifos, malathion, danitol

Establishing Bridgeheads in LA

- **Need to increase parasitoid releases in LA**
  - Bridgeheads from which the parasitoid can spread
  - 60-80 sites wanted from Riverside to LA
  - Areas with high HLB risk need targeting
  - ~ 3,000 ACP nymphs surveyed for parasitoids
    - No evidence of parasitism
- **Releases should be made simultaneously at the leading edges of the invasion**
  - Some modeling work suggests that natural enemies can slow invasion speed because propagule pressure is reduced


CDFA "Blue Line" Designating New Treatment Borders as of Feb 2012
CDFA sprays to contain or slow spread of ACP
Natural enemy releases inside the CDFA'S blue line
Shafter
Oildale
Bakersfield
Lamont
Arvin
Tehachapi
California City
Rosamond
Barstow
Mojave National Preserve
Bullhead City
Mohave Valley
Los Padres National Forest
Quartz Hill
Lancaster
Palmdale
Adelanto
Santa Clarita
Hesperia
Apple Valley
Goleta
Isla Vista
Santa Paula
Moorpark
Simi Valley
Angeles National Forest
San Fernando
Oxnard
Thousand Oaks
San Bernardino National Forest
Yucca Valley
Twentynine Palms
Highland
Yucaipa
Desert Hot Springs
Joshua Tree National
Inglewood
Torrance
Long Beach
Costa Mesa
Irvine
Orange
Santa Ana
Fullerton
Norco
Riverside
Moreno Valley
Perris
Banning
San Jacinto
Lake Elsinore
Wildomar
Temecula
San Juan Capistrano
Dana Point
Fallbrook
Oceanside
Vista
Encinitas
Solana Beach
San Diego
Chula Vista
Tijuana
Winter Gardens
Imperial
Calexico
Mexicali
San Luis Río Colorado

Tamarixia Release Summary

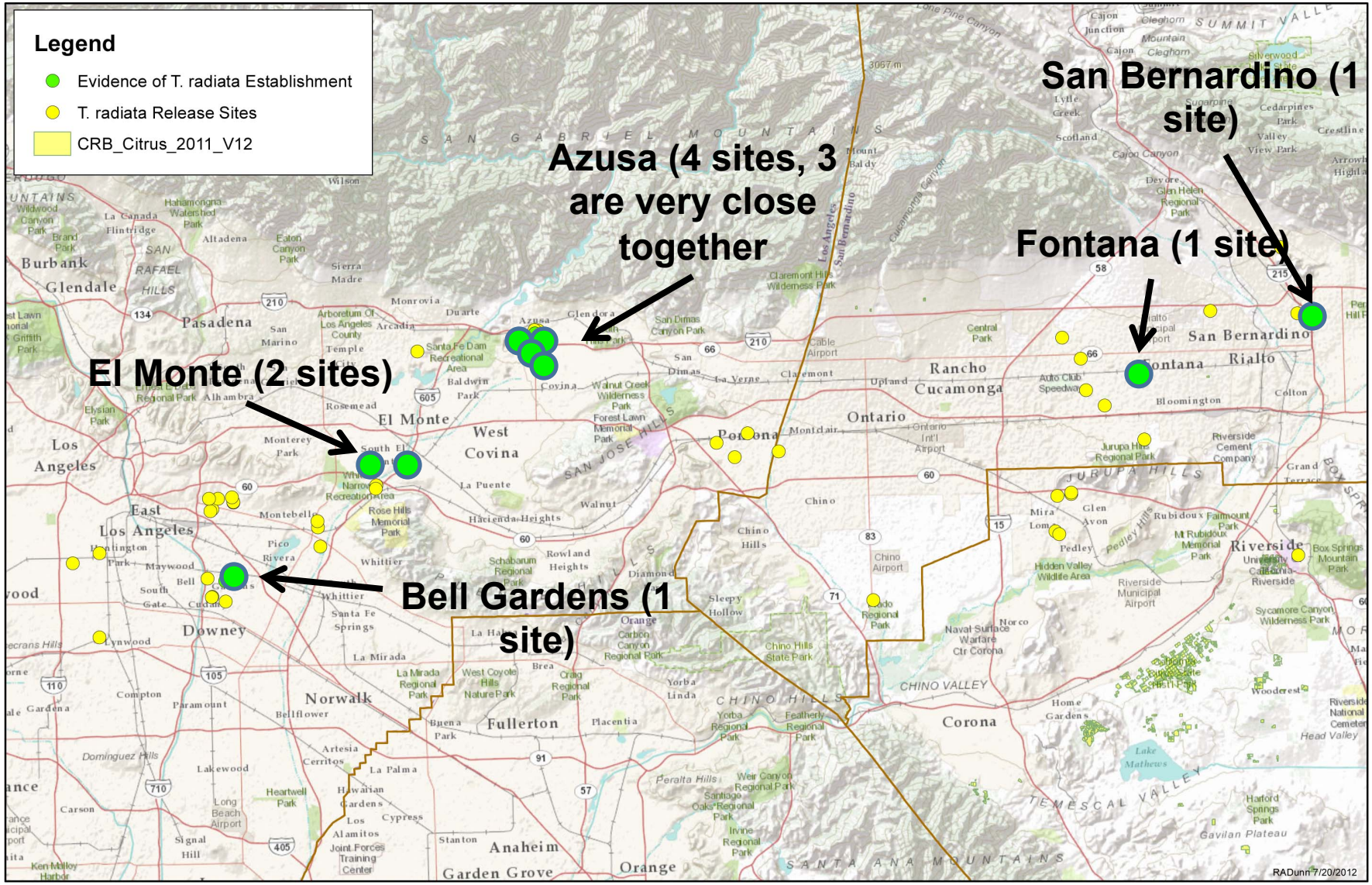
- As of Sept 19 2012
  - **Parasitoids Released:** 17,428 (75% female)
    - Release numbers: 22-938 per site
    - 62% of sites received > 100 *Tamarixia*
  - **Number of Release Sites:** 67 (24 zip codes, 24 cities)
  - **Locations:** Azusa, Bell Gardens, Cabazon, Calimesa, Chino, Duarte, Fontana, Los Angeles (90003, 90011, 90022, 90026, 90059), Mira Loma, Montclair, Ontario, Pico Rivera, Pomona, Rancho Cucamonga, Redlands, Rialto, Riverside, San Bernardino, South El Monte, and Whittier

Citrus Pest & Disease Prevention Program

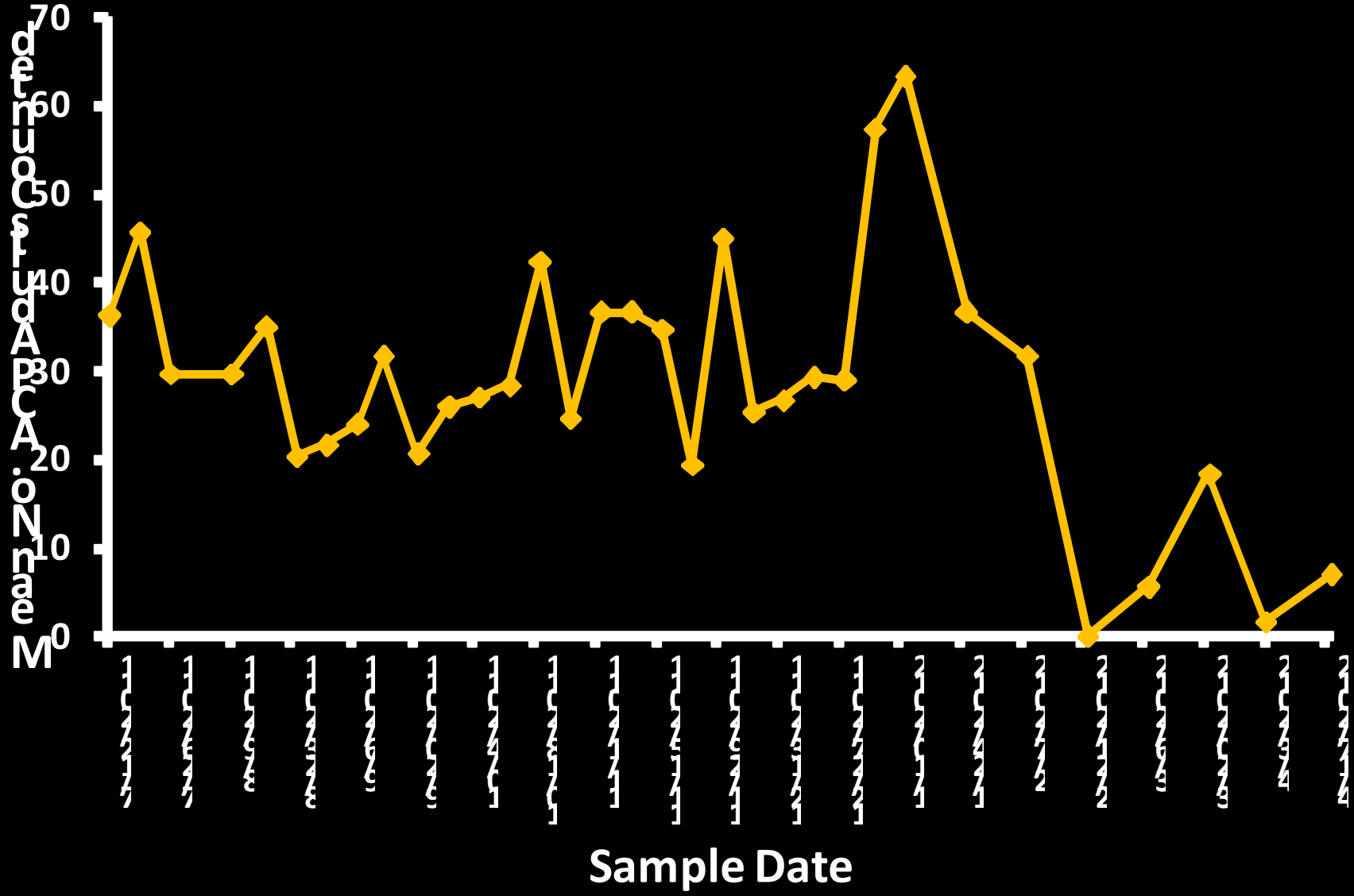
ACP Biocontrol Project

67 ~~46~~ release locations as of ~~7/13/2012~~ **Sept 17 2012**

Released 17,428 *T. radiata*



Adult ACP Population Trends in LA



Ants tending ACP nymphs

ACP mummies from which *Tamarixia* has emerged in Bell Gardens

Tamarixia female foraging on a patch of ACP in Azusa

Tamarixia parasitizing ACP in the field in Los Angeles

Other Biocontrol Agents

- *Diaphorencyrtus aligarhensis*
  - Two cultures in Quarantine
    - Uni-parental line
    - Bi-parental isolines started from last trip to Pakistan (June 2012)
- Host specificity testing
  - Two non-target species completed with uniparental line
    - Need to repeat with bi-parental strain
      - Delay releases by 3 months
  - Tentative completion dates and submission of EAR Fall 2013

Industry & UCR Cooperation

- CRB is backing the biocontrol program for urban areas behind the CDFA's "blue line"
  - Mass rearing committee
  - Release strategy committee
  - Establishment and impact evaluation committee
- Ramping up plant production major bottleneck to program
- Funding needs are being met by the CRB

Summary

- Spray programs likely unsustainable for SoCal
- ACP continuing to spread despite monitoring and pesticide treatments
- Greater use of natural enemies needed in urban and public areas where sprays are hard to make
- Pakistani *Tamarixia* likely established and spreading in SoCal
- IMPACT……?????




More Information on ACP and the Biocontrol Program

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