Biological Control of the Asian Citrus Psyllid in Southern California

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Background of Pest

- *Diaphorina citri* Kuwayama
- Native to Asia from Pakistan to Vietnam
- Introduced to Florida in 1998
- First detected in Texas in 2001
- Detected in California in 2009
- Attack most species of citrus
  - Lemon, Lime, orange, and grapefruit
Asian Citrus Psyllid Life cycle

- Adults emerge and lay eggs on new flush
- 5 nymphal instars
- In warm weather (> 80 degrees) has a two week life cycle
Asian Citrus Psyllid (ACP)
Citrus Greening Disease

- Huanglongbing (HLB)
  - Yellow Dragon disease
- First discovered in Florida in 2005
- Detected in Texas and Southern California in 2012
- Spread by Asian Citrus Psyllid (ACP)
  - Also spread by grafting infected branches
- Can take years for infected trees to develop symptoms
- Fatal to trees
Citrus Greening disease
Citrus Greening Disease
Citrus Greening Disease

**Economic Costs**

- In Florida has led to 3.63 billion dollars in lost revenue
- 6,611 jobs lost by reducing orange juice production
Citrus Industry in California

- Citrus estimated as a 2 billion dollar industry in California
- California produces 80% of the nation's fresh citrus
  - Main source of the nation's fresh market oranges and lemons
- 50-70% percent of homeowners have a citrus tree in their backyard
Initial control measures

- Establish area wide spray program
- Quarantine on moving citrus
- Remove infected HLB tree
Biological Control Agent

- *Tamarixia radiata*
  - Hymenoptera: Eulophidae
- Native to Pakistan
- Ectoparasitoid
- Sting late instar nymph
- Two week life cycle
Collection of *Tamarixia*
How do we mass rear *Tamarixia*?
Establishment of natural enemies for classical biological control

- Introduce genetic variation so that the local conditions can select for the locally optimal genotypes
- Once selection has taken place locally optimal genotypes can be retrieved from the field and used for mass rearing (but see later)
- Initial releases are extremely important because if a suboptimal population establishes it will be time consuming and difficult (sometimes impossible) to improve the already established population by releasing additional genetic variation.
Experiments with *Drosophila melanogaster*


*Figure 1.* Expected relationships between reproductive fitness and populations size ($N_e$) due to inbreeding depression, mutational accumulation and genetic adaptation to captivity. Combined represents the net effects of all factors. The effects are shown for populations maintained ~ 50 generations under (a) benign captive conditions, and (b) for these populations when translocated to the wild environment.
What is known about effect of mass rearing in biocontrol?

- In biocontrol projects natural enemies are often reared for long periods in lab.
- Not much known about effect of mass rearing on biocontrol success.
- Myers and Sabath, 1980 find negative correlation between time in rearing and success of weed biocontrol agents.
- Little to no experimental evidence of effect of mass rearing on field performance of natural enemies.
• Selection for rearing circumstances
  • Which traits?
    • Development time
    • With that often circadian rhythm
      • Results in changes in mating time
    • Size

Fig. 2. Relationship between generation and the mean and standard deviation of larval development time in the mass-reared melon fly, *B. cucurbitae*.
• Relaxed selection because of benign environment
  • For parasitoids?
    • Host finding?
    • Mate finding?
    • Temperature?
    • Humidity?

Fig. 1. Graphical depiction of directional- and relaxed-selection hypotheses. The solid curve represents the distribution of a behavioral trait in a wild population and the dashed curve represents a captive-bred population. (a) Directional selection: the mean shifts, but the shape of the distribution does not change. (b) Relaxed selection: the mean remains the same, but the distribution flattens out, including more values at either extreme.
What does this mean for mass rearing?

• Important to do it right the first time you don’t get a second chance
  • But how come some biocontrol projects are successful without really taking care of this?
  • How come so many biocontrol projects are not successful?
• How can we assure the proper introduction of genetic variation into the field without losing it during the prolonged mass rearing phase?
<table>
<thead>
<tr>
<th>Stage</th>
<th>Goal</th>
<th>Solution</th>
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<tbody>
<tr>
<td>Founding sample</td>
<td>Genetically variable population by size</td>
<td>Bigger is better. Sample ( N &gt; 1000 ) or several samples of ( N &gt; 100 )</td>
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<td></td>
<td>Genetically variable population by mixing (optional)</td>
<td>Danger of outbreeding depression. Sample from several populations separated by a small genetic distance</td>
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<td></td>
<td>Population adapted to field release site</td>
<td>Sample on or near the field site. If not possible, sample from climatically and ecologically similar area</td>
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<td>Establishment in captivity</td>
<td>Maintain genetic variation</td>
<td>Avoid “crash-recovery” bottleneck. Initially establish many subpopulations</td>
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<td>Captive rearing: the</td>
<td>Maximize “effectiveness” [Eq. (1)] of future field release</td>
<td>Options:</td>
</tr>
<tr>
<td>optimization strategy</td>
<td></td>
<td>1. Replace populations about every 10 generations</td>
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<td></td>
<td>2. Continuously incorporates new wild-caught individuals</td>
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<td>3. Incorporate rearing strategies that maintain field-related fitness</td>
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<td>Captive rearing: the</td>
<td>Avoid “domestication” during captive rearing</td>
<td>Isofemale lines—ideally maintain ( &gt;100 ) separate lines</td>
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<tr>
<td>avoidance strategy</td>
<td></td>
<td>Lines must be intercrossed prior to field release</td>
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Two approaches to optimize genetics of *Tamarixia*: 1. Mass-rearing

1. Maintaining genetic variability and hybrid vigor of wasps in the mass rearing

- 1, 2, 3, 4, 5, 6, 7
- 1x2, 2x4, 5x1, 6x7

Mating cage where individuals from isocages mate with each other

Mass rearing cage where hybrid offspring for field releases is produced

Hybrid females for release in field
TWO APPROACHES TO OPTIMIZE GENETICS OF TAMARIXIA: 2. FIELD INSECTARIES

- Collect large number of Tamarixia in Pakistan
- Allow each individual female to produce offspring in Quarantine
- Collect mother to make sure
  - She is a T. radiata
  - She is not infected with HLB
- If T. radiata and free of HLB use her offspring for field insectaries
- Field insectaries consist of caged trees that have many ACP on them
- Release offspring of each mother in each of the field insectaries
- Allow them to produce offspring in these cages and collect offspring (TX 15,000 wasps per field insectary) for distribution throughout the area
Conclusions

• Important to introduce sufficient genetic variation into the field in the initial release for Classical Biological Control. Improving upon the first release is time consuming once a suboptimal population is established.

• Maintaining initial genetic variation best done by maintaining populations with little genetic variation- these are not able to adapt to the lab circumstances and no selection for mass rearing conditions will take place. Isofemale lines (how many the more the better but ~20 isofemale lines will capture most of the common genetic variation present in the source population.

• Mix populations before release so as to restore genetic variation in population and release the hybrid individuals.

• Once population is established and selection has taken place do mass rearing with field selected individuals, but avoid adaptation to the lab, max rearing of one population 10 generations.
Rearing of *Tamarixia radiata*

- Rearing conducted to preserve genetic diversity
- Long term rearing in lab can lead to a loss of fitness
- Maintain 17 separate *Tamarixia* lines
- Mix 17 lines together in a mass sting cage prior to release
Rearing of ACP

- Rear Asian citrus psyllid nymphs on a single type of host plant
- Curry leaf (*Murraya koenigii*)
Mass Rearing: ACP

- Use twenty plants per cage
  - Curry leaf (*Murraya koenigii*)
- Inoculated young flush with adult ACP
  - Allow ACP adults to lay eggs for 5 days and then remove them
- Each plant averages 100 nymphs
  - 10 days old
Mass Rearing: *Tamarixia radiata*

- Receive 15-20 curry plants with ~100 nymphs per plant
- Collect wasps from all 16 lines to total 1 female for every 30 nymphs
- Collect adult wasps 10-12 days after first sting
Host Feeding

The consumption of host body fluids
Why Host Feed?

- To mature eggs
- To increase longevity
- To increase fecundity
Question 1: How does egg load vary between Host Fed and Non-Host Fed wasps over time?
Methods: *T. radiata* egg load

- Established two cages each with one curry plant
  - Host Fed (plant with 150 ACP nymphs)
  - Non-Host Fed (empty curry plant)
- All treatments contained a honey source
- Added 50 wasps to each treatment
- Removed 10 wasps per day, dissected them, and counted egg load
Results: *T. radiata* egg load

<table>
<thead>
<tr>
<th>Day</th>
<th>Not Host Fed</th>
<th>Host Fed</th>
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<tbody>
<tr>
<td>Day: 1</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Day: 2</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Day: 3</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>Day: 4</td>
<td>6</td>
<td>14</td>
</tr>
</tbody>
</table>

* indicates significant difference.
Question 2: How does Host Feeding affect percent parasitism in mass rearing?
Methods: Host Feeding and Mass rearing

- Host Feed in similar manner to previous experiment
- Established two cages each with one curry plant
  - Host Fed (plant with 150 ACP nymphs)
  - Non-Host Fed (empty curry plant)
- All treatments contained a honey source
- Added 1 female wasp for every 30 ACP nymphs to each treatment (~50-60 females)
- Added 10 males to each treatment
- After 2 days, added twenty curry plants with ACP nymphs
Results: Host Feeding and Mass rearing

![Graph showing percent parasitism for Non-Host Fed and HostFed conditions. The HostFed condition shows significantly higher parasitism compared to the Non-Host Fed condition, marked with an asterisk.]
Monthly totals of *Tamarixia*

![Graph showing monthly totals of Tamarixia wasps]

- **Number of adult wasps**
- **Axes:**
  - Y-axis: Number of adult wasps
  - X-axis: Months (July to September)

- **Legend:**
  - Male
  - Female

- **Data Points:**
  - July: Low count (Male and Female)
  - August: Low count (Male and Female)
  - September: High count (Female)
Release of *Tamarixia radiata*

- Parasitoids are released directly onto ACP infested branches
- Parasitism has been witnessed in the field
- To date over 135,000 parasitoids have been released primarily in LA County
- Release, establishment, and spread taken over by CDFA
Predators observed consuming ACP in Southern California

- Green Lacewings
- Coccinelids
- Spiders
- Predatory Mites
- Brown Lacewings
- Syrphid Flies
Ants and ACP

- Recently observed ants tending psyllids in the field
- Observed ants attacking parasitoids
Natural enemy complex in Asia

- Ladybeetles (Coleoptera: Coccinellidae)
- Syrphid flies (Diptera: Syrphidae)
- Lacewings (Neuroptera: Chrysopidae, Hemerobiidae)
- Spiders (Araneae)

How do native predators affect ACP survival?
Methods: Predator Study

- Four Treatments
  - Cage (+Tangle Foot)
  - Open Cage (+Tangle Foot)
  - No Cage (+Tangle Foot)
  - No Cage (-Tangle Foot)
- Counted ACP cohorts every Monday, Wednesday, Friday
- Number of ACP in early, late, and adult stages recorded
Results: Predator study

Percent Survival

- March
- April
- May
- June
- July
- August

- No Cage
- No Cage (+TF)
- Open Cage
- Cage

Graph showing the percent survival of predators over months with different cage conditions.
Results: Predator Study
Future work: Predator Study

- Examine the gut contents of predators using molecular methods
- Have been collecting predators bi-monthly from March to present
- Further Ant control work
Future Research

- Test the efficacy of Nu-lure (parasitoid protein supplement) in mass rearing
- Impact of augmentative releases
  - Examine interaction between predators and *Tamarixia* wasps
Summary

- Imperative to protect the citrus trees of Southern California
- To date have produced over 130,000 wasps in Southern California area
- Important to understand the genetics in mass rearing of wasps for biological control
- Host fed *Tamarixia* wasps have significantly more eggs than non-host fed wasps
- Host feeding has significantly increased mass production of *Tamarixia radiata* wasps
- Native predators are having an impact on ACP densities throughout the year
- Further research is necessary to see how *Tamarixia* wasps affect densities in augmentative releases
Acknowledgements

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Questions?