Development of Sterile Insect Technique for Navel Orangeworm in California Orchards

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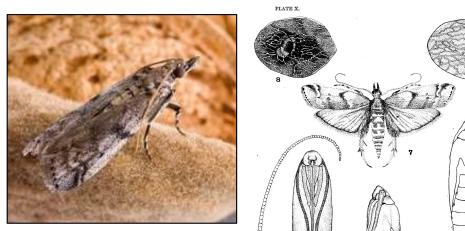






Navel Orangeworm Origins + Arrival in CA

Species Name Order: Lepidoptera Family: Pyralidae Species: Amyelois transitella



Arrival in California

1800s – Reported in Mexico, Caribbean, Central America, South America

1900s – Reported on citrus in AZ ("navel orange worm")

1940s – Reported on walnuts and almonds in CA

1970s – Reported on pistachio in CA

Navel Orangeworm on Walnuts

infestations in northern California orchards dependent on population overwintering in past crop's waste left in field

_____ A. E. Michelbacher

the novel orange-worm indexision in 1958 waht cross in northern Calls the 1958 waht cross in northern Calls here the downward trend will conuse in 1956 is unknown. The pert was found in only one of the specimental orthonic where the 1955 that orchard was at Modelso where the set caused some damage in 1956, One alf of the orchard received no winter Mixedian, and it was in this position of the orchard received no winter historian, and it was in this position rangeworm survived the winter. The any ruts on the ground and a few left the trees furnished excellent breeding the trees for the pest. These samora allowed arrest as non-very rendered in a suittle condition to be infrated. navel orangeworm, as compared to 0.16% in the plots where DDT was applied to control the coding moth. The navel orangeworm infestition in the event of the transformed processing the countered in 1954. This night he explained—in part—by the fact that the coding moth infestation in 1055 was considerably less than that in the preceding year.

No Spray Program

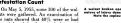
Infere is no known spray program that will directly control the navel orange worm. Control of the pest in the field is largely dependent upon preventive measures: 1. Effective control of the colling moth where it is a pest 2. Early harvest 3. Good general orchard sanitation practices

Because the navel orangeworm is a scavenger, uncontrolled infestations of the coding moth encourage attack by the pest. Nuts infested by the coding moth turnish a source of food upon which the navel orangeworm can increase. As a Some INSECT PESTS OF SALT RIVER VALLEY. 289

has been found in New Mexico that sweet-corn planted later than usual escapes the ravages of the worm, the moths having already laid their eggs in the earlier plants. On the same principle, in the South, corn is planted ahead of the cotton and after a while destroyed, with the result that the cotton is less injured,

THE NEW ORANGE-WORM.

On October 23, I found near Phoenix two oranges on the same tree, which were affected by a worm or caterpillar. The oranges had turned color prematurely, and dropped off at the least touch. From the apex hung in each case a small amount of debris, proclaiming the presence of a worm, and distinguishing them from the fruits affected by the black rot. On opening one of the oranges, the worm was found to occupy a small space near the apex, living on the soft tissues. It resembles in appearance the codling-worm of the apple, which, however, does



nuts showed that 40% were or had infested by larvae of the navel grownym There was an ourgence of 18 taken into the laboratory on August

Navel Orangeworm

Current Pest Status

Extremely Low Tolerance for Damage (<2%)

High Crop Value

- Yield/quality x price of course...
- Infestation leads to increased processing time/costs
- Carryover of infested remnant/mummy nuts to following year





Navel Orangeworm

Current Pest Status

Extremely Low Tolerance for Damage (<2%)

- Aflatoxin
- Known human carcinogen, regulated in domestic/foreign markets
- Aspergillus flavus fungi produce aflatoxin
- NOW adults move Aspergillus around
- Larval feeding create opportunities for fungal growth on nuts

Spread of Aspergillus flavus by Navel Orangeworm (Amyelois transitella) on Almond

Jeffrey D. Palumbo, Noreen E. Mahoney, and Douglas M. Light, Plant Mycotoxin Research Unit, Western Regional Research Center, United States Department of Agriculture–Agricultural Research Service (USDA-ARS) Albany, CA 94710; Joel Siegel, USDA-ARS, San Joaquin Valley Agricultural Sciences Center, Parlier CA 93648-9757; and Ryan D. Puckett and Themis J. Michailides, University of California–Davis, Kearney Agricultural Research and Extension Center, Parlier 93648

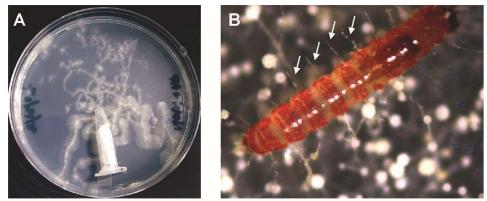


Fig. 1. A, Trails of Aspergillus flavus colonies, resulting from navel orangeworm (NOW) transport of conidia from microcentrifuge tube to potato dextrose agar medium. B, Accumulation of A. flavus conidia on setae (arrows) of NOW larva after crawling across plates containing sporulating A. flavus colonies.

Seasonal Phenology

- Overview
 - Overwinter as larvae/pupae in remnant "mummy" nuts
 - Adults emerge in the spring
 - 3-4 generations per year, depending on weather and host quality
- Populations develop more rapidly as the season progresses
 - Warmer weather
 - Develop more rapidly on new crop vs. mummy nuts
 - Increased host availability (hull split / hull slip)

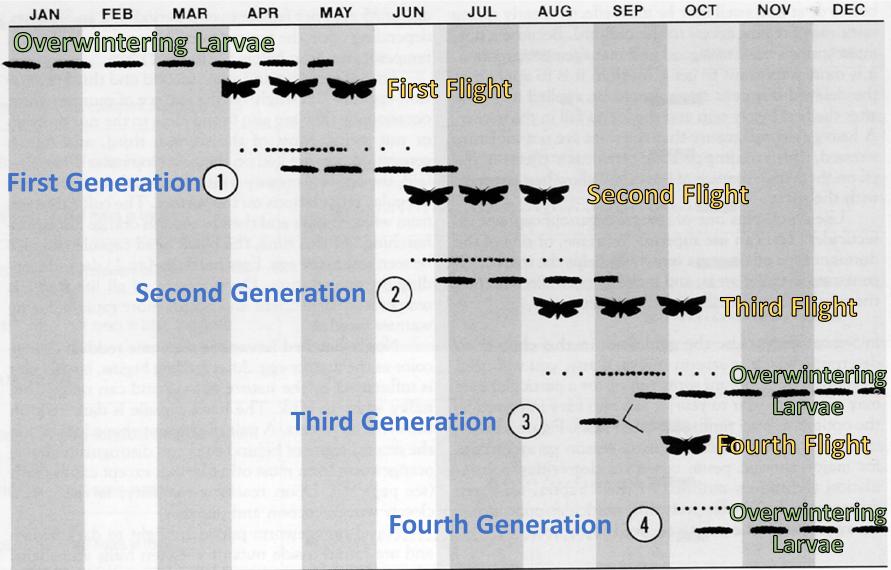


Mummy vs. New Crop Nuts



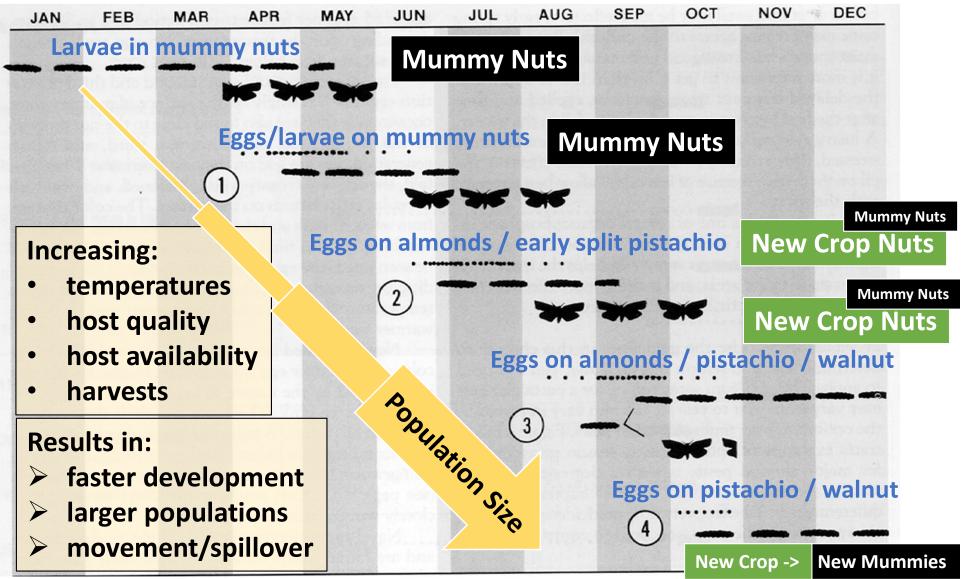
Increasing host availability as hull integrity declines

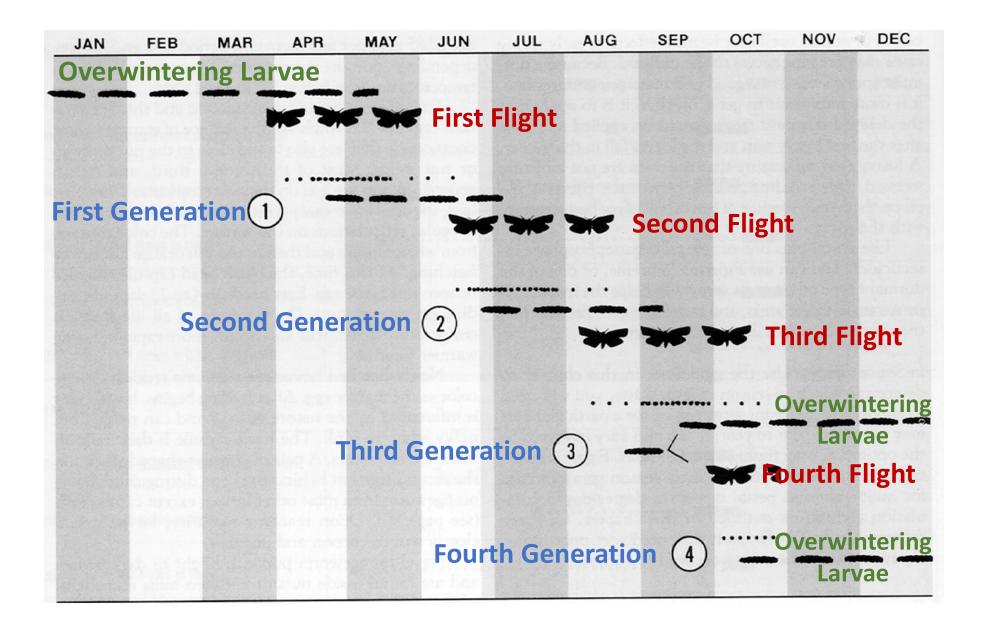
Seasonal Phenology Overview

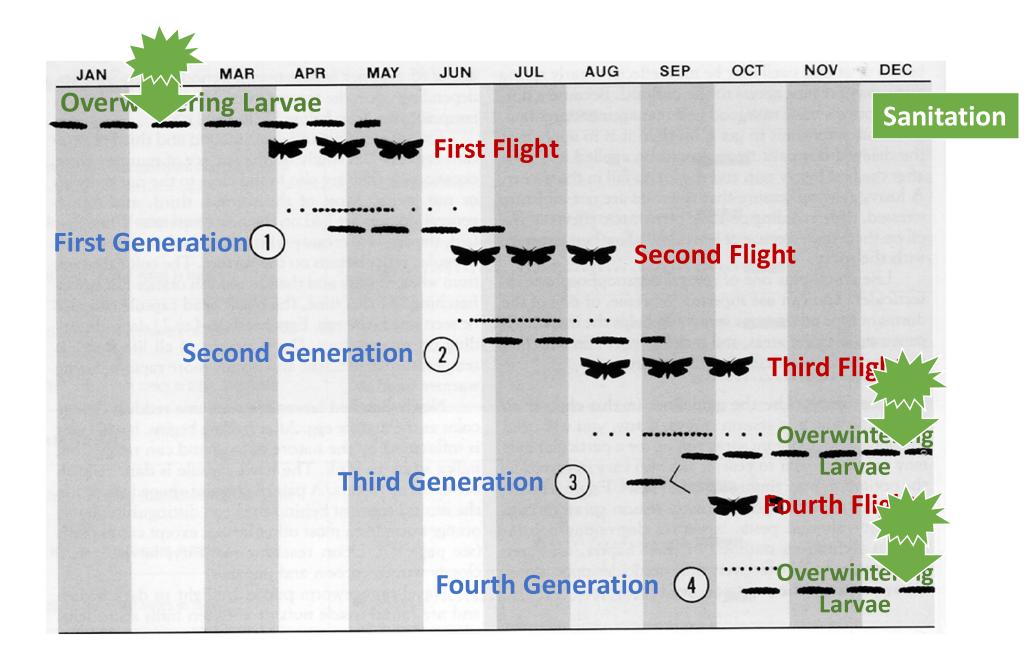


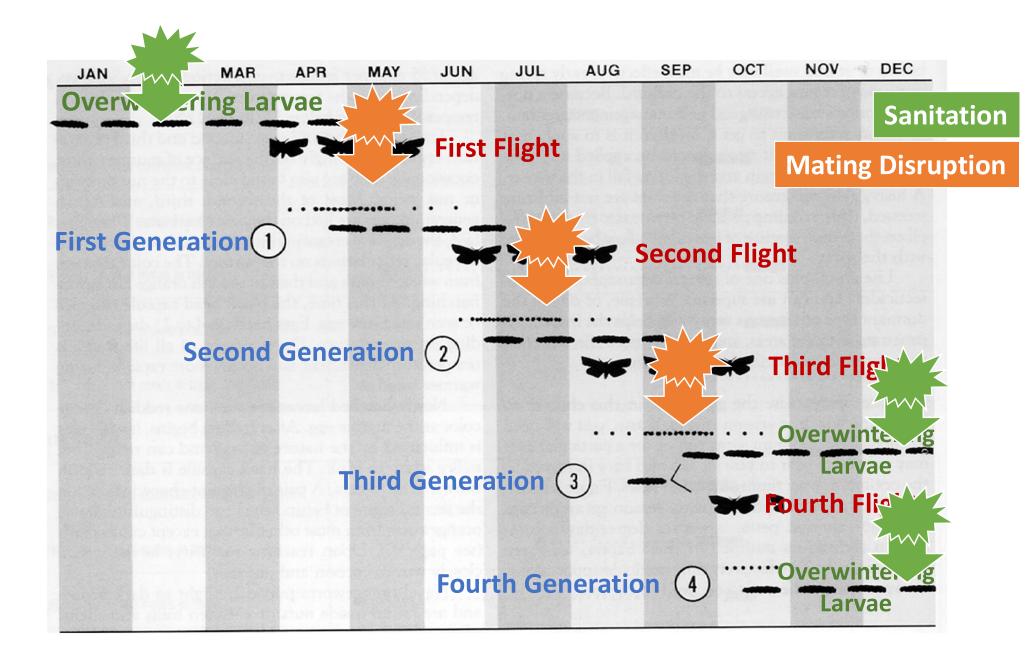
IPM for Almonds, UC ANR Publication #3308

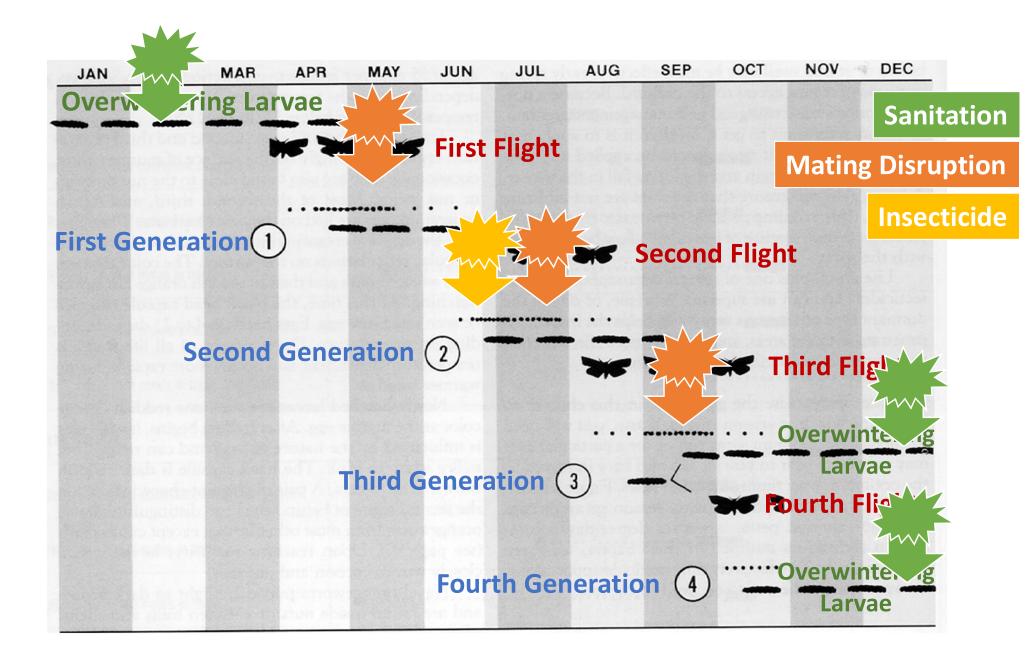
Seasonal Phenology Increasing Populations Over Time

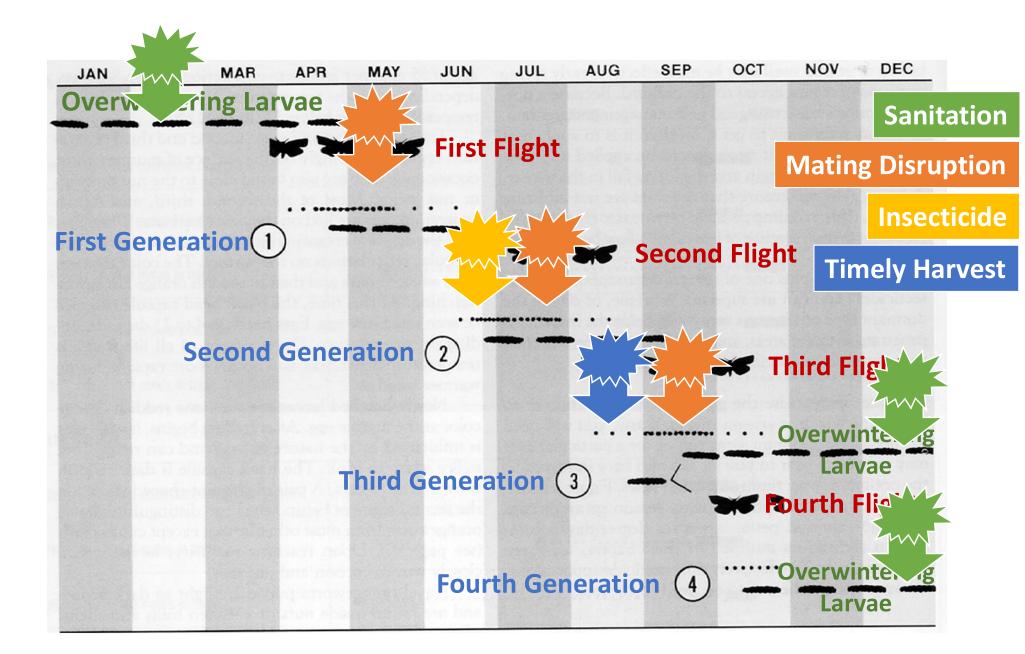


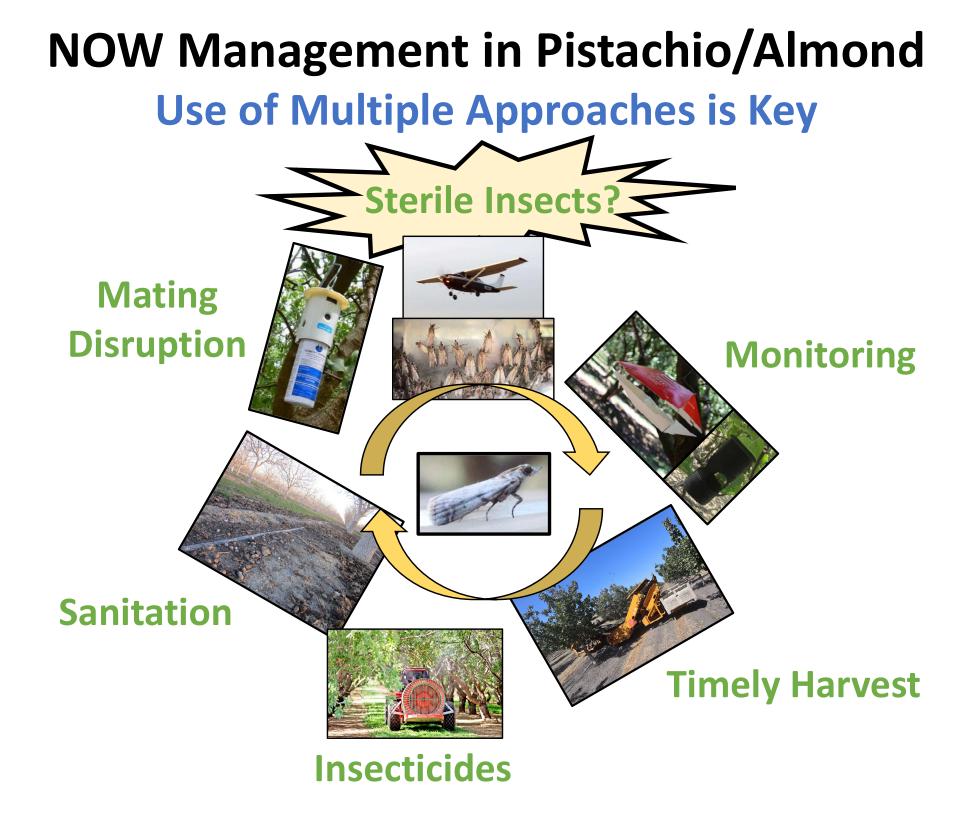












Sterile Insect Technique for Navel Orangeworm

Key Aspects and Progress to Date



SIT for NOW - Background

Phoenix Irradiation Facility Presents Novel Opportunity

USDA Pink Bollworm Rearing Facility

- Phoenix, AZ
- Operating since 1960s

Pink Bollworm Eradicated

• October 2018

Can the facility be repurposed for NOW?

 Pistachio Industry + USDA-APHIS



UNITED STATES DEPARTMENT OF AGRICULTURE Office of the Secretary Washington, D.C. 20250

ERADICATION OF PINK BOLLWORM

By the Secretary of Agriculture of the United States of America

A PROCLAMATION

WHEREAS cotton production is vital to the U.S. economy, accounting for nearly \$27 billion in products and services annually according to industry estimates, providing hundreds of thousands of jobs across many sectors, and supplying nearly one-third of the raw cotton that is traded globally; and

WHEREAS for more than 100 years the United States has been battling the pink bollworm, one of the most destructive cotton pests in the world, which has cost U.S. growers tens of millions of dollars annually in control costs and yield losses; and



Egg Production





Rearing Larvae/Pupae



Adult Emergence in Vacuum System



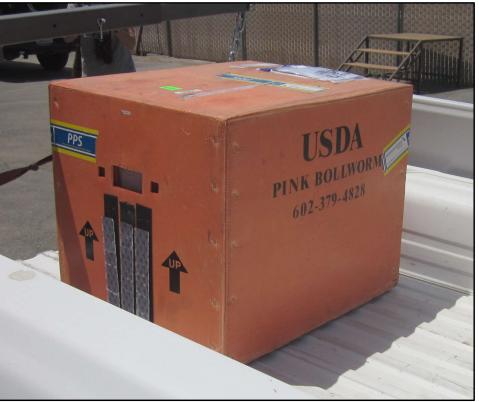
Adults Collected in Cold Chilled "Cyclones"





Moth Irradiated, Packaged and Shipped Out





SIT for NOW - Background Moth Production/Transportation Process Sterile Moth Shipped via Commercial Carrier Passive Cooling System



SIT for NOW - Background Moth Production/Transportation Process Released Using Modified Small Aircraft Cooling System + Released from Small Tube Below



SIT for NOW - Background Moth Production/Transportation Process When? Where? How Many?

Lots of moths...

• ~2,000,000 NOW/day

...but also lots of crops.

- 1.2M almonds, 300k pistachio, 250k walnuts
- Plus alternate hosts
- Where to deliver moths? When? How many?
 - Overflooding ratio
 - Delivery method, timing and location
 - Integration with existing IPM tools

Goal = develop a competitive sterile moth, and figure out how to best use it.

Sterile Insect Technique for NOW Project Project Summary 2018-2024

2018	2019	2020	2021	2022	2023	2024
Understanding						
the Problem						

Developing Alternatives

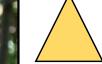
> Field Dispersal and Impacts on Wild NOW

> > Ecological/Economic Scenario Modeling

> > > Areawide IPM West Fresno County

Research Summary: 2018-2021 Monitoring Techniques and Assays





Pheromone Traps

- Synthetic pheromone lure
- Attracts males
- Large trapping radius (captures lots of moths)



Ovibait Traps

- Pistachio/almond bait
- Attracts mated females
- Smaller trapping radius (captures fewer moths)

Research Summary: 2018-2021 Monitoring Techniques and Assays



Female calling (emitting pheromone) at night



NOW mating

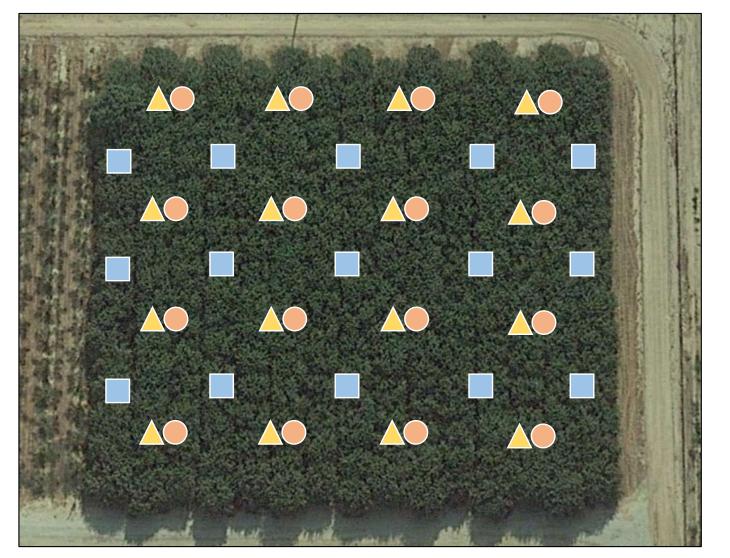
Mating Tables

- Sentinel virgin female with wings clipped
- Exposed overnight
- Check at dawn for paired male
- Dissect moths to determine...
 - Male is sterile vs wild
 - Female is mated

Sentinel Females Used

- Mendota Colony = control moth
 - Can sterile males locate females?
- Phoenix Facility
 - Can sterile females attract wild males?

Research Summary: 2018-2021 Field Release Sites – <u>Two Small Pistachio/Almond Orchards</u> Grid of Traps and Mating Tables



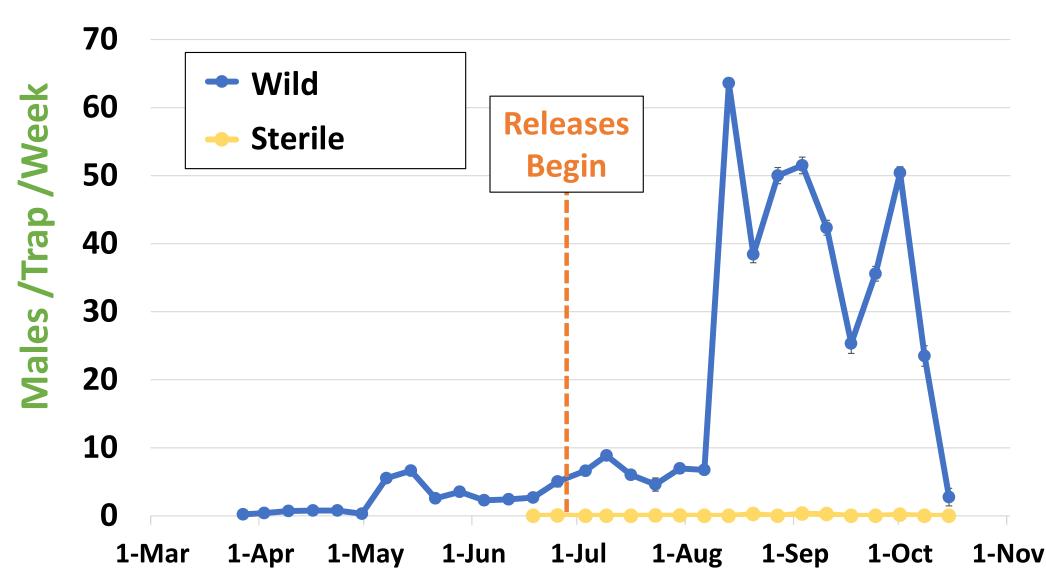
Pheromone
Ovibait
Mating Table

1-3 acre blocks

Crop Year 2018 What does moth recapture rate look like?

Crop Year 2018 Poor Recovery of Sterile Males

Flight Traps at Kearney

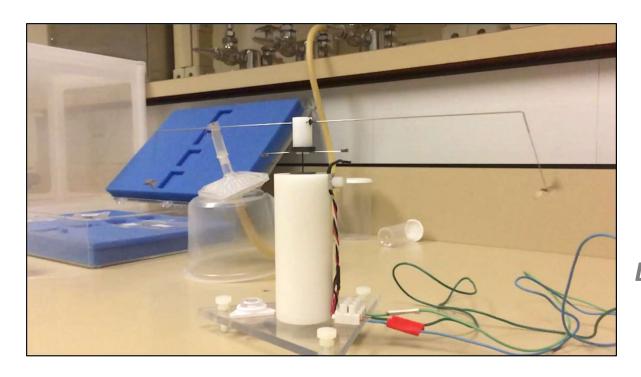


Crop Year 2019 *Poor recovery in 2018 Can males even fly and respond to pheromone?*

Crop Year 2019 Why Such Poor Recovery of Males?

Can they even fly?

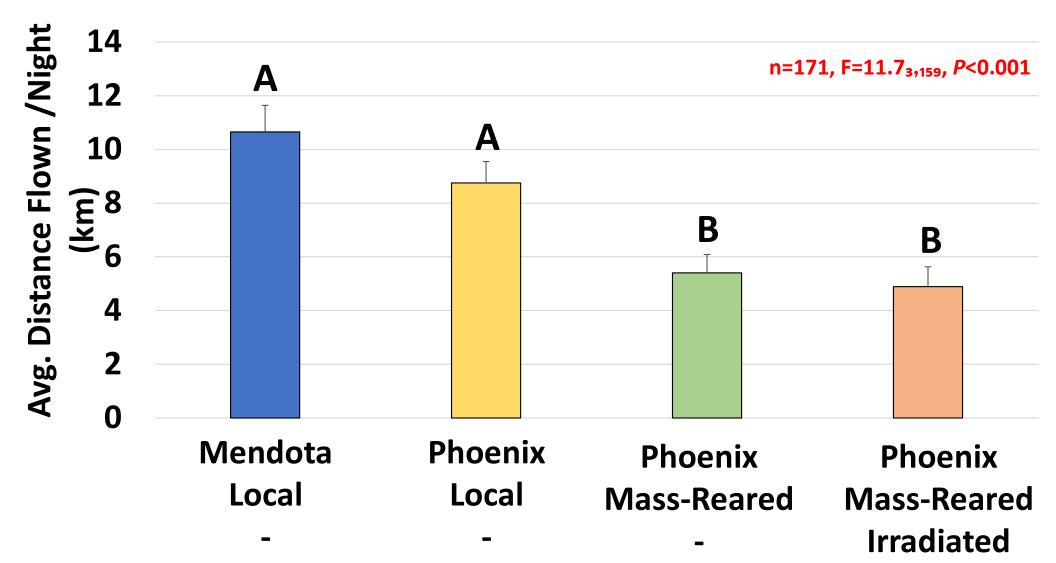
- Flight mill assays
- Do they respond to pheromone?
- Wind tunnel assays



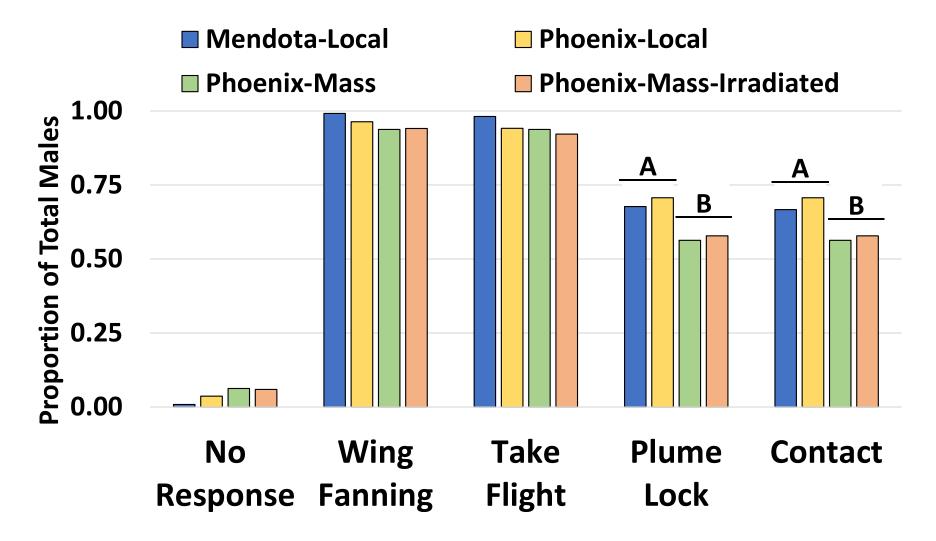
Tandem NOW on a flight mill

Video courtesy of Joshua Reger, Ph.D. Student Dept. Entomology, UC Riverside

Crop Year 2019 Evaluating the Phoenix Strain Males Males can fly – but not as well



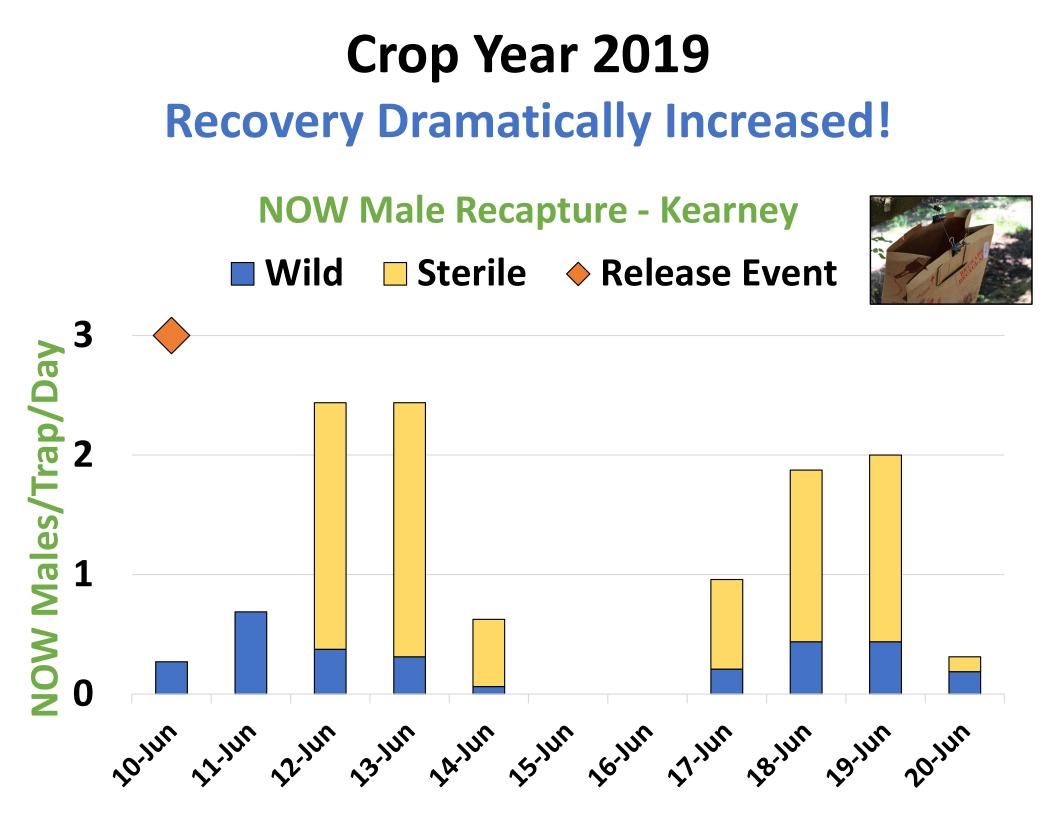
Crop Year 2019 Evaluating the Phoenix Strain Males Males do respond to pheromone



n=372, χ²=4.09, *P*<0.05

Crop Year 2019 New Release System Provisions Vertical Space Grocery Bags with Paper Tubes





Crop Year 2020-2021

YES - males fly and respond to pheromone BUT – can they mate in the wild?

ALSO - release system is important SO - can we improve it further? Crop Year 2020-2021 YES - males fly and respond to pheromone BUT – can they mate in the wild?

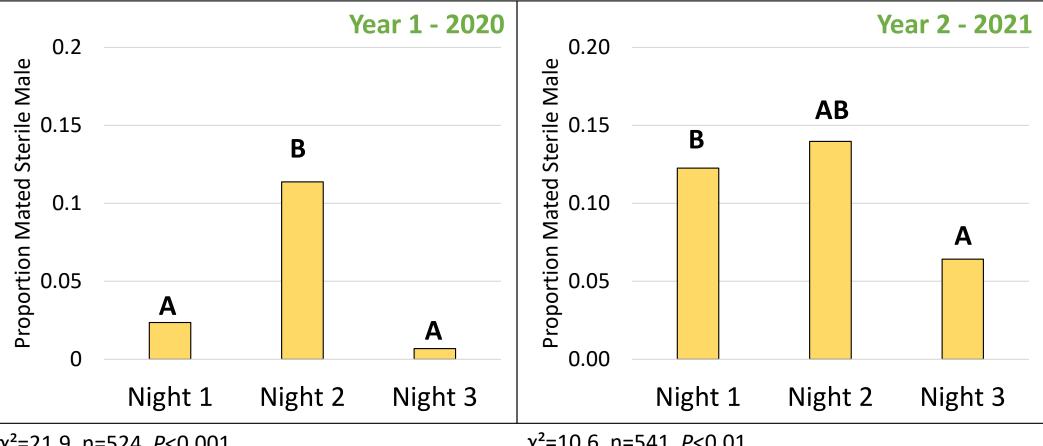
ALSO - release system is important SO - can we improve it further?

Crop Year 2020-2021 Recovery Improved – But Can They Mate?



Crop Years 2020-2021 Mass/Sterile Males Can Locate Sentinel Females

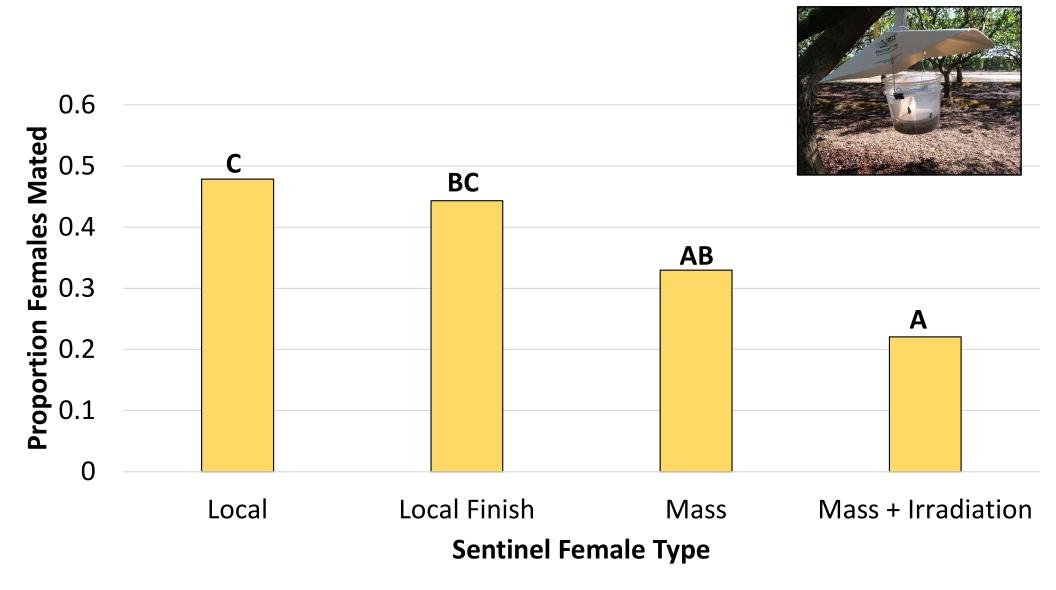




χ²=21.9, n=524, *P*<0.001

χ²=10.6, n=541, P<0.01

Crop Years 2020-2021 Mass/Sterile Females Can Attract Wild Males



χ²=44.0, n=1,115, *P*<0.001

Crop Year 2020-2021

YES - males fly and respond to pheromone BUT – can they mate in the wild?

ALSO - release system is important SO - can we improve it further?

Crop Year 2020-2021 Primary Focus on Transport/Release Methods

Transport

Shipped via UPS



Driven in refrigerated cooler

M3



Vehicle: M3 Agriculture

Agriculture Technologies

Release



Paper Bag + Tubes

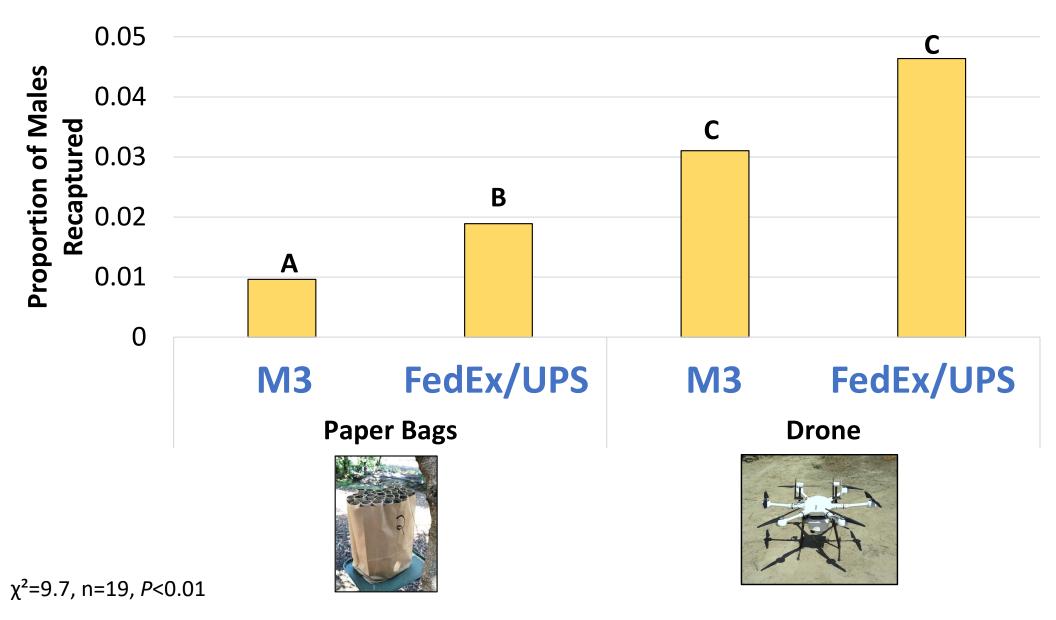


UAV/ Drone

Drone: M3 Agriculture

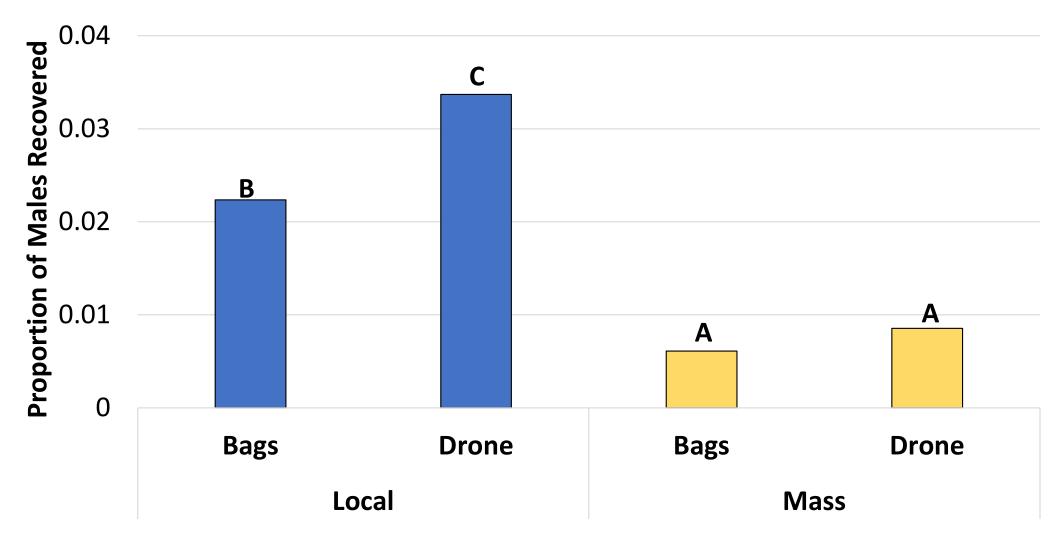
Crop Years 2020-2021

Transport and Release Does Influence Performance Mass-Rear Moths Only



Crop Years 2020-2021

Mass Rearing Negatively Impacts Field Performance Regardless of Release Device or Transport



χ²=352.2, n=40, *P*<0.01

Crop Years 2021-2023

Improved Recovery from the Airplane How do they disperse in large blocks? Can they impact wild NOW?

Mass-rearing/handling impacts SO - new "MCS" strain

Where should moths be allocated? Ecological-economic scenario modeling

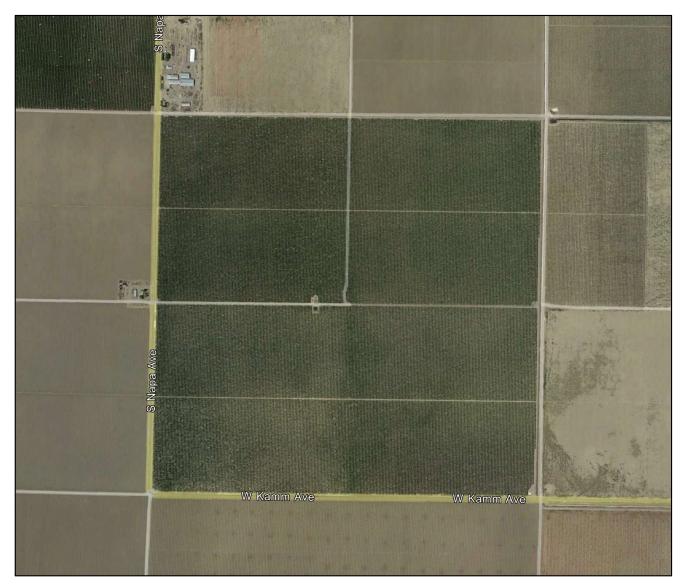
Crop Years 2021-2023

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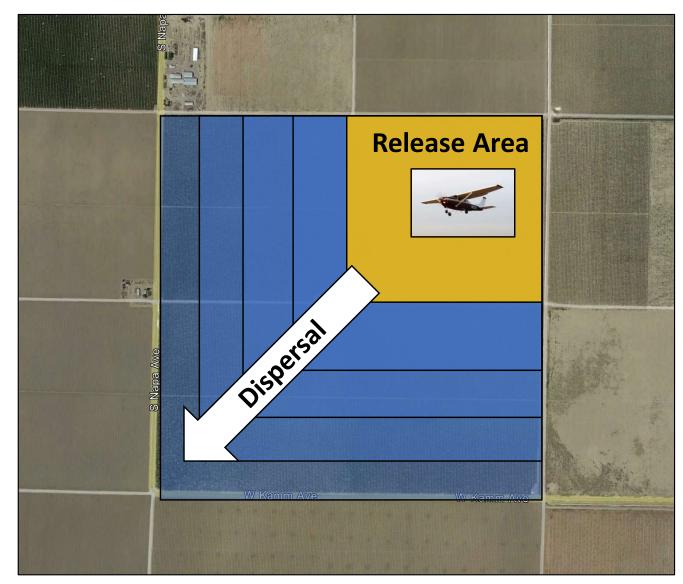
Crop Years 2021/2022 Dispersal in Large Block Setting - Pistachios Weekly Release with Grid of Traps



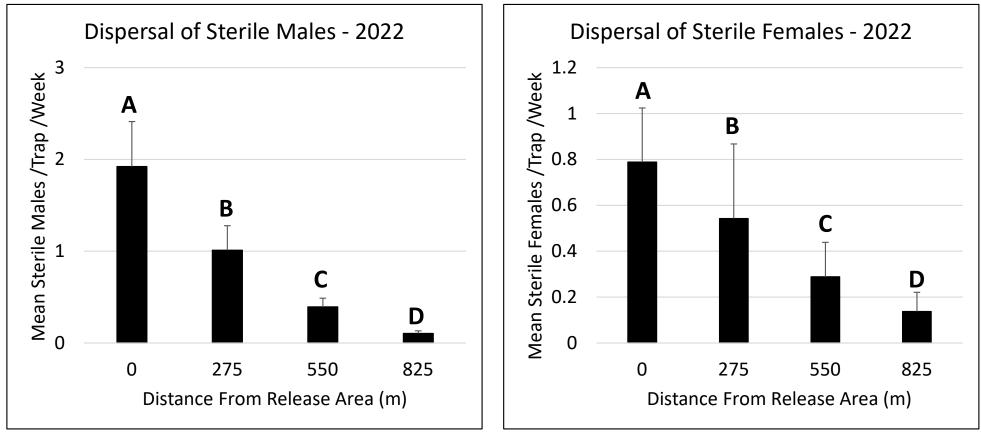
Crop Years 2021/2022 Dispersal in Large Block Setting - Pistachios Weekly Release with Grid of Traps



Crop Years 2021/2022 Dispersal in Large Block Setting - Pistachios Weekly Release with Grid of Traps



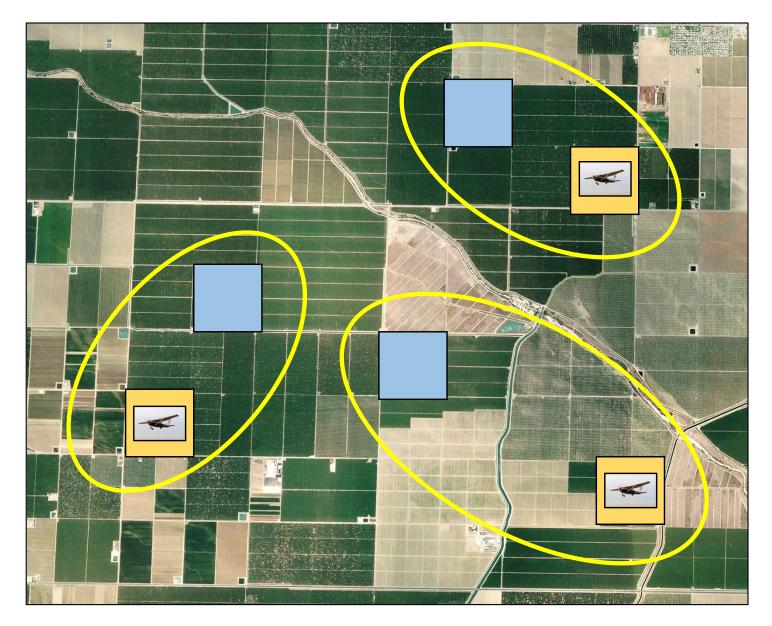
Crop Years 2021/2022 Dispersal in Large Block Setting - Pistachios ~50% remain in the release area – otherwise disperse out



χ²=300.4, n=459, *P*<0.001

χ²=53.9, n=350, *P*<0.001

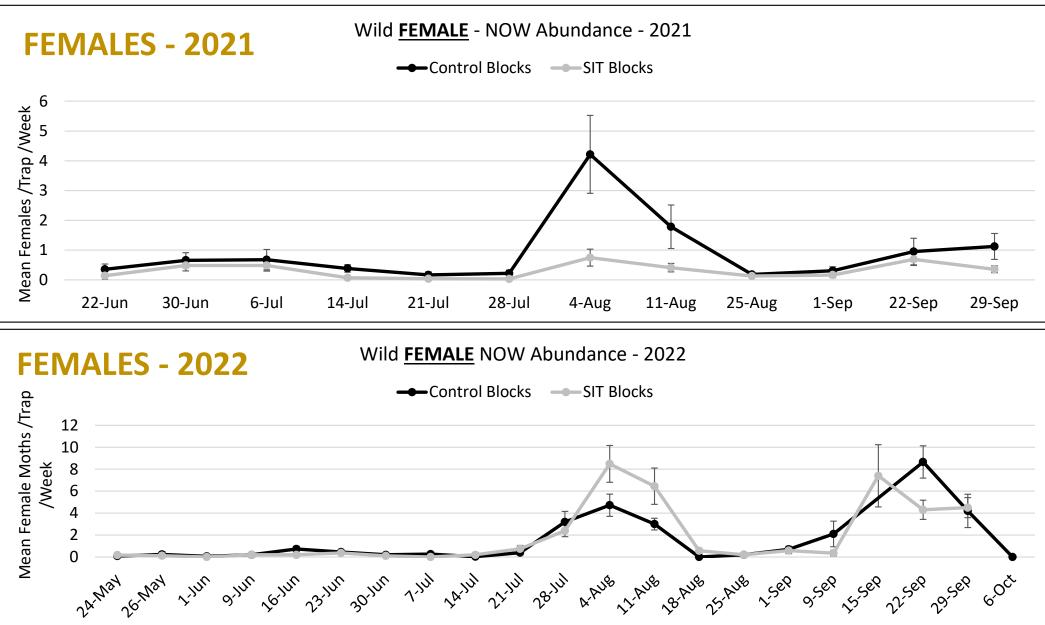
Crop Years 2021/2022 Impact on Wild Populations – Almonds Weekly Release in Paired Plots



Crop Years 2021/2022

Impact on Wild Populations - Almonds

So Far - Inconsistent Impacts on Wild Populations and Crop Damage



Crop Years 2021-2023

Improved Recovery from the Airplane How do they disperse in large blocks? Can they impact wild NOW?

Mass-rearing/handling impacts SO - new "MCS" strain

Where should moths be allocated? Ecological-economic scenario modeling

Crop Years 2022-2023

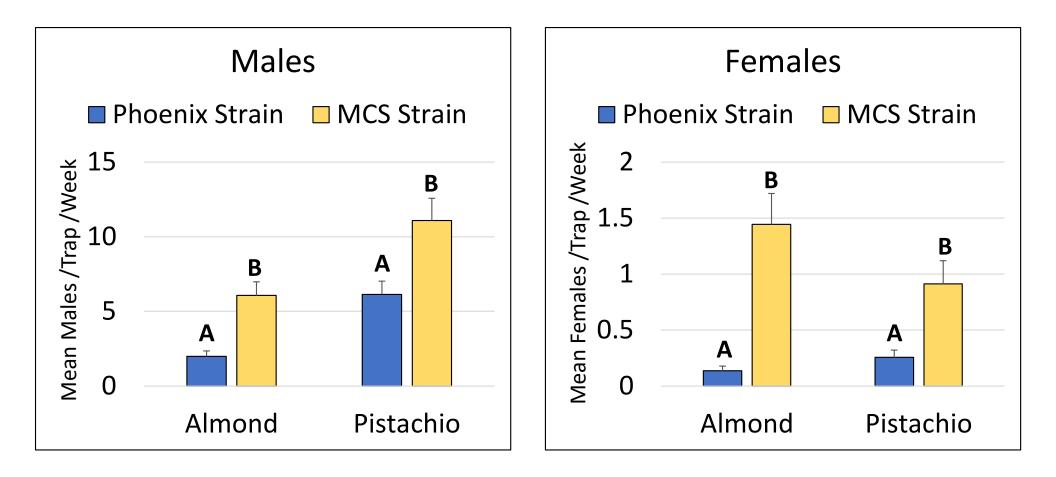
Comparison of Improved Strain for Mass Rearing 'MCS' Strain Selected for Rearing Conditions





Crop Year 2022

Comparison of Improved Strain for Mass Rearing Small Plot Work - So Far Looks Promising...

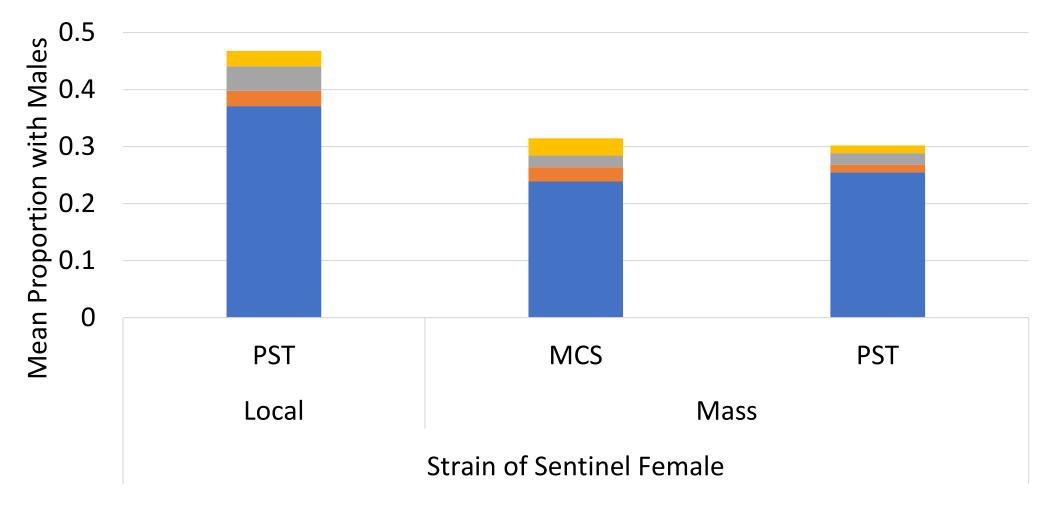


Crop Year 2023

Comparison of Improved Strain for Mass Rearing Mating Tables – sterile females can attract wild NOW

Performance of Sentinel Females

Wild Unknown MCS PST

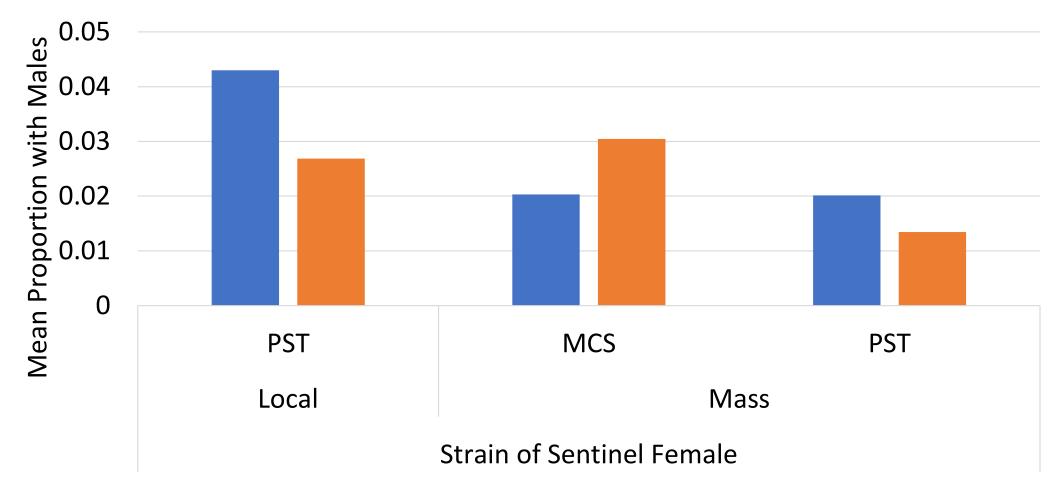


Crop Year 2023

Comparison of Improved Strain for Mass Rearing Mating Tables – sterile males can find sentinel females

Performance of Sterile Males

MCS PST



Crop Years 2021-2023

Improved Recovery from the Airplane How do they disperse in large blocks? Can they impact wild NOW?

Mass-rearing/handling impacts SO - new "MCS" strain

Where should moths be allocated? Ecological-economic scenario modeling

Crop Years 2021-2023

Ecological/Economic Scenario Modeling

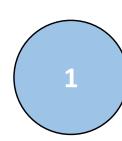
Can we determine if/when/where sterile NOW makes sense?

Models incorporate various features to make them realistic, such as data on the distribution and arrangement of tree nut orchards, pesticide use and tree phenology.

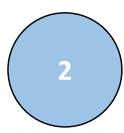


Co-PIs: Dr. Ran Wei (UC Riverside) and Dr. Brittney Goodrich (UC Davis)

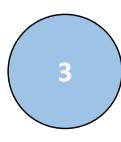
Crop Years 2021-2023 Ecologica/Economic Scenario Modeling General Process



Predefined scenarios include different combinations of management practices.

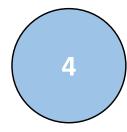


Scenarios then generate different population curves for NOW in a given region.



Those NOW populations then have differential impacts on crop damage.

	Alm	ond	Pistachio		
% Damage Rate	Nonpareil	Monterey	1st-Harvest	2nd-Harvest	
1-Pesticide	0.04	0.04	0.06	0.73	
Sanitation + 1-Pesticide	0	0.09	0.01	0.1	
Sanitation + MD + 1-Pesticide	0	0	0	0	
Sanitation + SIT-Low Rate	1.35	0	1.38	2.42	
Sanitation + SIT-High Rate	0	0	0	0	

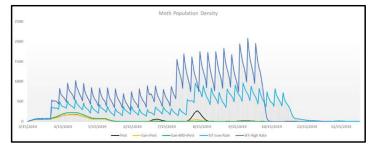


Management efforts and crop damage can then be used to estimate economic costs/benefits under each scenario.

IPM	Percent Damage	IPM	I Cost Per Acre	Reve	enue Per Acre	Net E	Benefit Per Acre
Pesticide	0.04	\$	65.46	\$	3,998.50	\$	3,933.04
Sanitation + Pesticide	0	\$	421.00	\$	4,000.10	\$	3,579.10
Sanitation + Pesticide + MD	0	\$	531.00	\$	4,000.10	\$	3,469.10
Sanitation + Low SIT	1.35	\$	355.54	\$	3,902.50	\$	3,546.96
Sanitation + High SIT	0	\$	355.54	\$	4,000.10	\$	3,644.56

Example Scenarios

- 1. Pesticides Only
- 2. Sanitation + Pesticides
- 3. Sanitation + Mating Disruption (MD) + Pesticides
- 4. Sanitation + SIT at Low Rate
- 5. Sanitation + SIT at High Rate



2018

• Poor initial recovery, likely due to release method

2018

• Poor initial recovery, likely due to release method

2019

- Confirmed male flight and pheromone response
- Improved release method (paper bags) for field experiments

2018

• Poor initial recovery, likely due to release method

2019

- Confirmed male flight and pheromone response
- Improved release method (paper bags) for field experiments

2020-2021

- Determined that mass-rearing has a clear negative impact
- That said sterile M/F can locate/attract wild F/M in current form
- New transport and release methods can improve field performance

2022-2023

- Initial work with 'MCS' strain definitely performs better
- Starting to better understand field dispersal
- Very mixed impacts on wild NOW and crop damage
- Figuring out the ecological/economic scenario modeling

2022-2023

- Initial work with 'MCS' strain definitely performs better
- Starting to better understand field dispersal
- Very mixed impacts on wild NOW and crop damage
- Figuring out the ecological/economic scenario modeling

2023-2024 and beyond...

- Continued work on dispersal, impacts of wild NOW, modeling etc.
- New markers for sterile male spermatophores
- Exploring X-ray and eBeam as alternatives
- Improving the cold collection and handling system
- New external markers for field studies

Sterile Insect Technique for NOW Project Project Summary 2018-2024

2018	2019	2020	2021	2022	2023	2024
Understand	ling					
the Problen	n					

Developing Alternatives

> Field Dispersal and Impacts on Wild NOW

> > Ecological/Economic Scenario Modeling

> > > Areawide IPM West Fresno County

Thank You!

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[Collaborators] Chuck Burks, Joel Siegel (USDA ARS), Davis/Claus/Andress/Garvey/Walton (APHIS), Nathan Moses-Gonzales et al. (M3 Agriculture) [Lab Assistants] Sarah Meierotto, Reva Scheibner, Jessica Maccaro, Javi Herrera, Tyler Colombero, Victoria Morelos, German Camacho, Lino Salinas, Anisabel Guzman, Hector Jacome-Saenz, Celeste Lara [Postdocs / Grad Students] Dylan Tussey, Jean Liu, Nathalie Baena Bejarano, Kadie Britt, Joshua Reger [Funding] <u>CA Pistachio Research Board</u> + Almond Board of CA + APHIS PPA 7721 + CDFA SCBG







UCRIVERSITY OF CALIFORNIA USDA







Development of a Pheromone Lure for Leaffooted Plant Bug (Coreidae: Leptoglossus zonatus)







Houston Wilson^{1,2}, Sarah Meierotto^{1,2}, Reva Scheibner^{1,2}, Sean Halloran¹, Jocelyn Millar¹, Kent Daane^{2,3} ¹Dept. Entomology, UC Riverside ²Kearney Agricultural Research and Extension Center ³Dept. Enviro. Science Policy and Management, UC Berkeley

Leaffooted Bugs (LFB) Key Species in California

Leptoglossus zonatus

- Two distinct yellow marks on pronotum
- Most common species currently

Leptoglossus clypealis

- Distinct clypeus points outward from head
- Used to be abundant, now less common

Leptoglossus occidentalis

- No marks, no clypeus
- Rare, mostly a forest/conifer pest





Leaffooted Bugs (LFB) Current Pest Status

- **Pierce-suck feeder, primarily seeds**
- Can attack a wide range of hosts
- Directly attack pistachio nuts Early season (Apr – Jun)
- Epicarp lesion
- Nut drop/abortion
- Late season (Jun-Sept)
- Kernal necrosis
- "Achilles heel"

Transmit Pathogens

- Stigmatomycosis
- Botryosphaeria
- Shoot blight



Epicarp Lesion



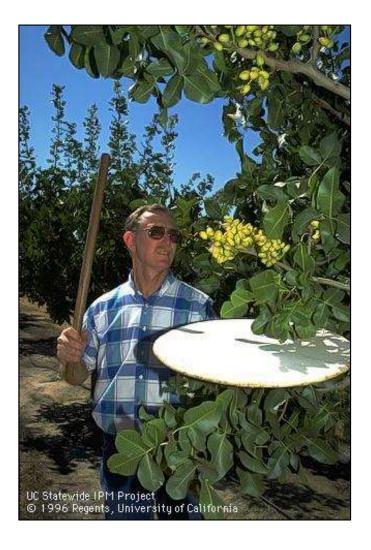
Kernal Necrosis



Damage through stem base

Current Monitoring Beat Sampling, Visual Searching

- **Monitoring Techniques (~April)**
- **1. Beat sampling**
 - Early in the morning, bugs less active
 - Hold tray below, strike clusters
 - Examine what falls out
- 2. Look for egg masses
- 3. Look for damage (small, black nuts)



Current Monitoring Beat Sampling, Visual Searching

Monitoring Techniques (~April)

Significant Limitations

- Monitoring targets "after the fact" signs
- Time intensive, active monitoring
- No clear thresholds
- Arrival can be difficult to predict



New Traps and Pheromone Lures for LFB Developing a Passive Sampling System



Active, time intensive sampling of tree canopy

Passive trapping with pheromone lures



Kent Daane (UC Berkeley)



Jocelyn Millar (UC Riverside)



Houston Wilson (UC Riverside) New Traps and Pheromone Lures for LFB Current Project Goals and Objectives Goal = Develop a Trap/Lure System for LFB

Key Project Objectives

- 1. Find a trap that works
- 2. Identify key pheromone compounds
- 3. Synthesize these chemicals
- 4. Evaluate lures in the field
- 5. Develop reliable monitoring



Photo: Kent Daane

LFB Pheromone **Trap/Lure Project Progress to Date Objective 1** Find a Trap that Works

LFB Pheromone Trap/Lure Project Finding a Trap that Works Trap Comparison Study – 2017



4-ft



Pyramid 2-ft



Clear Sticky

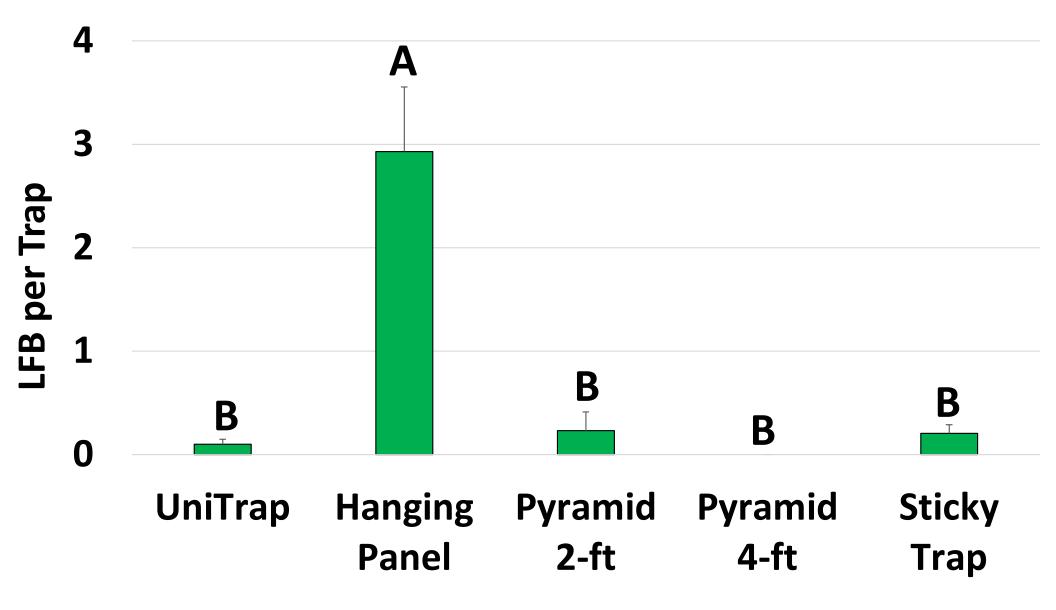


Hanging Panel

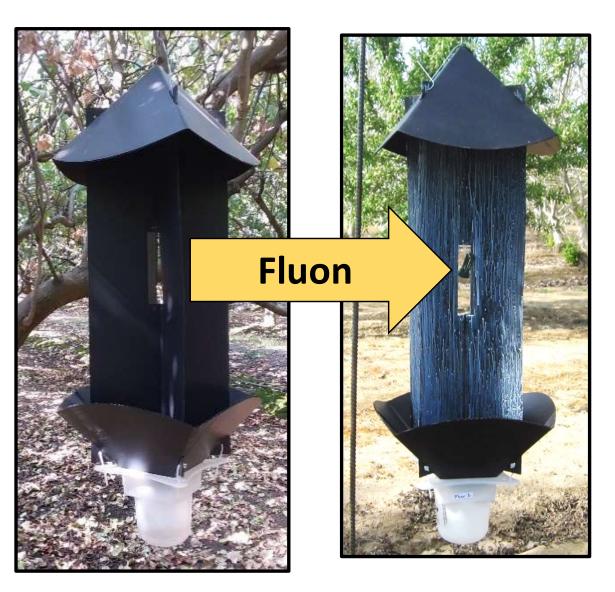


UniTrap

LFB Pheromone Trap/Lure Project Finding a Trap that Works Trap Comparison Study



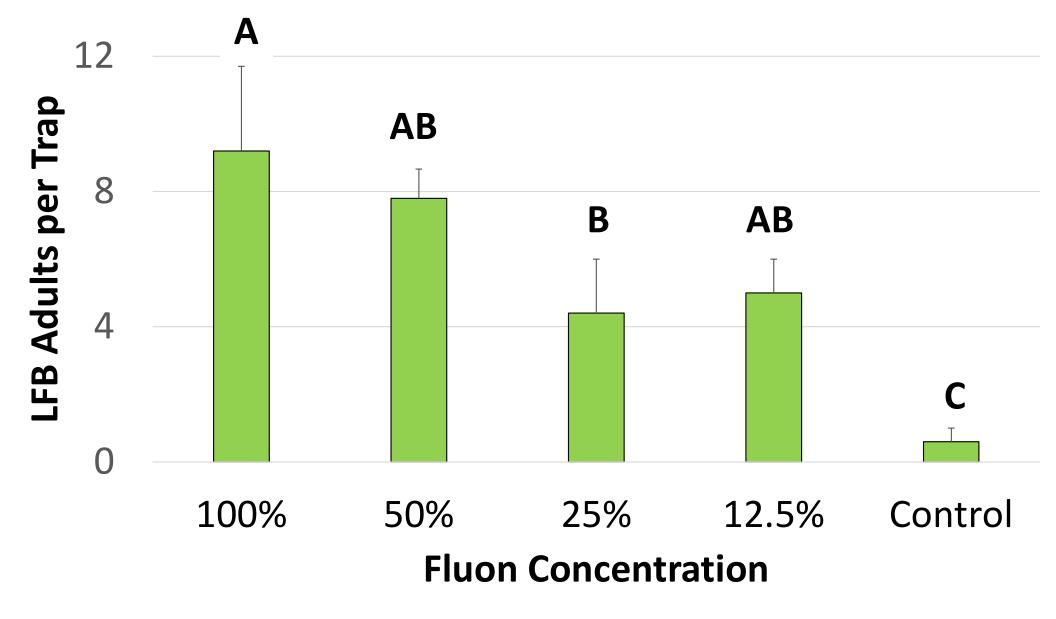
LFB Pheromone Trap/Lure Project Finding a Trap that Works Fluon to Improve Trap Catch



Setup:

- 1 site
- 4 dilutions x 5 reps
- Trap check 2x/month
- Nov. 13 Dec. 4

LFB Pheromone Trap/Lure Project Finding a Trap that Works Fluon to Improve Trap Catch



LFB Pheromone Trap/Lure Project Finding a Trap that Works Effect of Trap Color





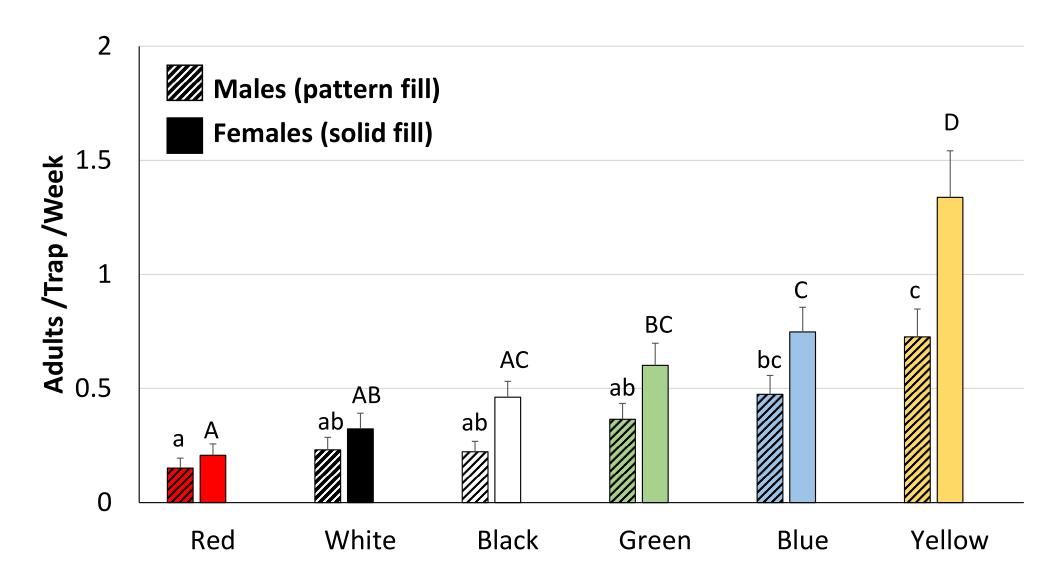








LFB Pheromone Trap/Lure Project Finding a Trap that Works Effect of Trap Color



LFB Pheromone **Trap/Lure Project Progress to Date Objectives 2-3** Identification and Synthesis of **Pheromone Compounds**

LFB Pheromone Trap/Lure Project

Found a Good Trap...





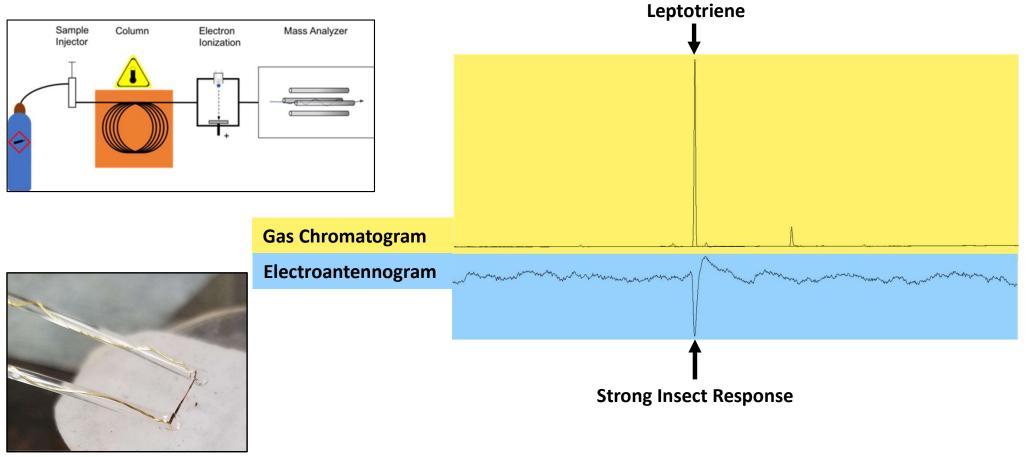
...But We Still Need a Lure!!

LFB Pheromone Trap/Lure Project Pheromone Characterization and Synthesis

General Approach

- Isolate reproductively mature males
- Capture and analyze pheromones
- Measure adult response to individual compounds





LFB Pheromone Trap/Lure Project Pheromone Characterization and Synthesis

Key Aspects of the Pheromone

- Composed of 9 compounds
- *Leptotriene* is entirely new to science!

Next Step – Synthesize the Compounds

- Multi-year effort
- Various elements assembled 2018-2021
- Finally have all components in place by summer 2021
- Work in 2022-2023 focused on improving efficiency/yield of compounds

Field Trials with the Candidate Compounds

- Kearney Ag. Center spring/fall 2021-2023
- Commercial orchards spring/fall 2022-2023





LFB Pheromone **Trap/Lure Project Progress to Date Objective 4** Lab/Field Tests of New Lures

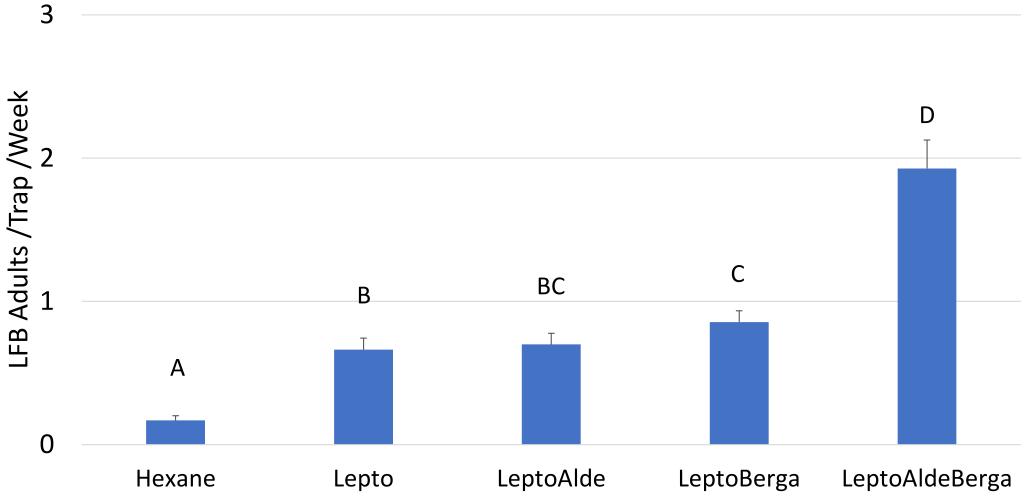
LFB Pheromone Trap/Lure Project Preliminary Candidate Pheromones Field Assays in Infested Orchards





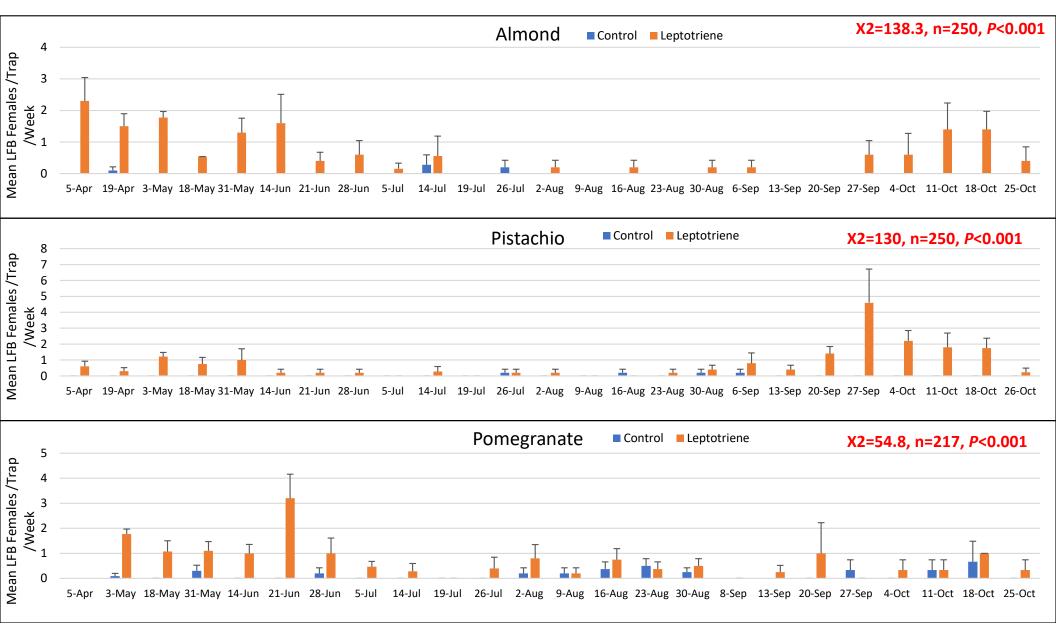
Comparison of Blends Almonds (spring) + Pomegranates (fall) Commercial Sites + Kearney Ag. Center 2021-2023





Season Long Attractancy of Lures Almond + Pistachio + Pomegranate @ Kearney Ag. Center 2022

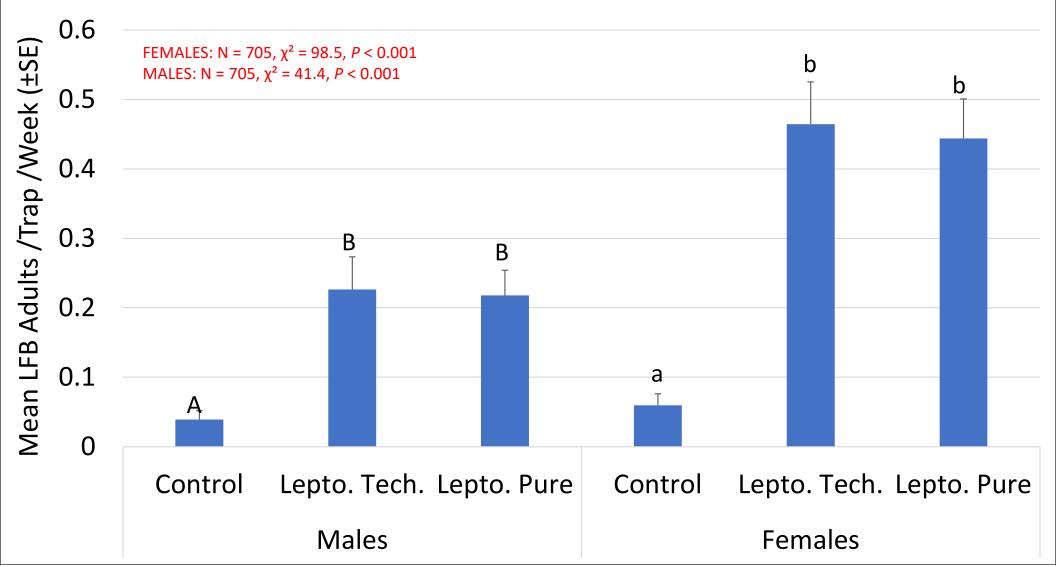
LFB Trap/Lure Project Season Long Attractancy of Lures LFB Attracted to Leptotriene All Year



Cheaper "Technical Grade" Leptotriene Almond + Pistachio + Pomegranate @ Kearney Ag. Center 2023

LFB Trap/Lure Project Cheaper "Technical Grade" Leptotriene Appears to be equally attractive

Leptotriene – Technical Grade vs. Pure



Evaluation of a Commercial Lure Produced by Sterling International (Spokane, WA) Spring 2023 (Almonds)

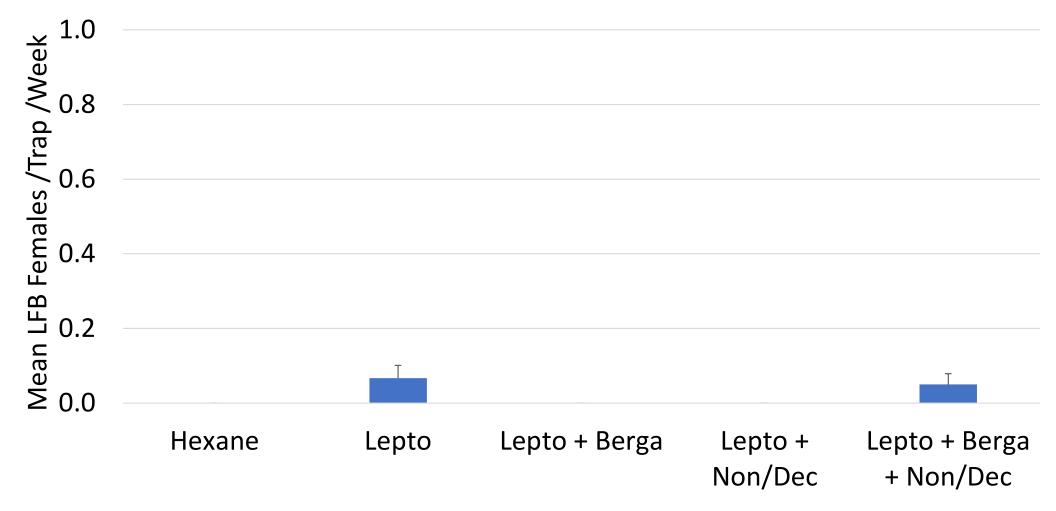
LFB Trap/Lure Project

Evaluation of a Commercial Lure – Sterling International

Not Quite There Yet...But More to Come

Note - these are NOT the UCR-made lures!

Exp 1 - Sterling Lures in Sterling Pyramid Traps



LFB Pheromone Trap/Lure Project Summary of Experiments – 2017-2022

LFB Traps

Hanging panel trap is best

- Yellow/blue most attractive
- Absolutely needs a lure

LFB Lures

Pheromones



- Identified and synthesized all 9 pheromone compounds
- Documented LFB response to certain blends leptotriene is key!
- Optimized synthesis 4.5x of leptotriene
- Demonstrated season-long attraction
- Developed a cheaper version of leptotriene ("technical grade")

LFB Pheromone Trap/Lure Project Summary of Experiments – 2017-2022

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- Developed a cheaper version of leptotriene ("technical grade")

Now starting to work with private companies to scale-up production

LFB Pheromone Trap/Lure Project Concluding Remarks

Key Project Objectives/Achievements

- \checkmark Find a trap that works
- ✓ Identify key pheromone compounds
- ✓ Synthesize these chemicals
- ✓ Evaluate lures in the field
- Develop reliable monitoring

Next Steps

- Further improve synthesis of compounds
- Quantify cost/time savings
- Develop a commercial lure
- Relate trap catch to orchard populations



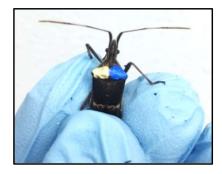


LFB Pheromone Trap/Lure Project Plans for 2024

Continue to refine the synthesis
 Revisit trap selection (with lure)
 Explore commercial lure production



Thank You!!



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