Update on Development of Sterile Insect Technique for Navel Orangeworm

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Sterile Insect Technique

How Does It Work?

Concept

- Introduce sterile insects into wild populations
- Mating with sterile = no reproduction / pop. declines

Process

- Mass-produce target pest
- Sterilize with radiation (or other means)
- Mass release over target area
- “Overflooding ratio” (sterile:wild ratio)

Images: postnatural.org

Sterile Insect Technique

Some Key SIT Programs

Group	Species	Region	Years
Flies (Diptera)	Screwworm	US / Latin America	1950s-present
	Mex. Fruit Fly	Central America	1960s
	Med. Fruit Fly	Global	1970s-present
	Melon Fly	Japan	1970-80s
	Onion Maggot	Netherlands	1980s-present
Beetles (Coleoptera)	Sweet Potato Weevil	Japan	1990s
	Boll Weevil	US	1970s
Moths (Lepidoptera)	Codling Moth	British Columbia	1990s-present
	Pink Bollworm	Southwest US	1970s-2018
	False Codling Moth	South Africa	2007-present

Sterile Insect Technique

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Moths (Lepidoptera)	Codling Moth	British Columbia	1990s-present
	Pink Bollworm	Southwest US	1970s-2018

Navel Orangeworm?

SIT for NOW - Background

Phoenix Irradiation Facility Presents Novel Opportunity

USDA Pink Bollworm Rearing Facility

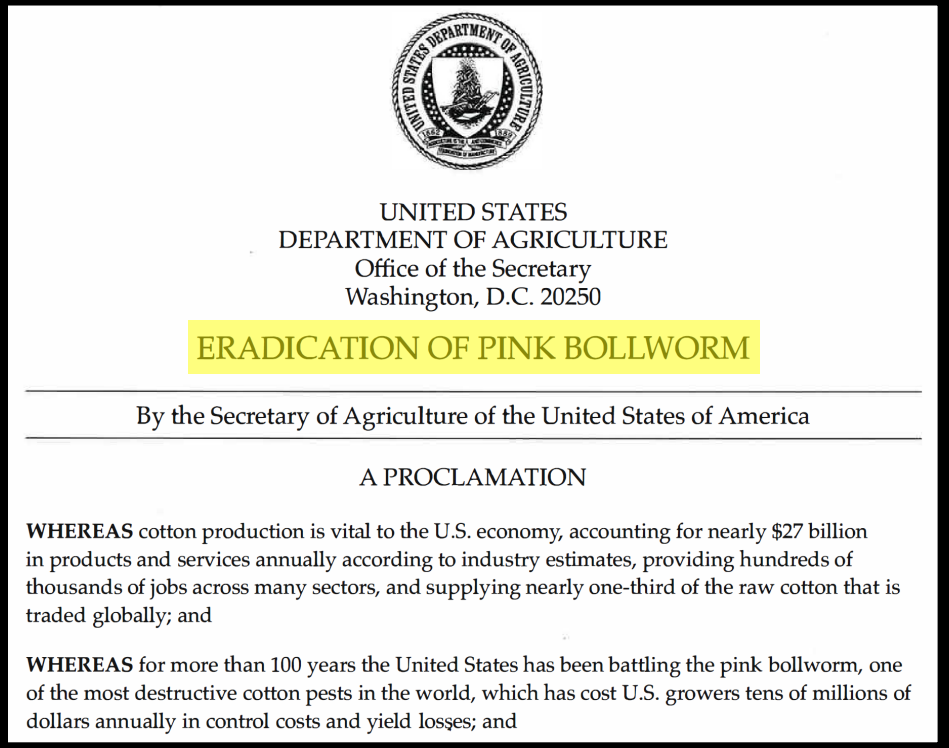
- Phoenix, AZ
- Operating since 1960s

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

SIT for NOW - Background

Moth Production/Transportation Process

Egg Production

SIT for NOW - Background

Moth Production/Transportation Process

Rearing Larvae/Pupae

SIT for NOW - Background

Moth Production/Transportation Process

Adult Emergence in Vacuum System

SIT for NOW - Background

Moth Production/Transportation Process

Adults Collected in Cold Chilled “Cyclones”

SIT for NOW - Background

Moth Production/Transportation Process

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

Small tube
below the plane

SIT for NOW - Background

Moth Production/Transportation Process

When? Where? How Many?

Lots of moths…

- **750,000+ NOW/day**

SIT for NOW - Background

Moth Production/Transportation Process

When? Where? How Many?

Lots of moths…

- **750,000+ NOW/day**

…but also lots of crops.

- **1.2M almonds, 300k pistachio, 250k walnuts**
- **Plus alternate hosts**

SIT for NOW - Background

Moth Production/Transportation Process

When? Where? How Many?

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Where to deliver moths? When? How many?

- **Overflooding ratio**
- **Delivery method, timing and location**
- **Integration with existing IPM tools**

SIT for NOW - Background

Moth Production/Transportation Process

When? Where? How Many?

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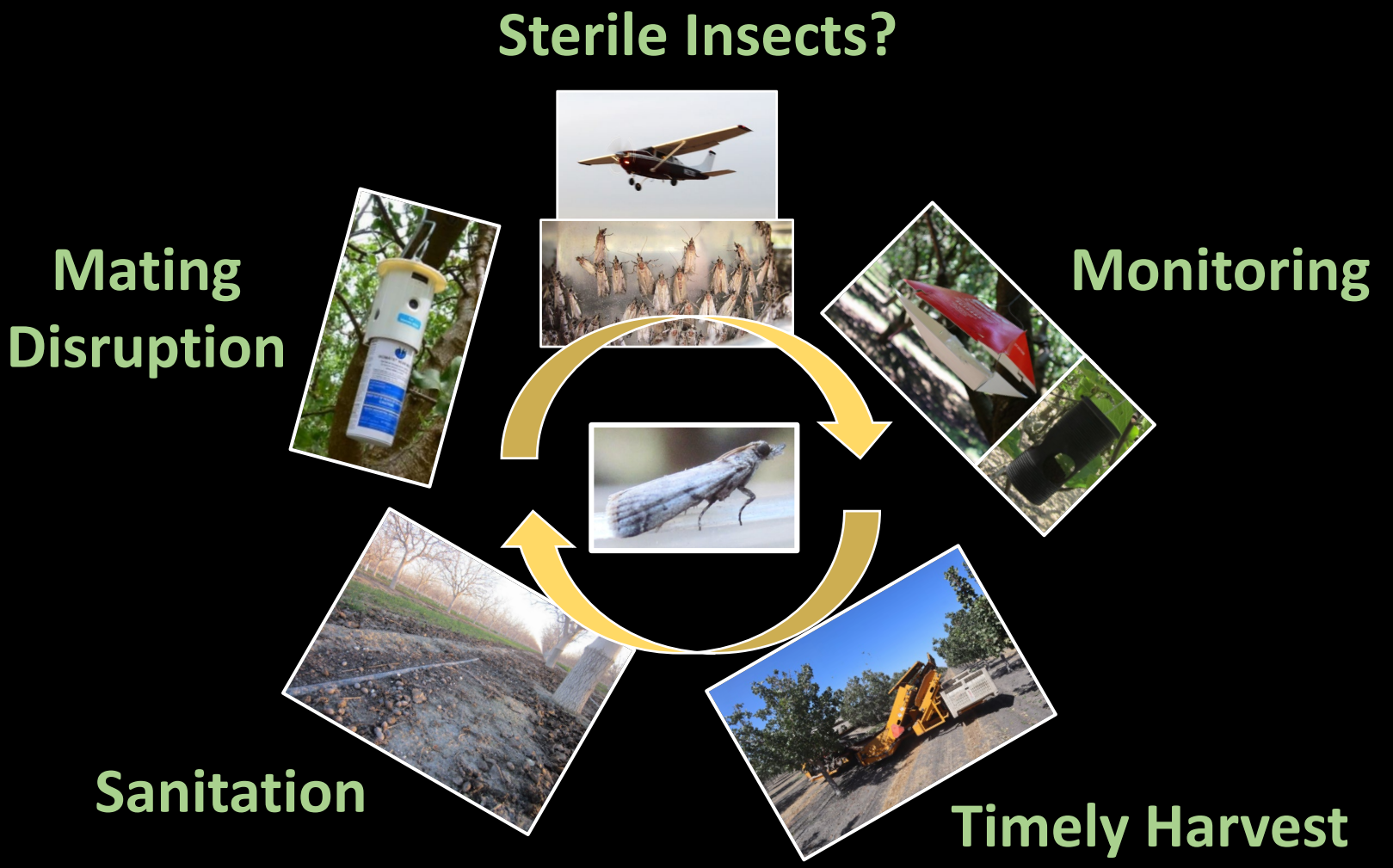
Where to deliver moths? When? How many?

- **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.

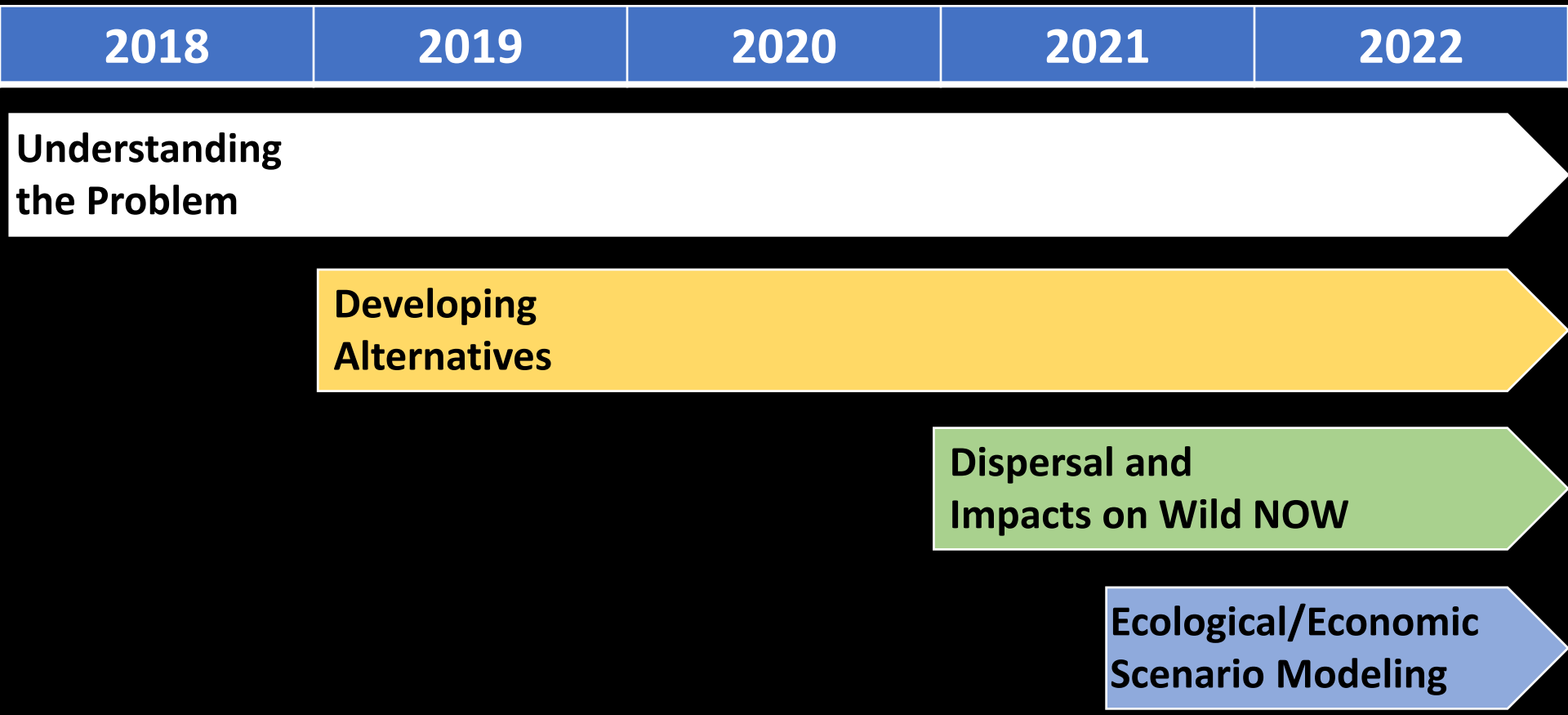
NOW Management in Pistachio/Almond

Use of Multiple Approaches is Key



Sterile Insect Technique for NOW Project

Project Summary 2018-2021



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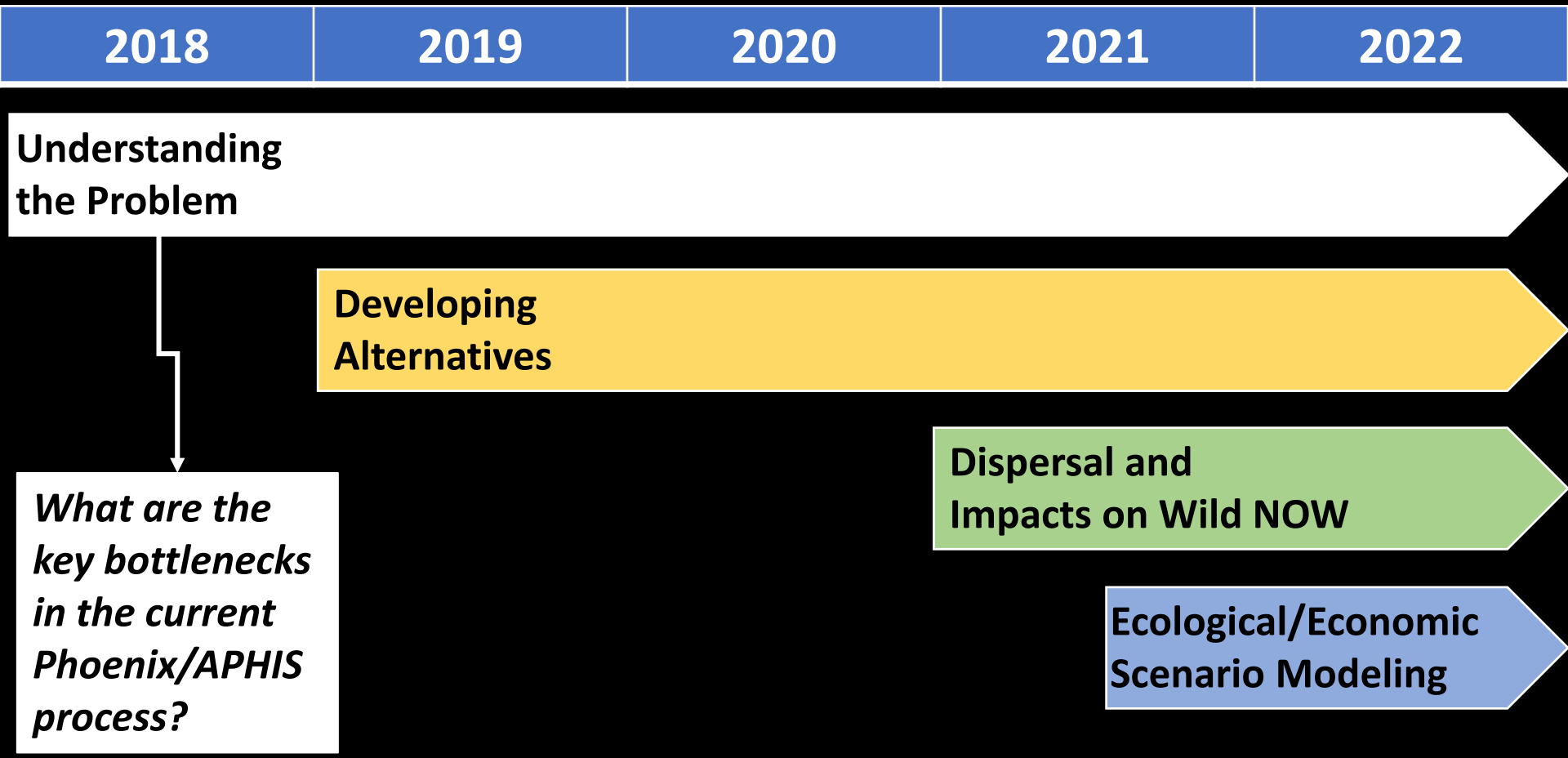


Research Summary: 2018-2021

Monitoring Techniques and Assays

Sterile Moths Internally Marked - Dye in Larval Diet

Research Summary: 2018-2021

Monitoring Techniques and Assays

Pheromone Traps

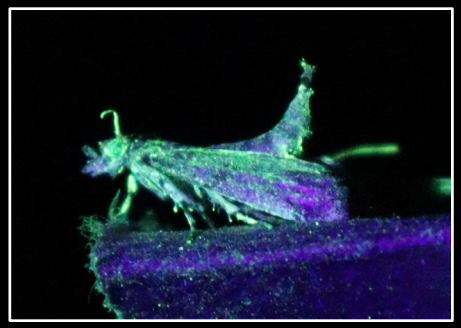
- Synthetic pheromone lure
- Attracts males
- Large trapping radius (captures lots of moths)

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

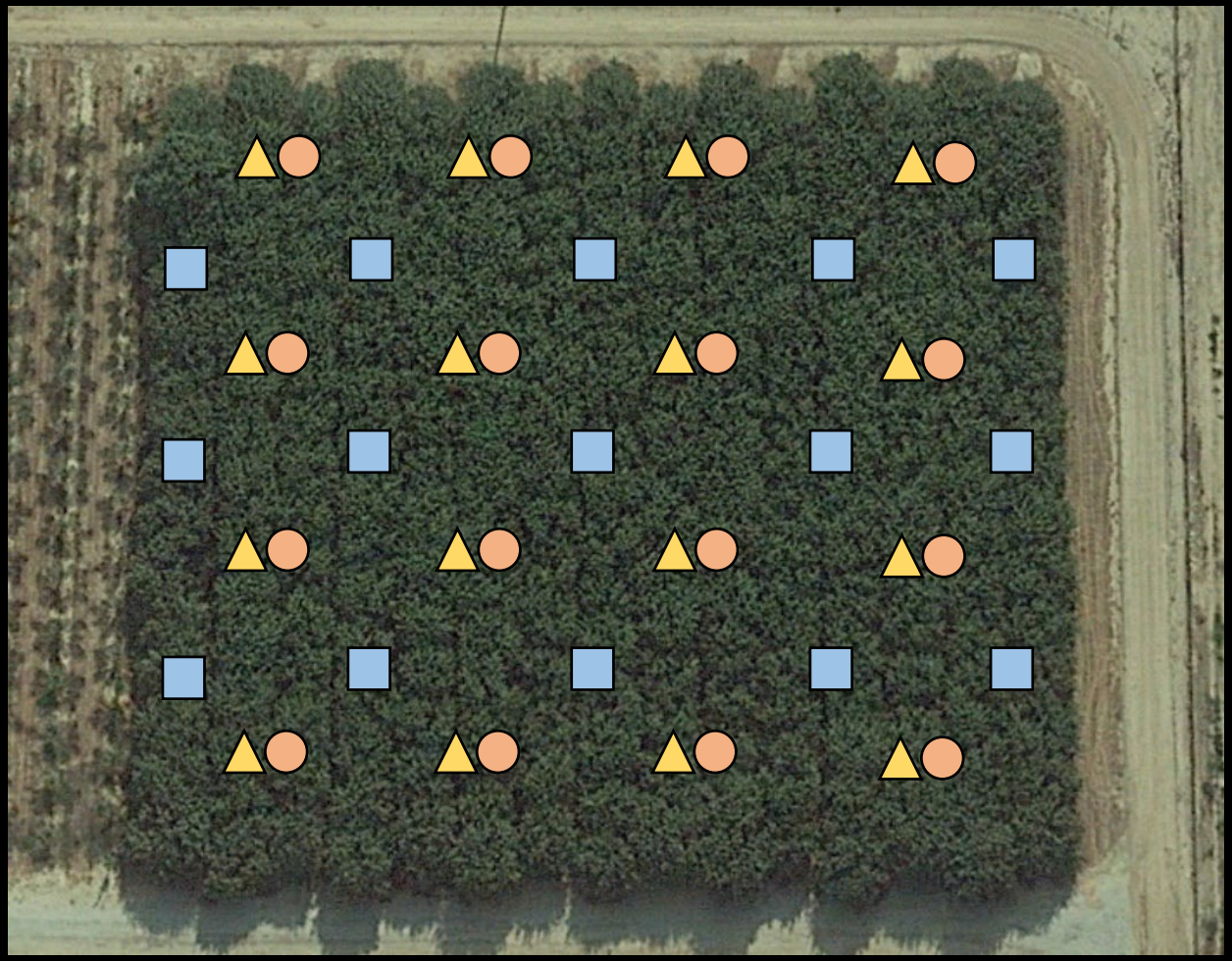
Sentinel Females Used

- Mendota Colony
  - can sterile males locate females?
- Phoenix Facility
  - can sterile females attract wild males?

Research Summary: 2018-2021

Field Release Studies – Small Orchards

Grid of Traps and Mating Tables



Pheromone

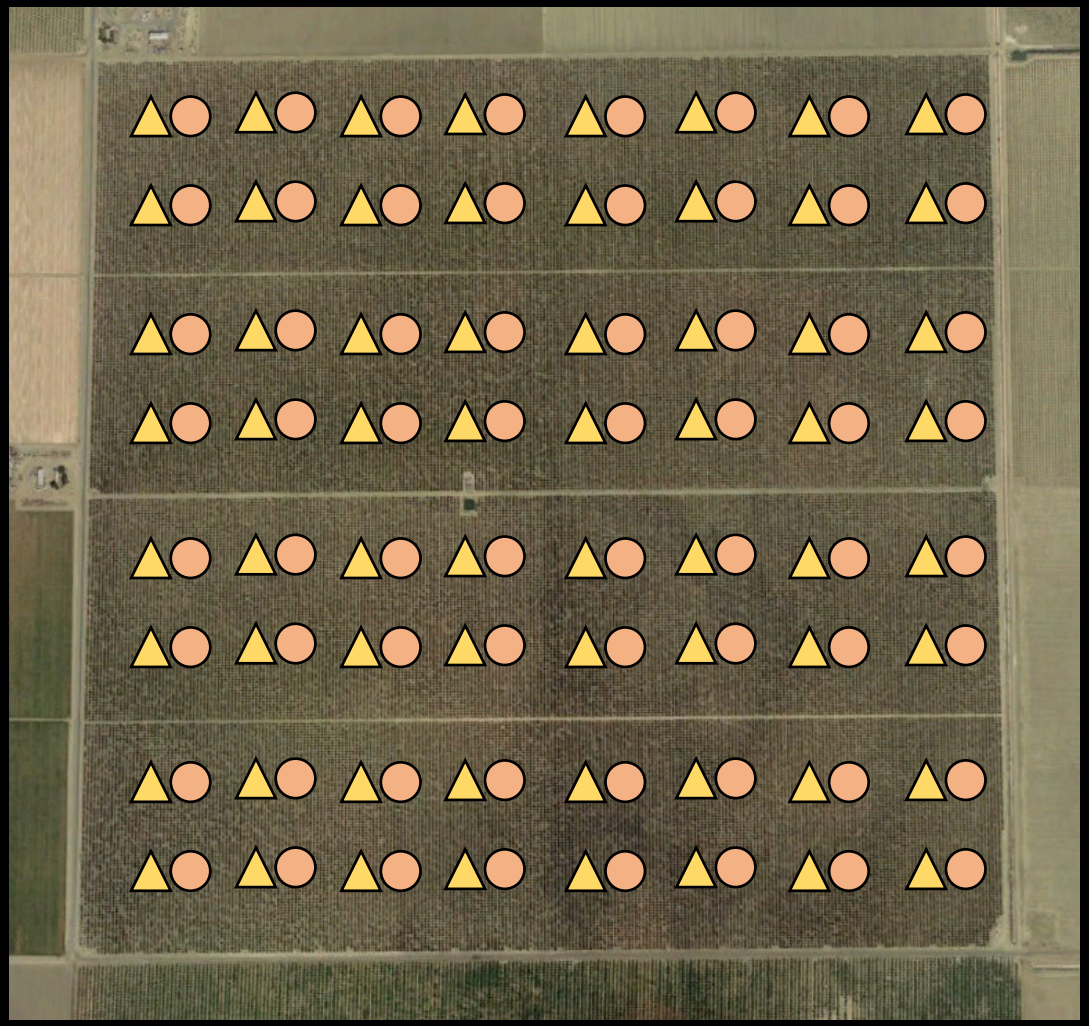
Ovibait

Mating Table

Research Summary: 2018-2021

Field Release Studies – Large Orchards

Grid of Traps Only



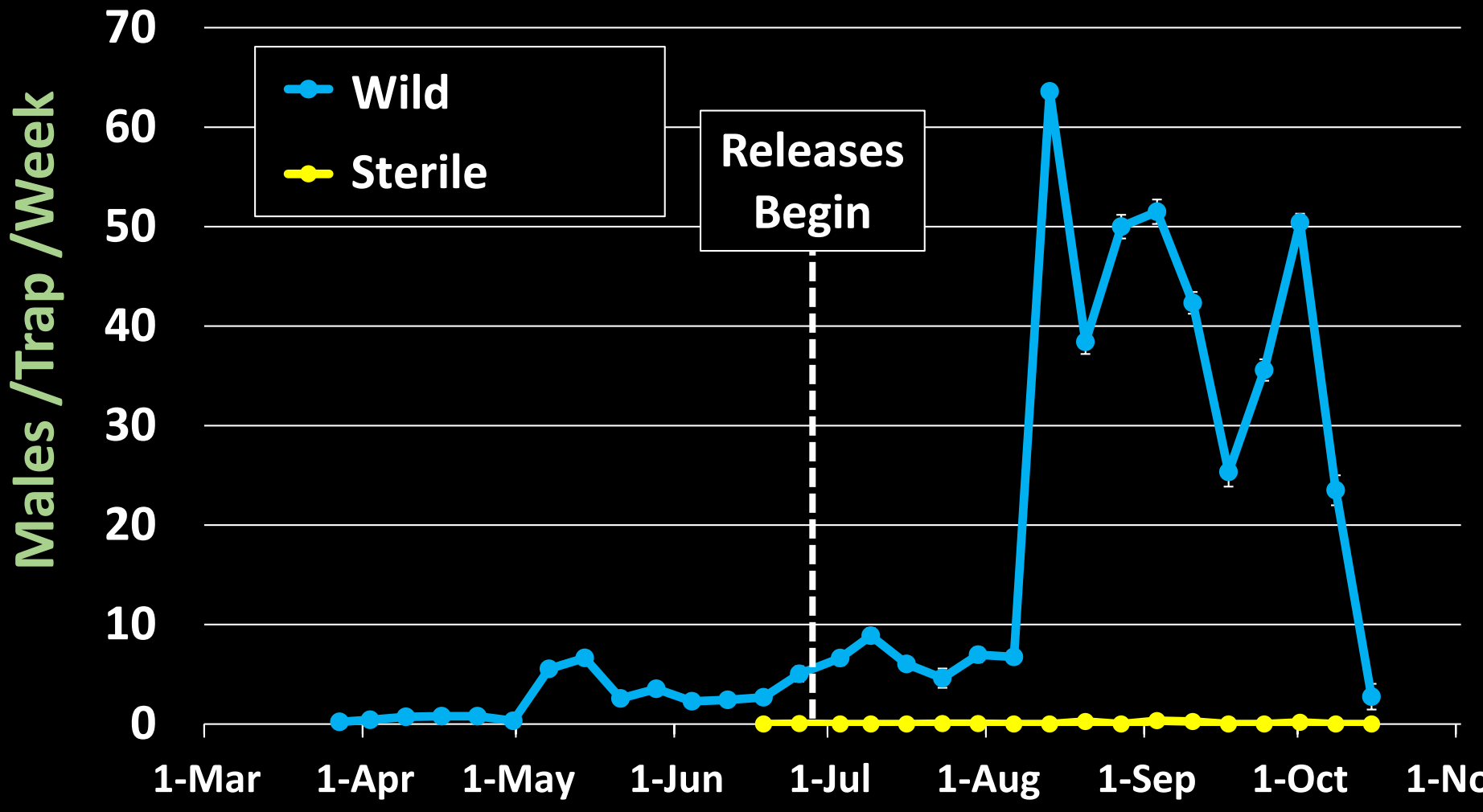
▲ Pheromone

● Ovibait

Crop Year 2018

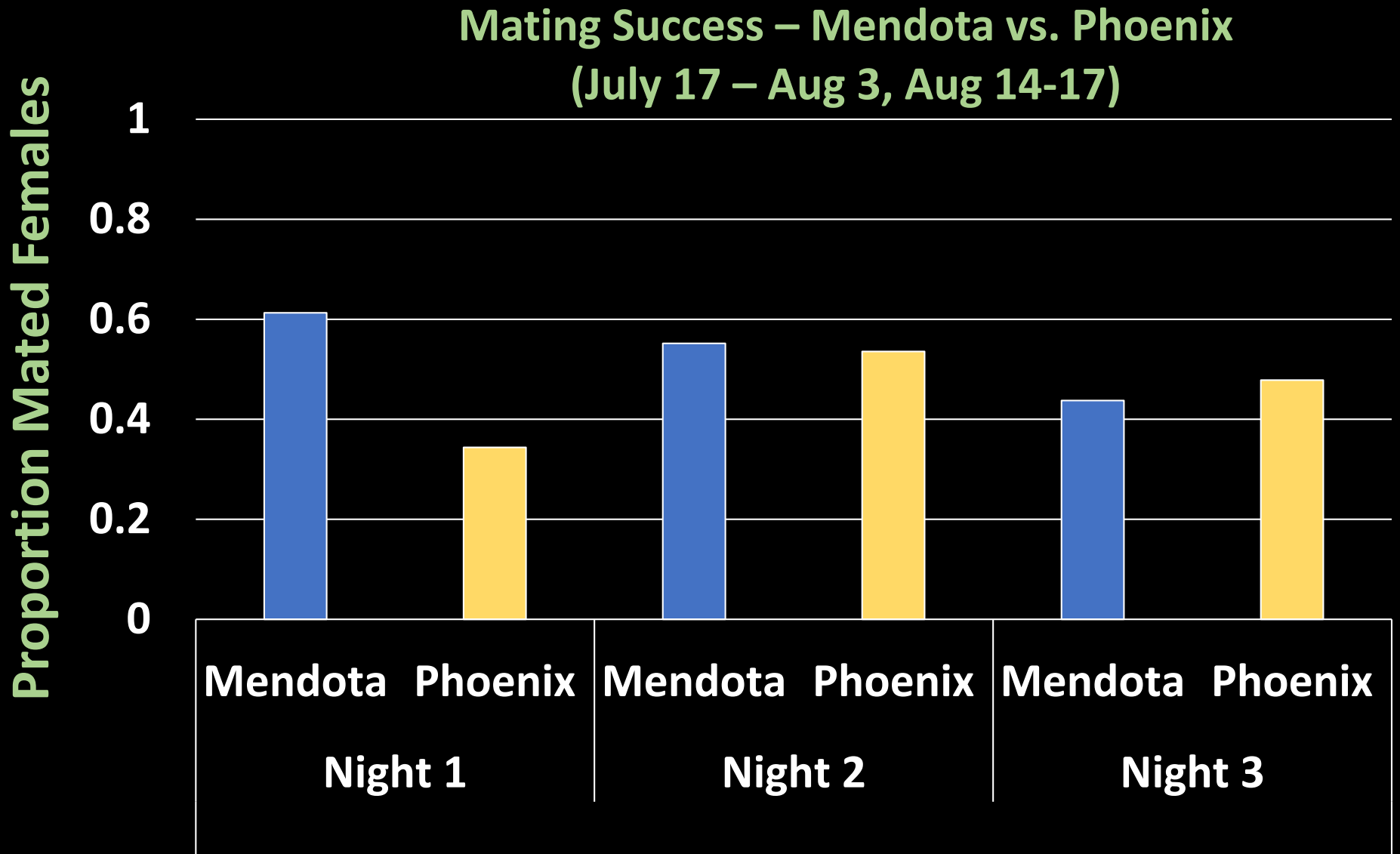
Poor Recovery of Sterile Males

Flight Traps at Kearney



Crop Year 2018

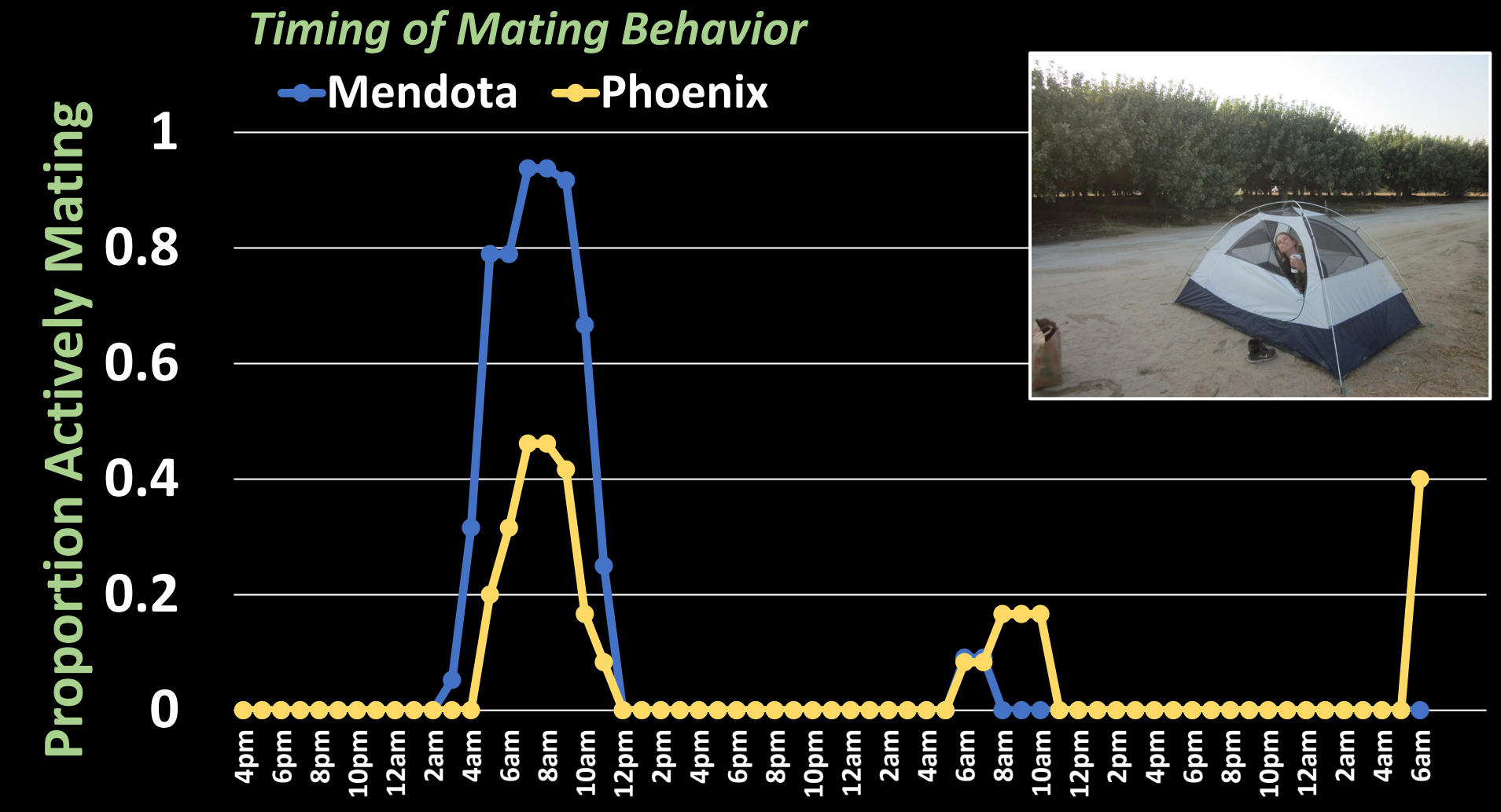
Sterile Females Attract Wild Males



Crop Year 2018

Sterile Females Call/Mate at the Right Time

…but Appear to Need Photoperiod Adjustment



Crop Year 2019

Flight Mill Assay

Phoenix Facility NOW – Are They Active Fliers?

Joshua Reger

MS Thesis @

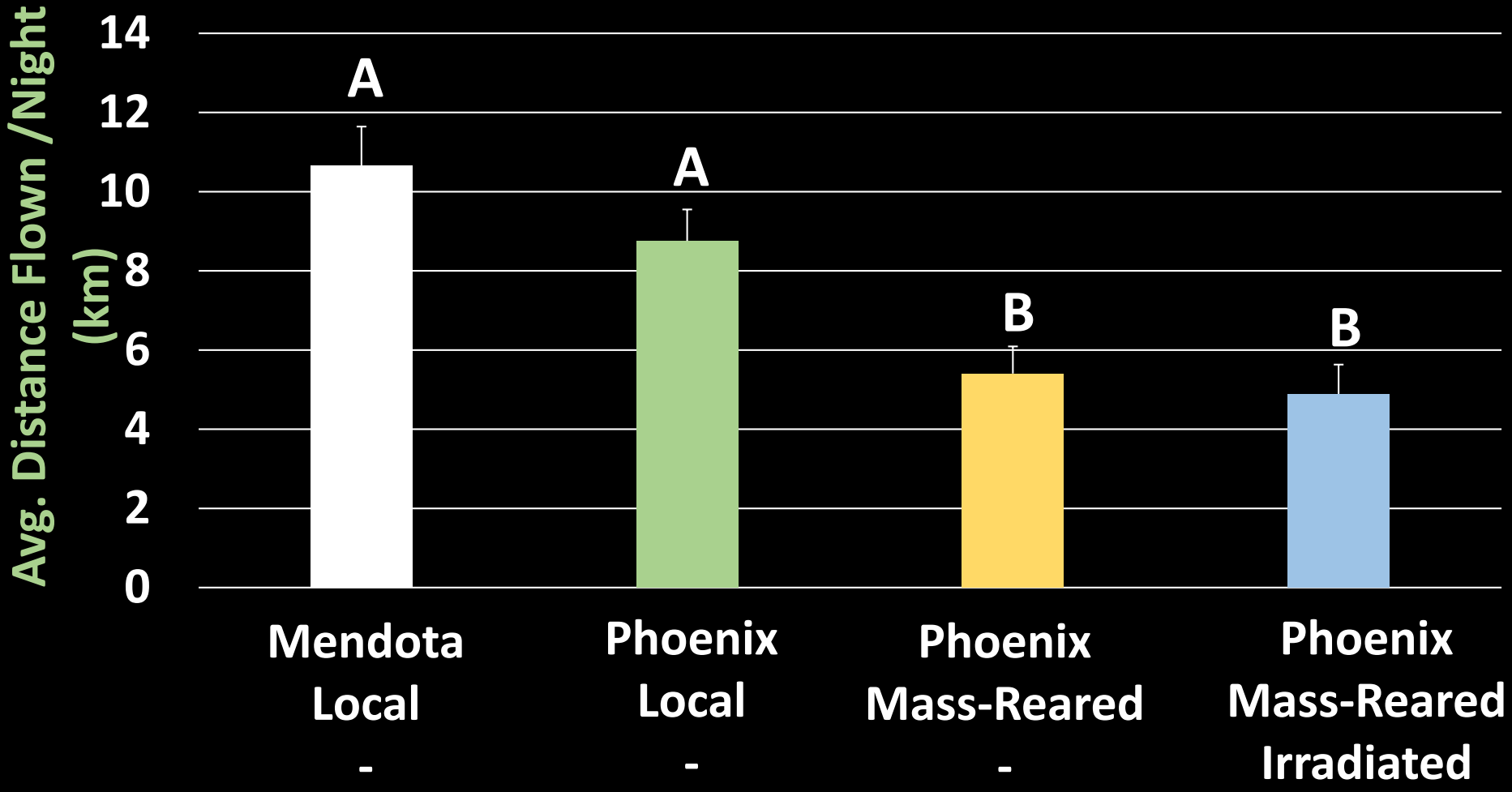
Fresno State

Crop Year 2019

Flight Mill Assay

Phoenix Strain Flies Well

…but Mass-Rearing + Transport Have Negative Impacts

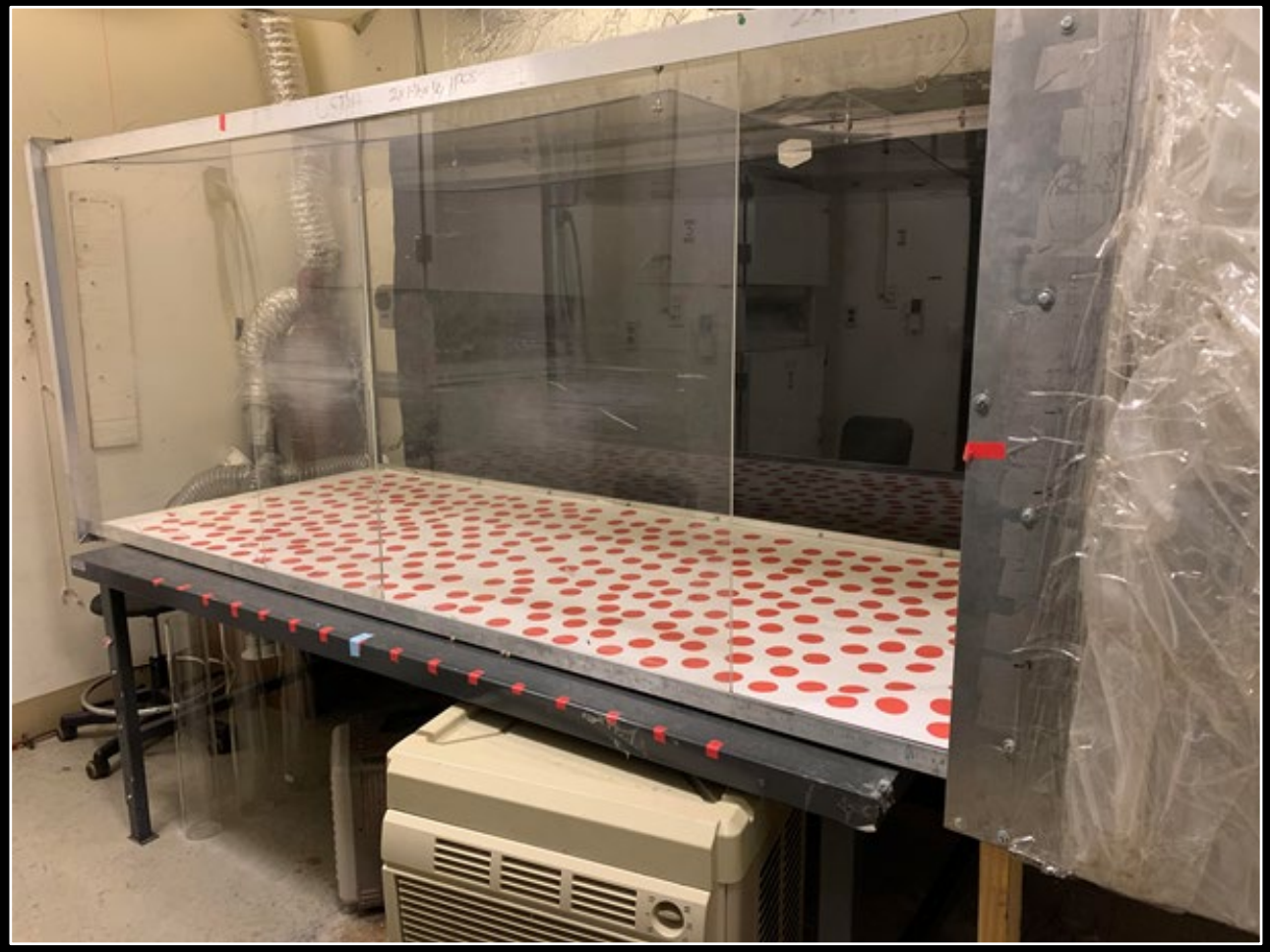


Crop Year 2019

Wind Tunnel Assay

Do Phoenix Strain NOW Respond to Pheromone?

Joshua Reger
MS Thesis @
Fresno State

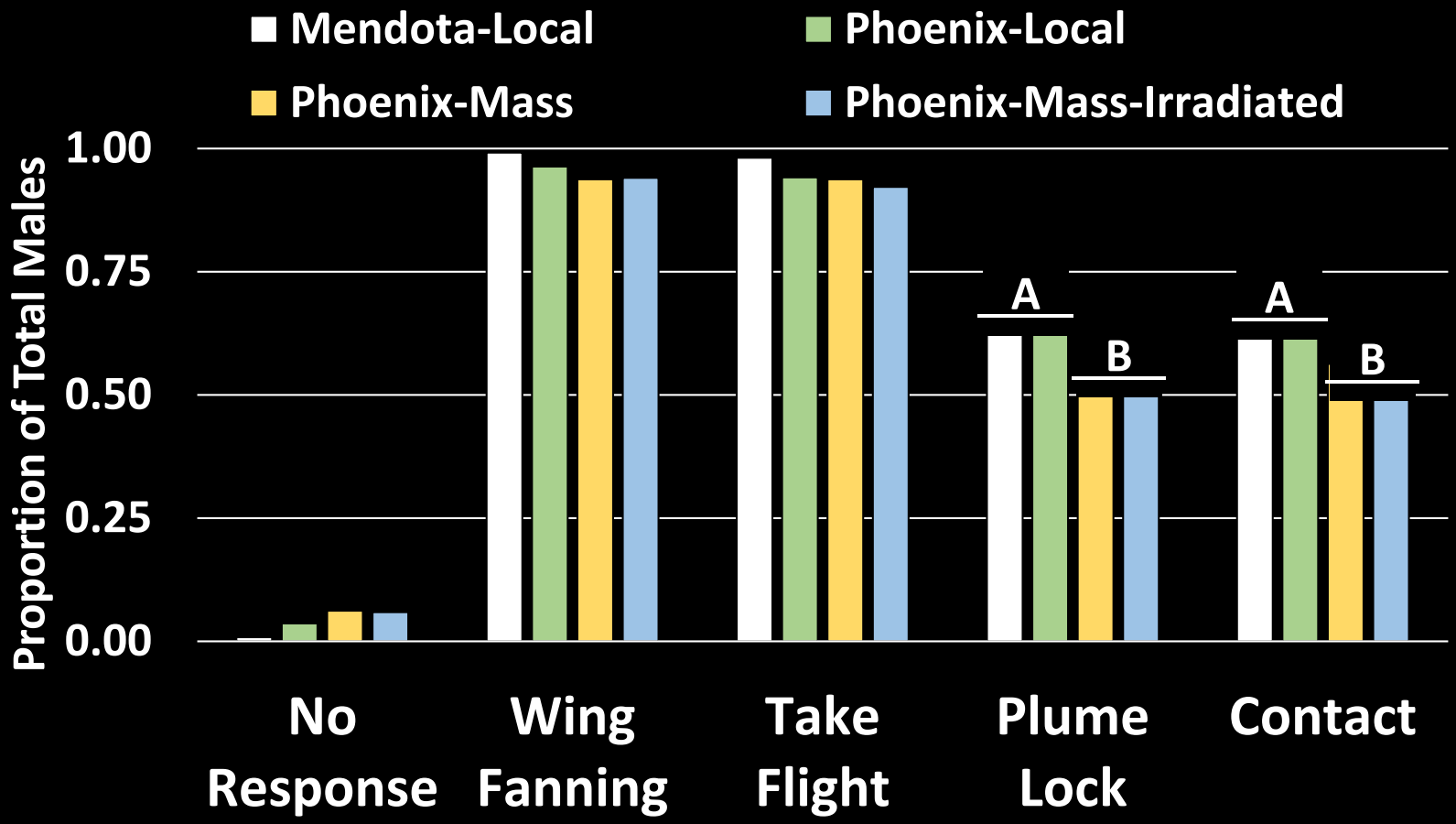


Crop Year 2019

Wind Tunnel Assay

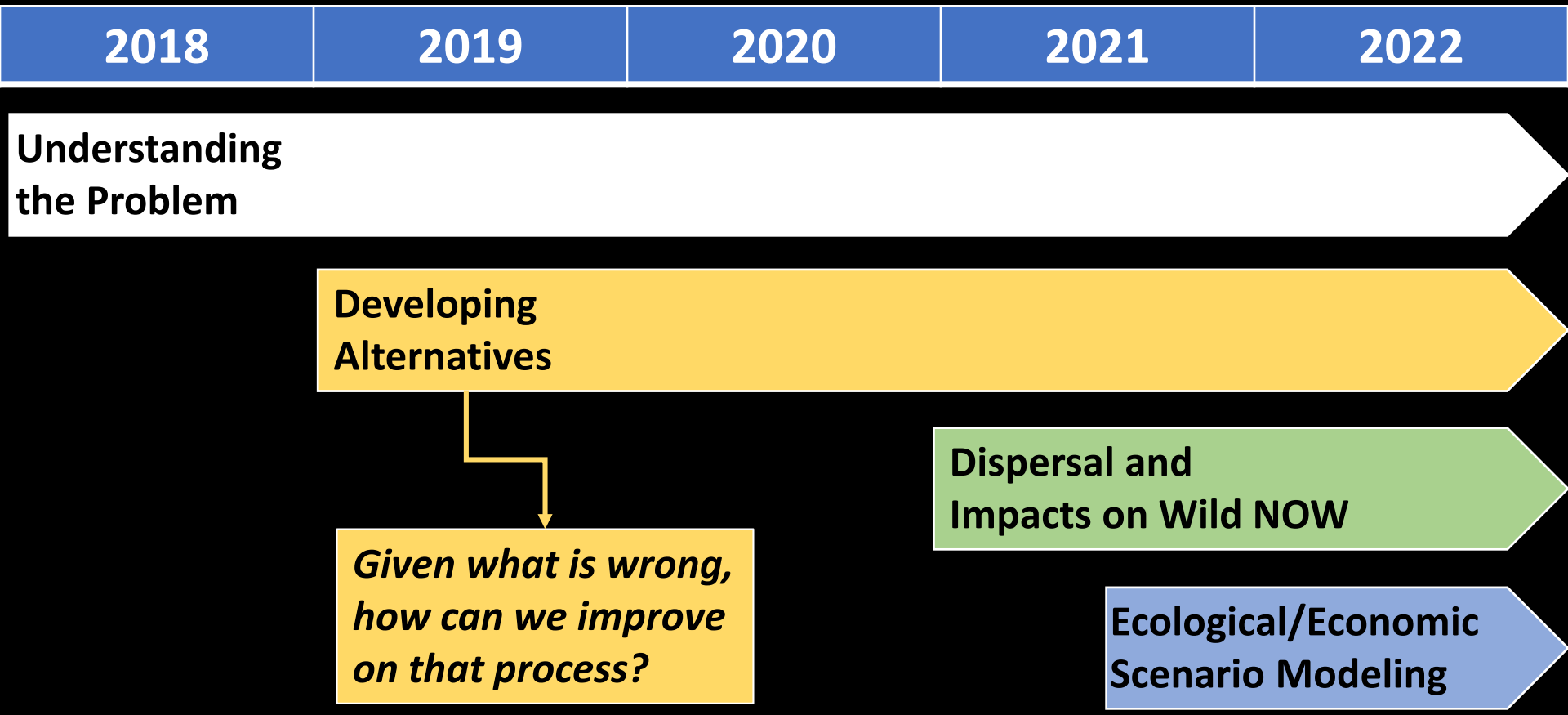
Yes - Phoenix Strain Responds to Pheromone

…but Mass-Rearing + Transport Have Negative Impacts



Sterile Insect Technique for NOW Project

Project Summary 2018-2021



Crop Year 2019

New Release System Provisions Vertical Space

Grocery Bags with Paper Tubes

Crop Year 2019

Recovery Dramatically Increased!

Crop Year 2019

Night Observations Confirm NOW Bag Activity

Crop Year 2020

Primary Focus on Transport/Release Methods

Transport

Shipped via UPS

Driven in refrigerated cooler

Vehicle: M3 Agriculture

Release

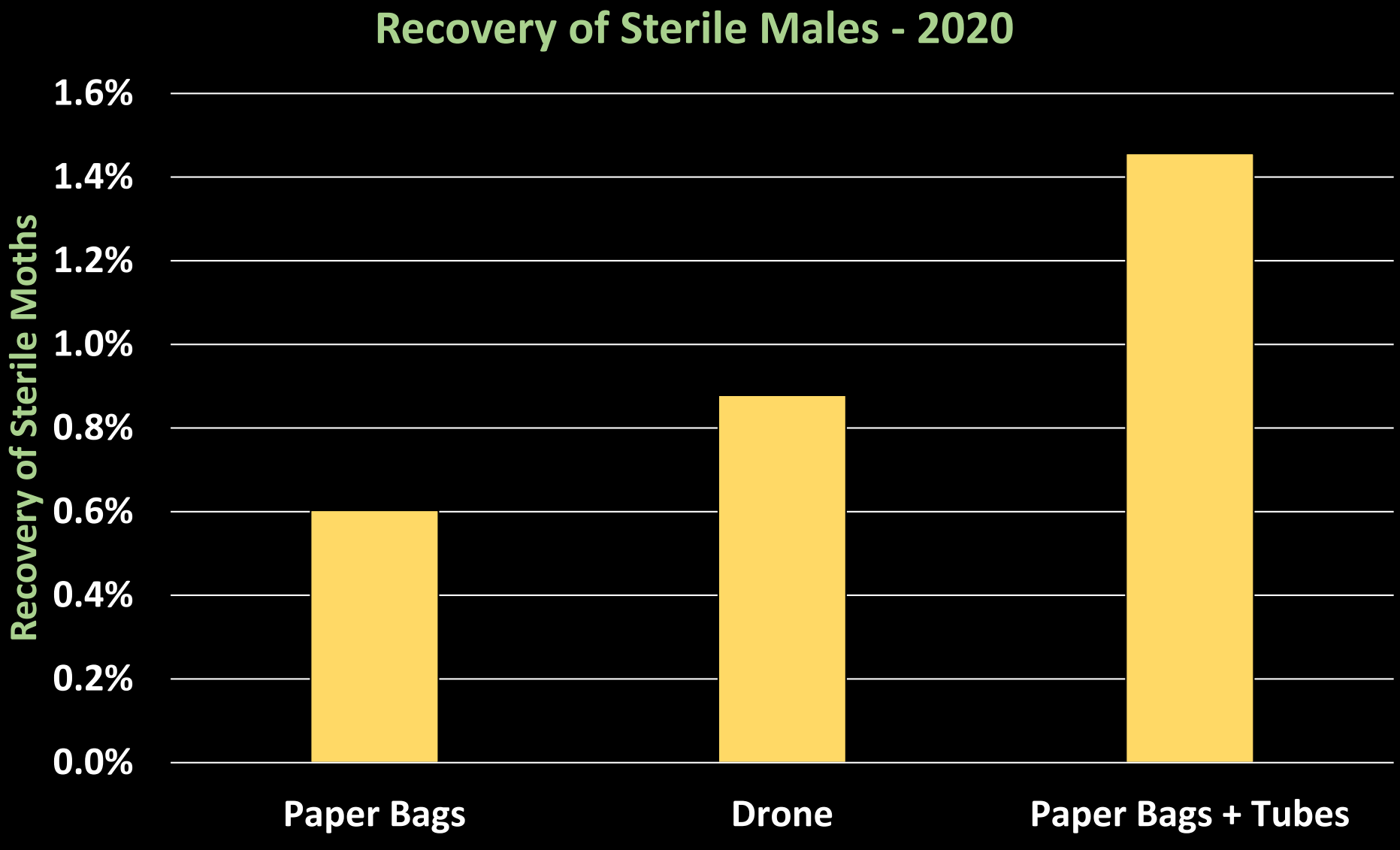
Paper Bag + Tubes

Drones

Drone: M3 Agriculture

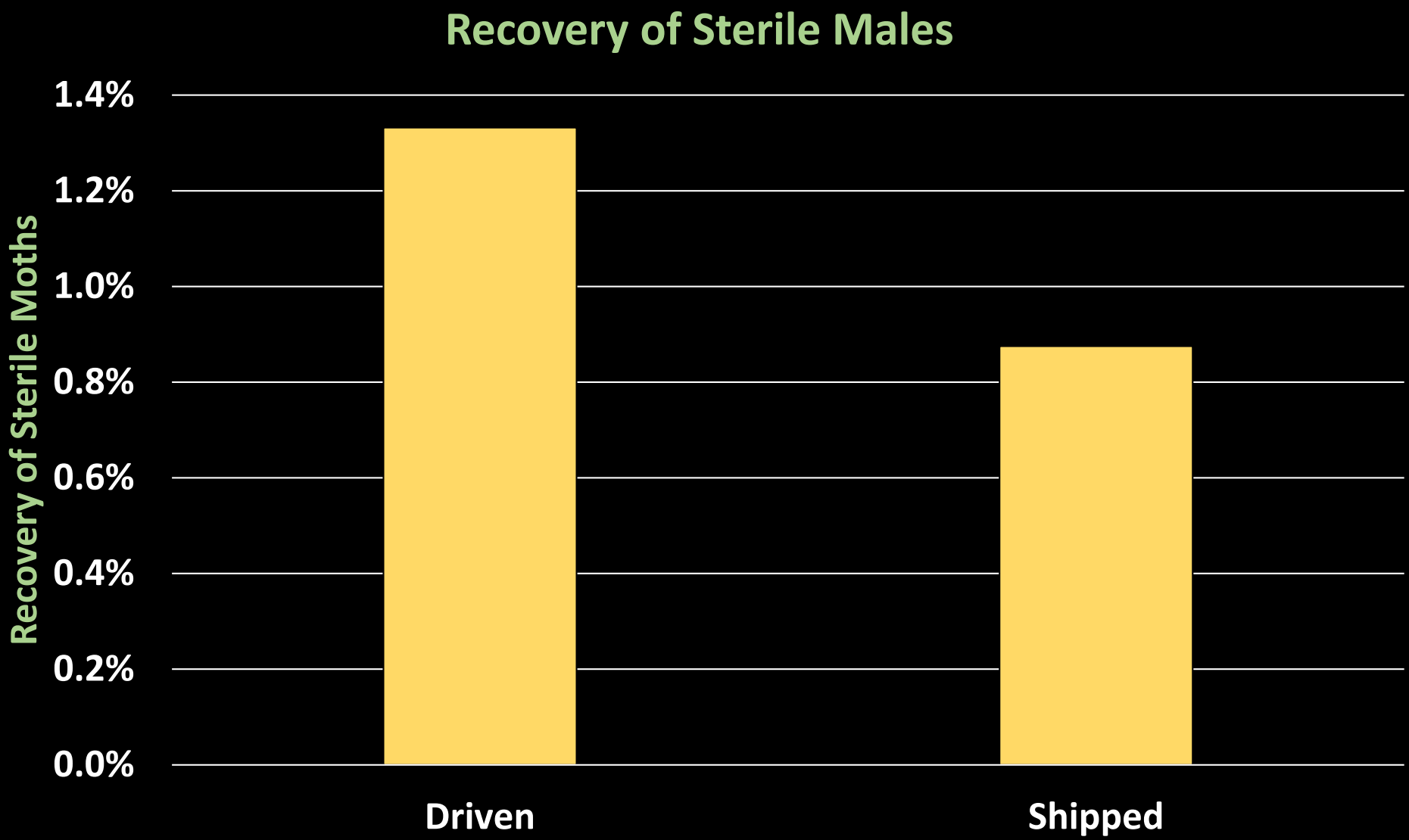
Crop Year 2020

Drone and Modified Paper Bag Both Perform Well



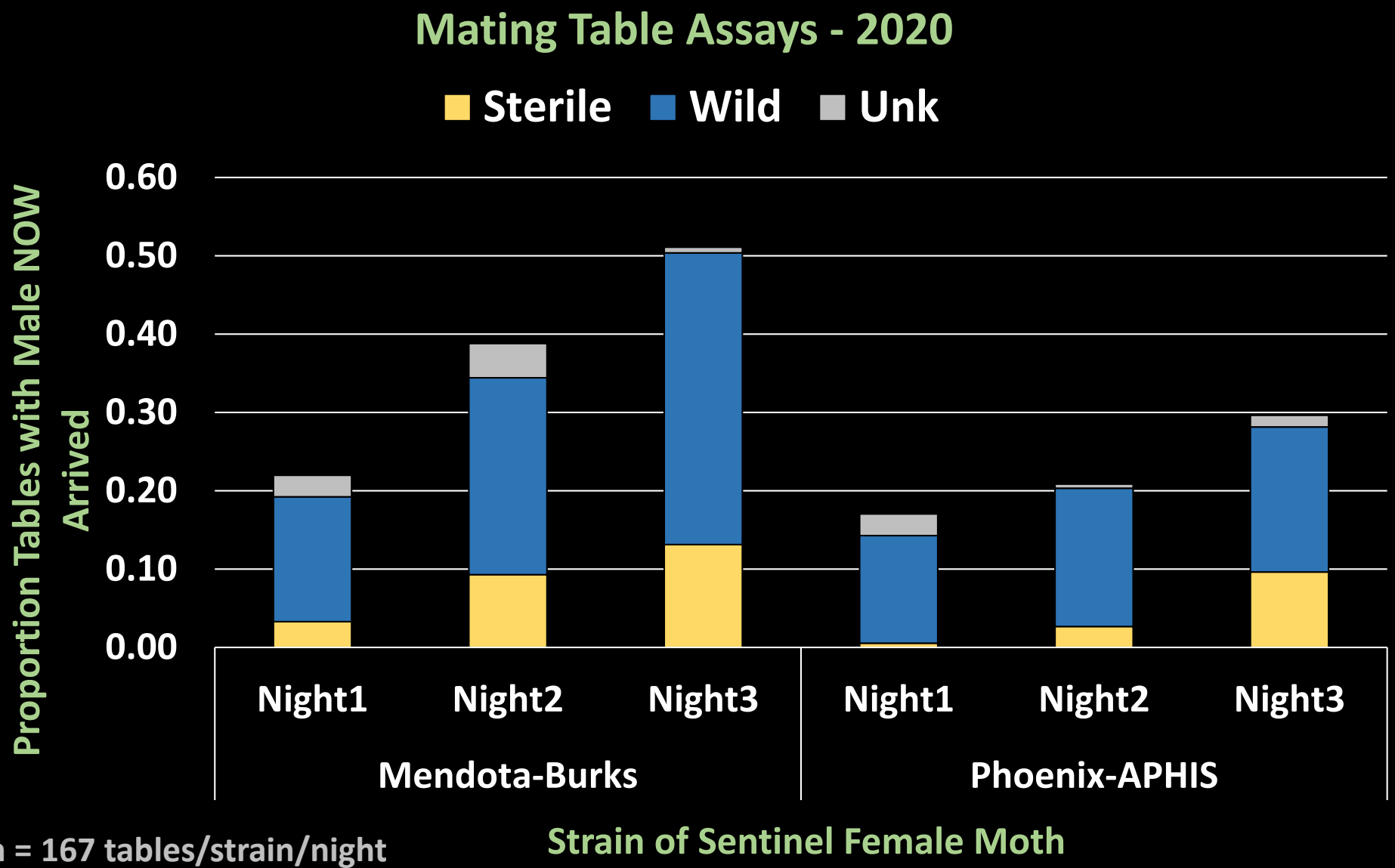
Crop Year 2020

Better Handling Can Also Improve Performance



Crop Year 2020

First Recovery of Sterile Males in Mating Tables



n = 167 tables/strain/night

RESEARCH IN 2021

Small Block Experiment

Kearney Ag. Center

Experiment Details

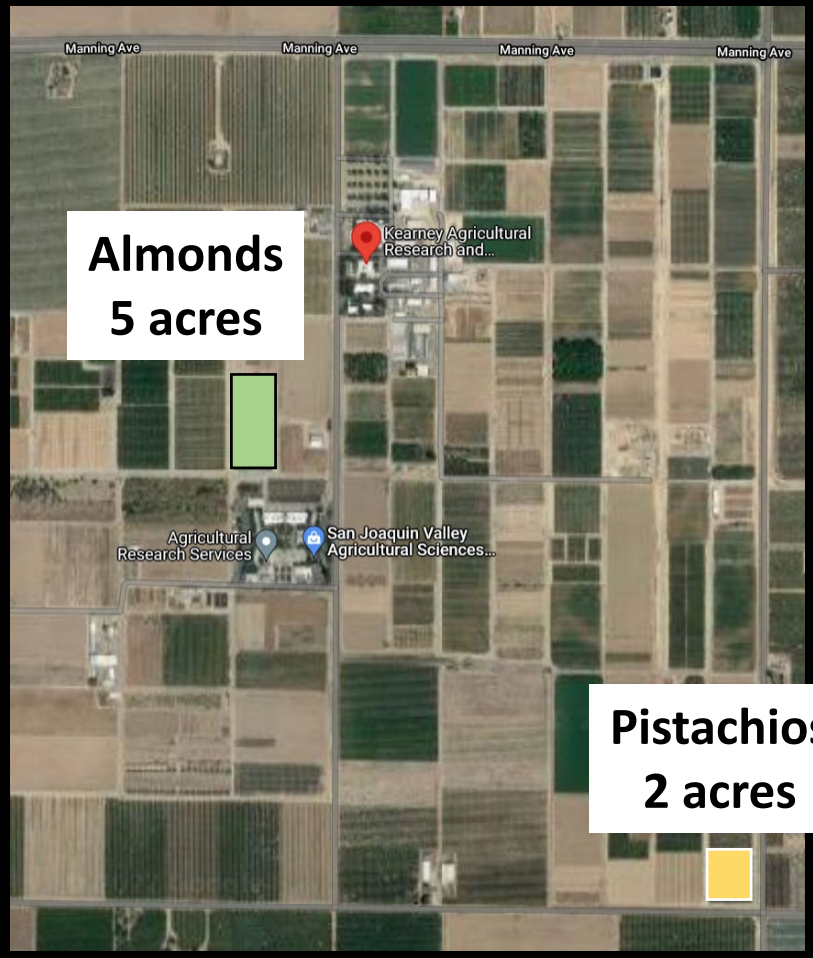
- Weekly moth releases (May 4 – Sept. 28)
- Replicate findings from 2020
- Determine baseline recovery rate

Treatment Comparisons

- Release Device: Paper Bags vs. Drone
- Rearing: Local vs. Mass-Rear

Measures

- NOW abundance
- Mating Tables



Small Block Experiment

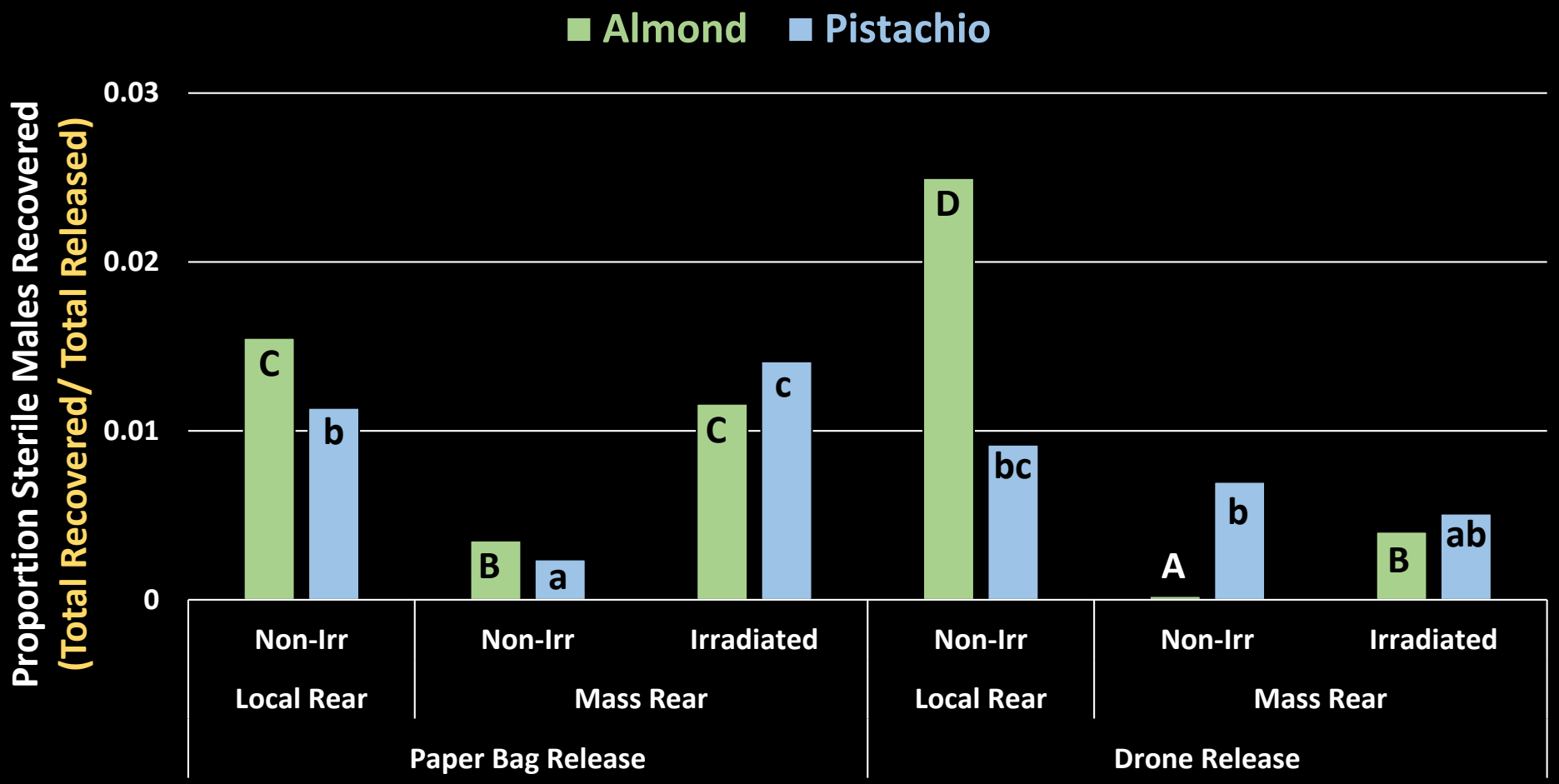
Pheromone Traps

Recovery of Sterile Males

Small Block Experiment – Pheromone Traps

Recovery of Sterile Males

Recovery of Mass-Reared Tended to be Lower



Small Block Experiment – Pheromone Traps

Recovery of Sterile Males

Key Points

Mass-reared tend to perform worse

- Local - 1.5% (range 0.9 – 2.5%)
- Mass-rear - 0.6% (range 0.0 – 1.4%)

Drone seems just as viable as paper bags

- Paper Bag – 1.4% (local moths)
- Drone – 1.7% (local moths)

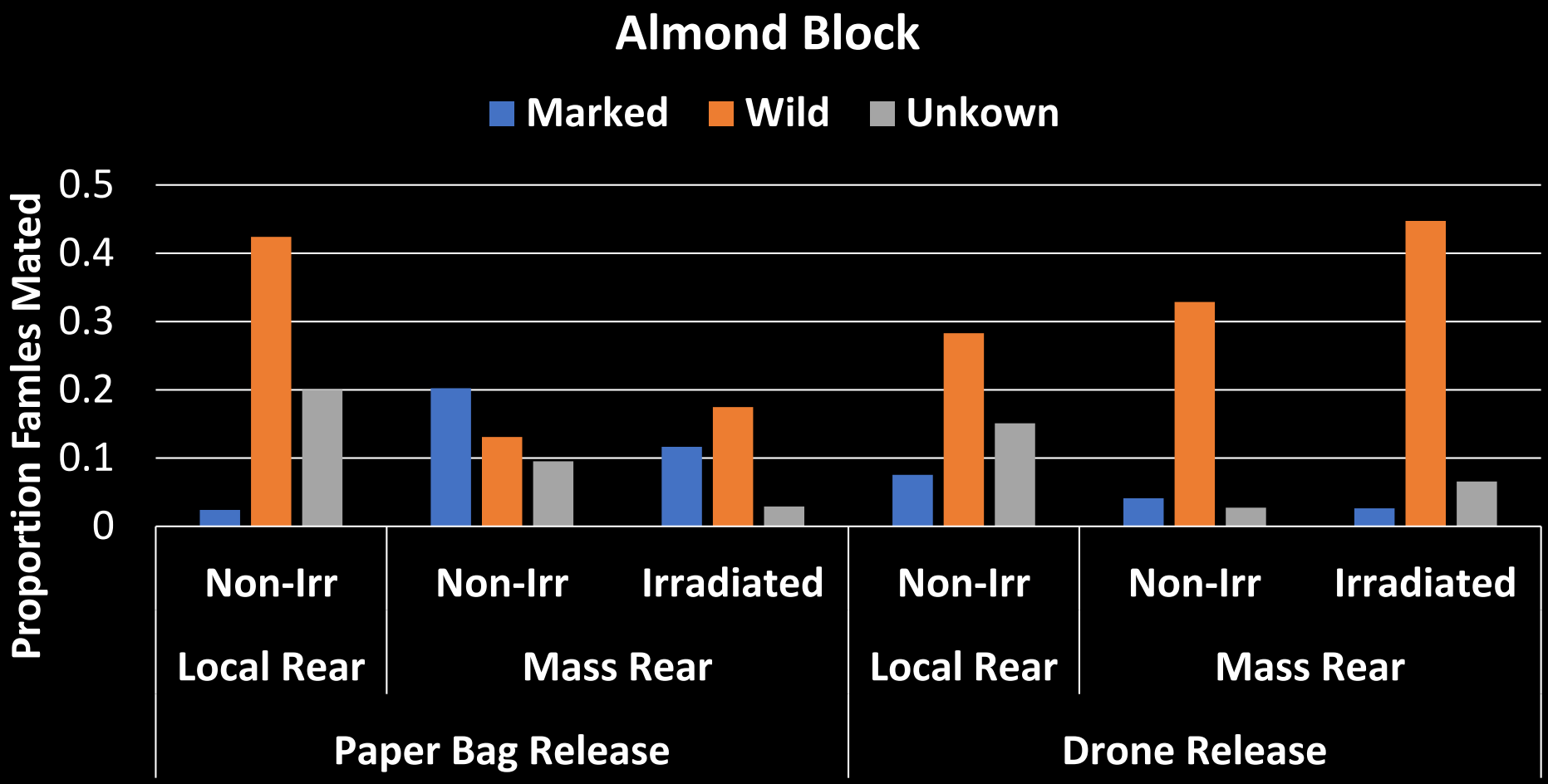
Small Block Experiment

Mating Tables

Small Block Experiment - Mating Tables

Sterile Males Finding Sentinel Females

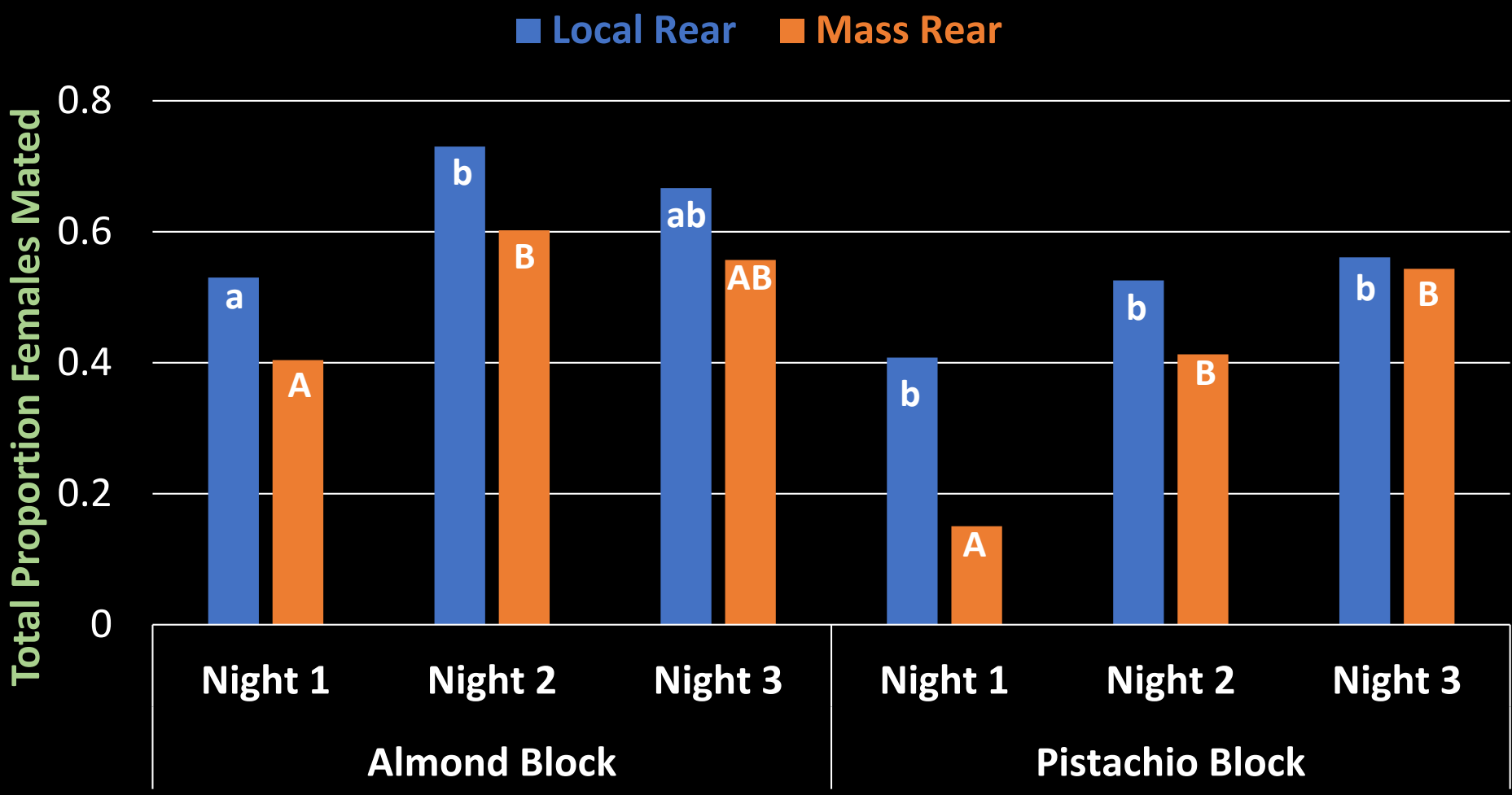
Too Many Spermatophores of Unknown Origin



Small Block Experiment - Mating Tables

Sterile Female Mating Success

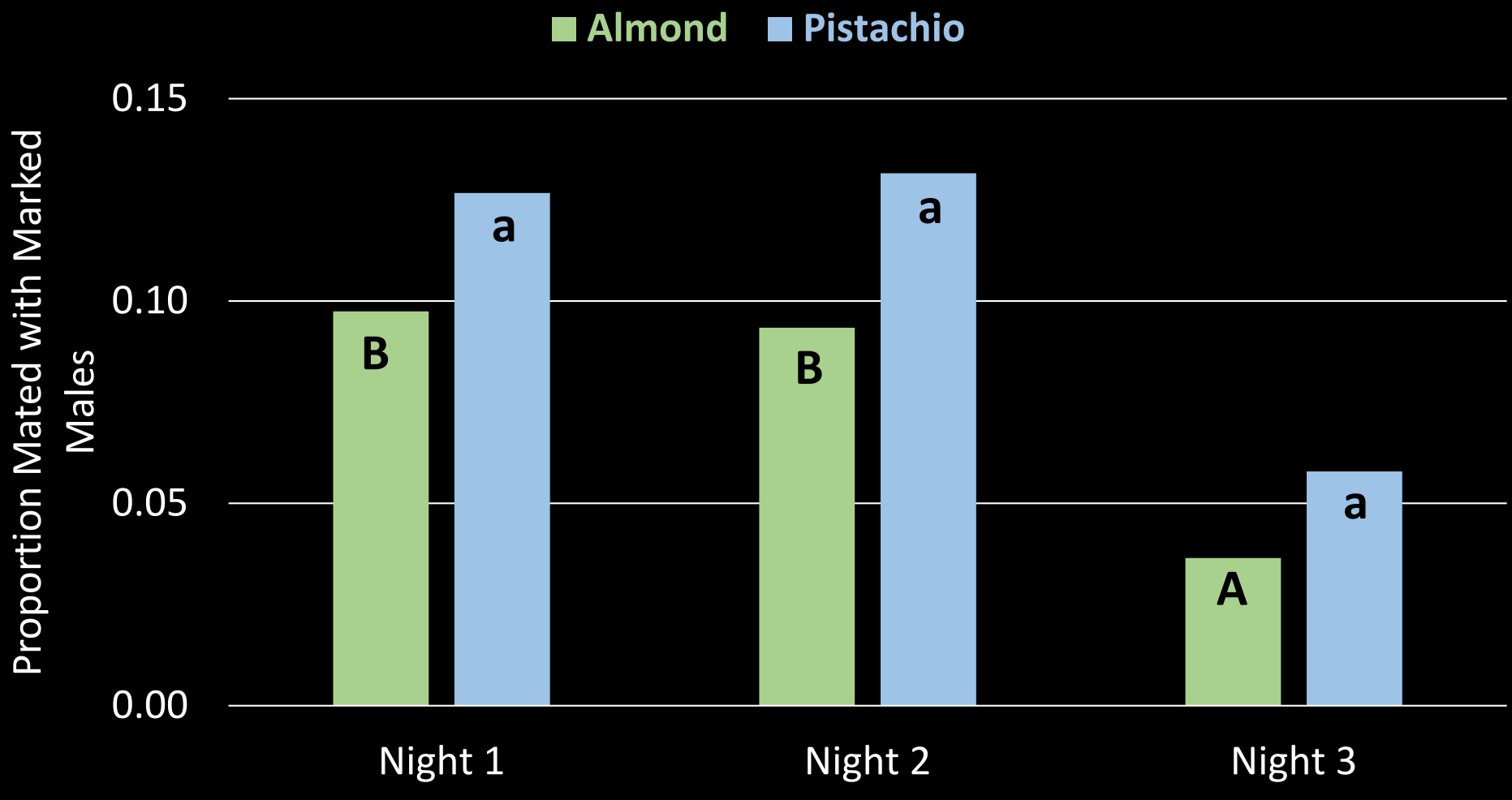
Some Mass-Rear Photoperiod Adjustment



Small Block Experiment - Mating Tables

Sterile Male Mating Success

Sterile Males Consistently Find Females



Small Block Experiment - Mating Tables

Sterile Moth Performance

Key Points

Sterile females consistently attract wild males

- Photoperiod adjustment sometimes delays activity

Sterile males consistently locate/mate with sentinel females

- Activity declines after first couple nights (dispersal?)

Too many "unknown" spermatophores

- Despite our best efforts, many of the males got away
- "Unknowns" severely cloud the data

Small Block Experiment - Mating Tables

Sterile Moth Performance

Developing Markers for Spermatophores

Multiple approaches in 2022

- Dyes, DNA markers, isotopes in diet etc.

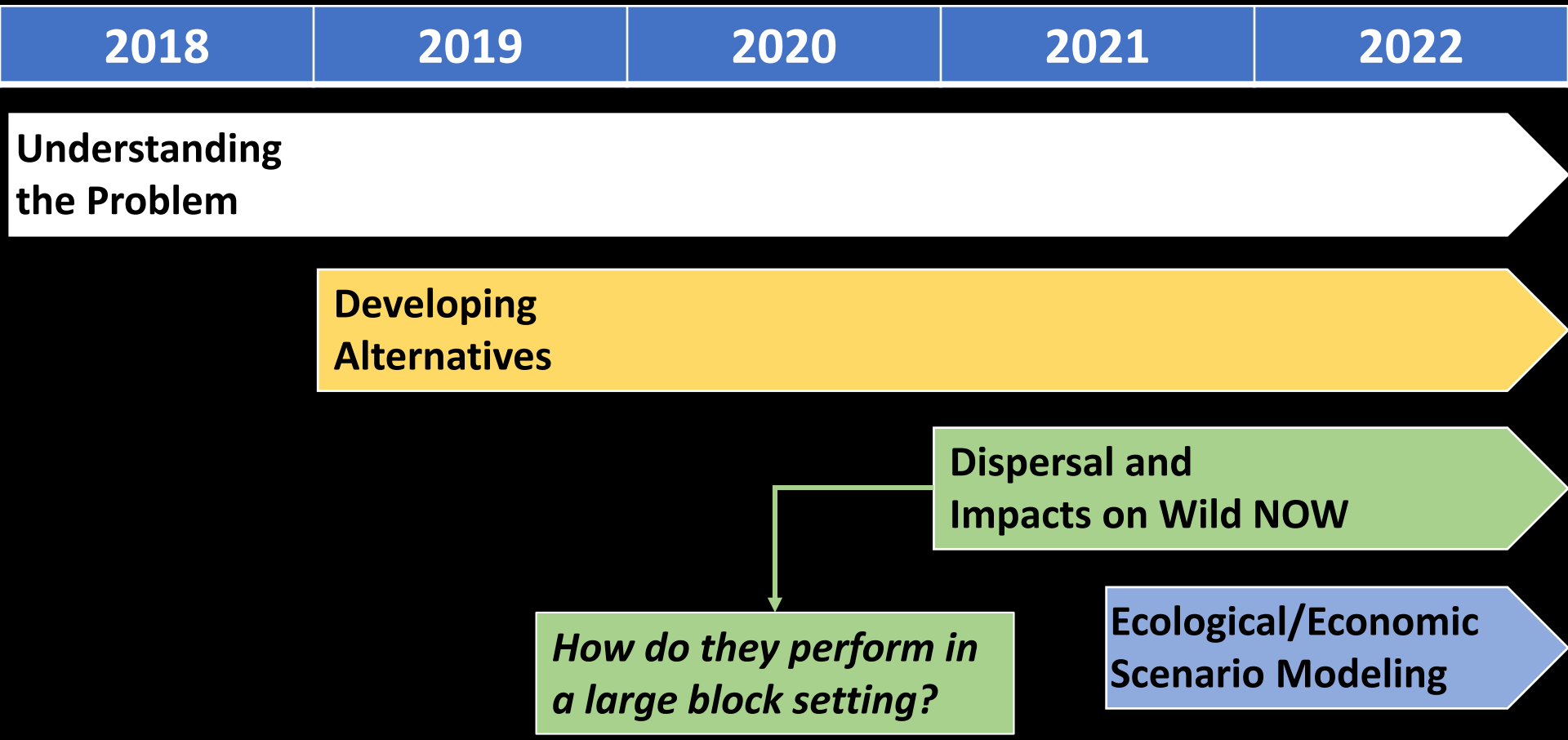
- Improves the accuracy (as well as logistics) of on-going mating table studies

Dyed spermatophore glows under UV light

Photo: Nicole Culbert (UCR)

Sterile Insect Technique for NOW Project

Project Summary 2018-2021



Large Block Experiment

Recovery of Sterile Males

Control Plots (no SIT)

SIT Plots – weekly release (Jun. 16 – Oct. 1)

Block Details

- Almonds >8 years old
- Paired 40 acre plots
- NonPareil + Pollenizers

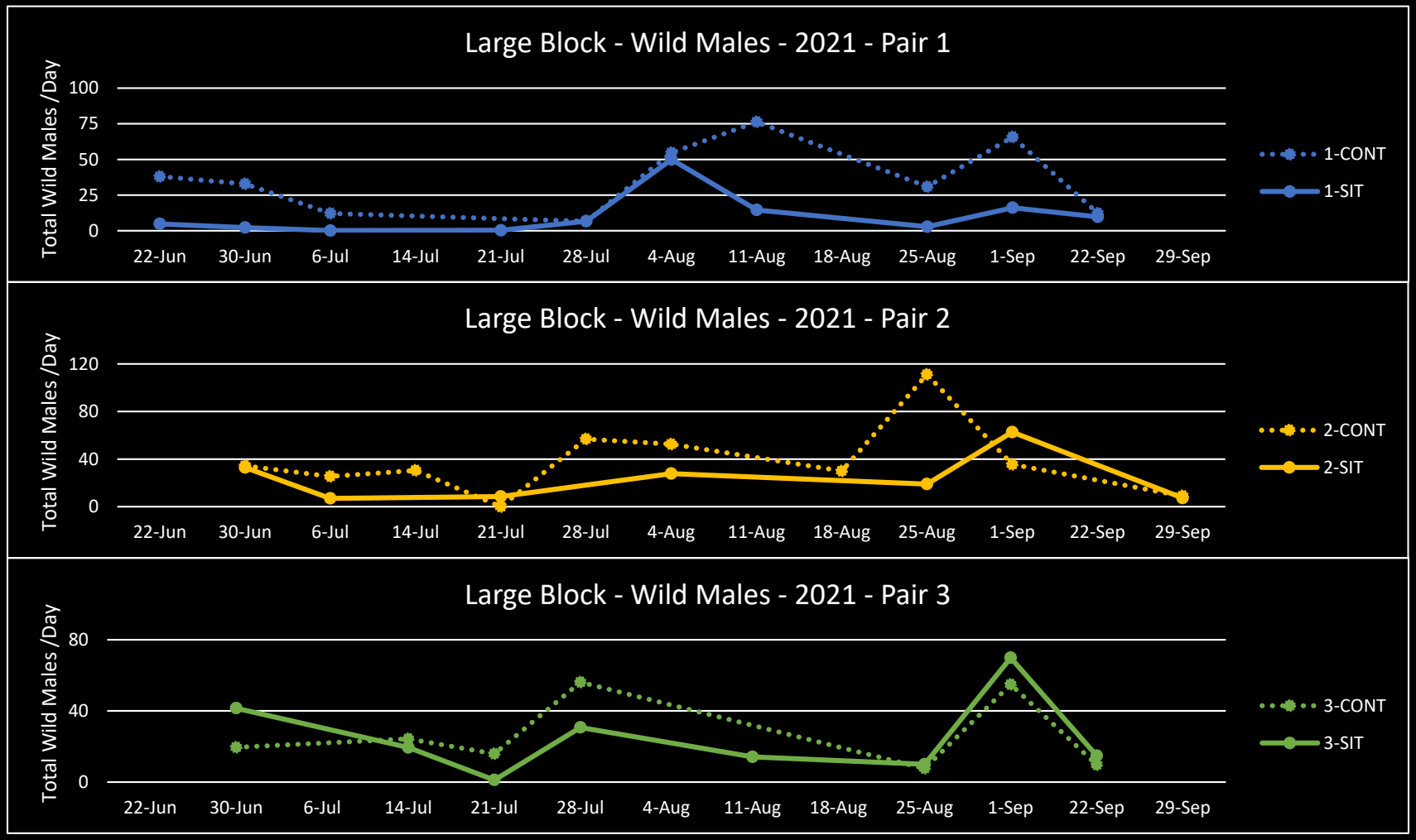
Measures

- NOW abundance
- Egg deposition
- Crop damage

Large Block Experiment

Wild Male Activity

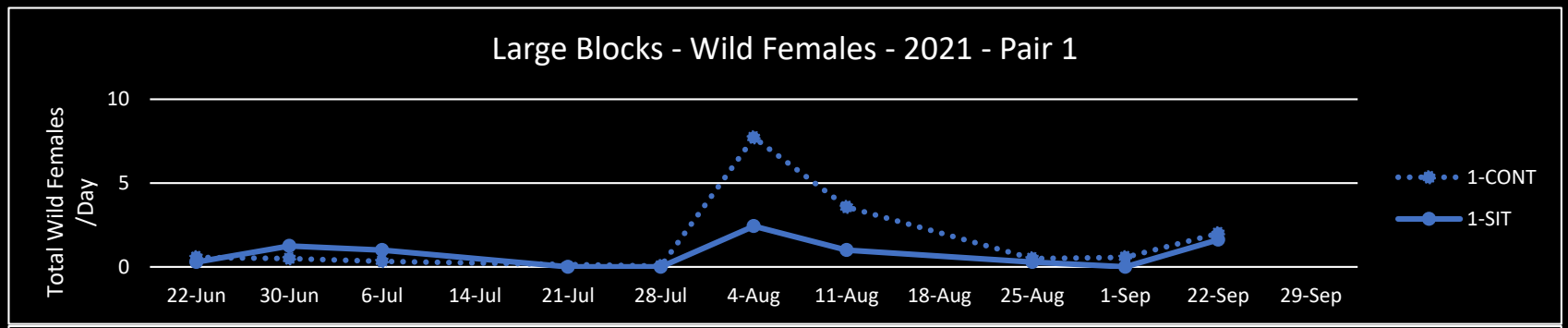
Reduced Wild Male Activity in the SIT Blocks

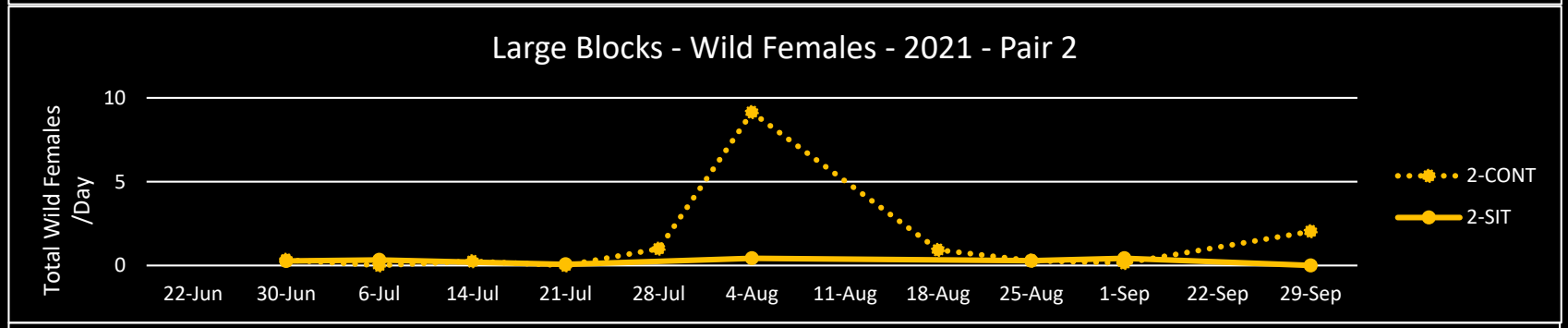


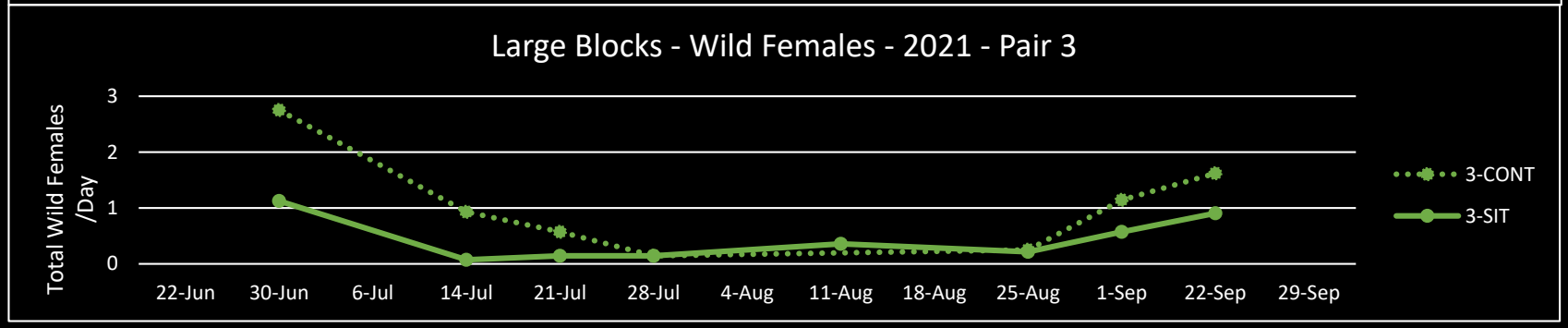
Large Block Experiment

Wild Female Activity

Reduced in the SIT Blocks



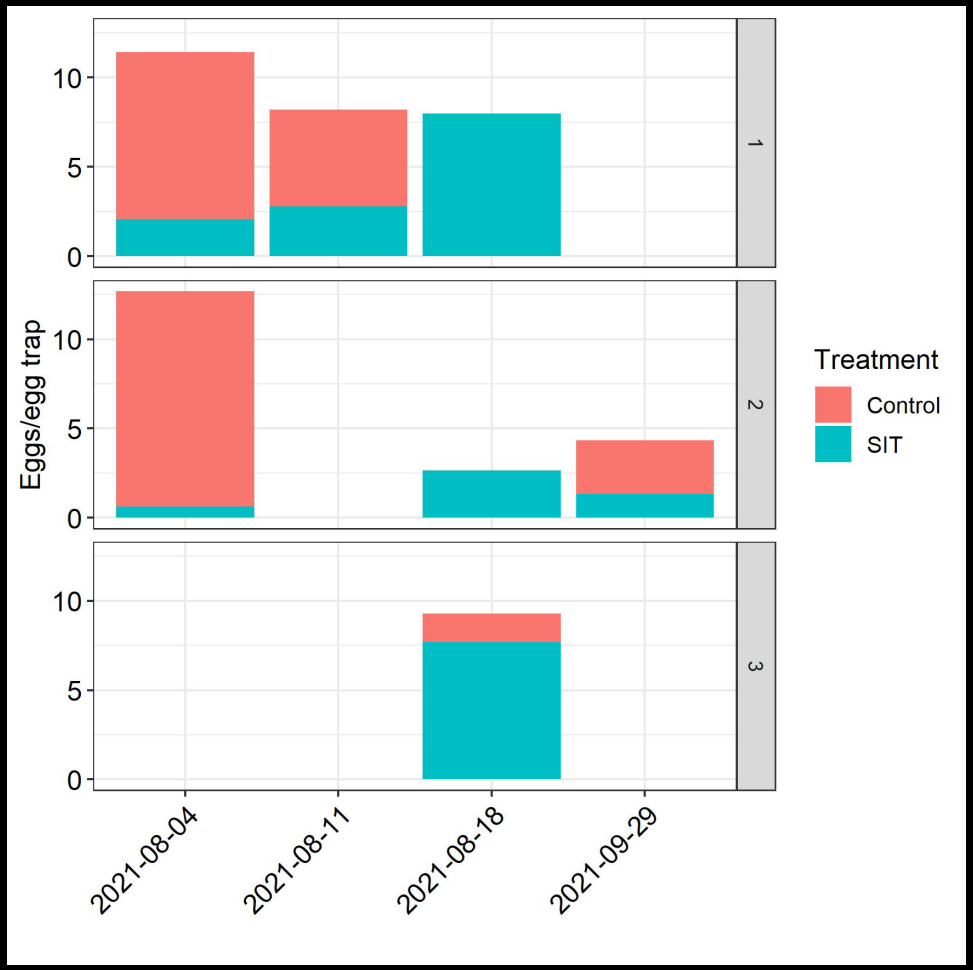




Large Block Experiment

Egg Deposition

Trend is More Eggs in Control Blocks



Egg Traps

Key Points

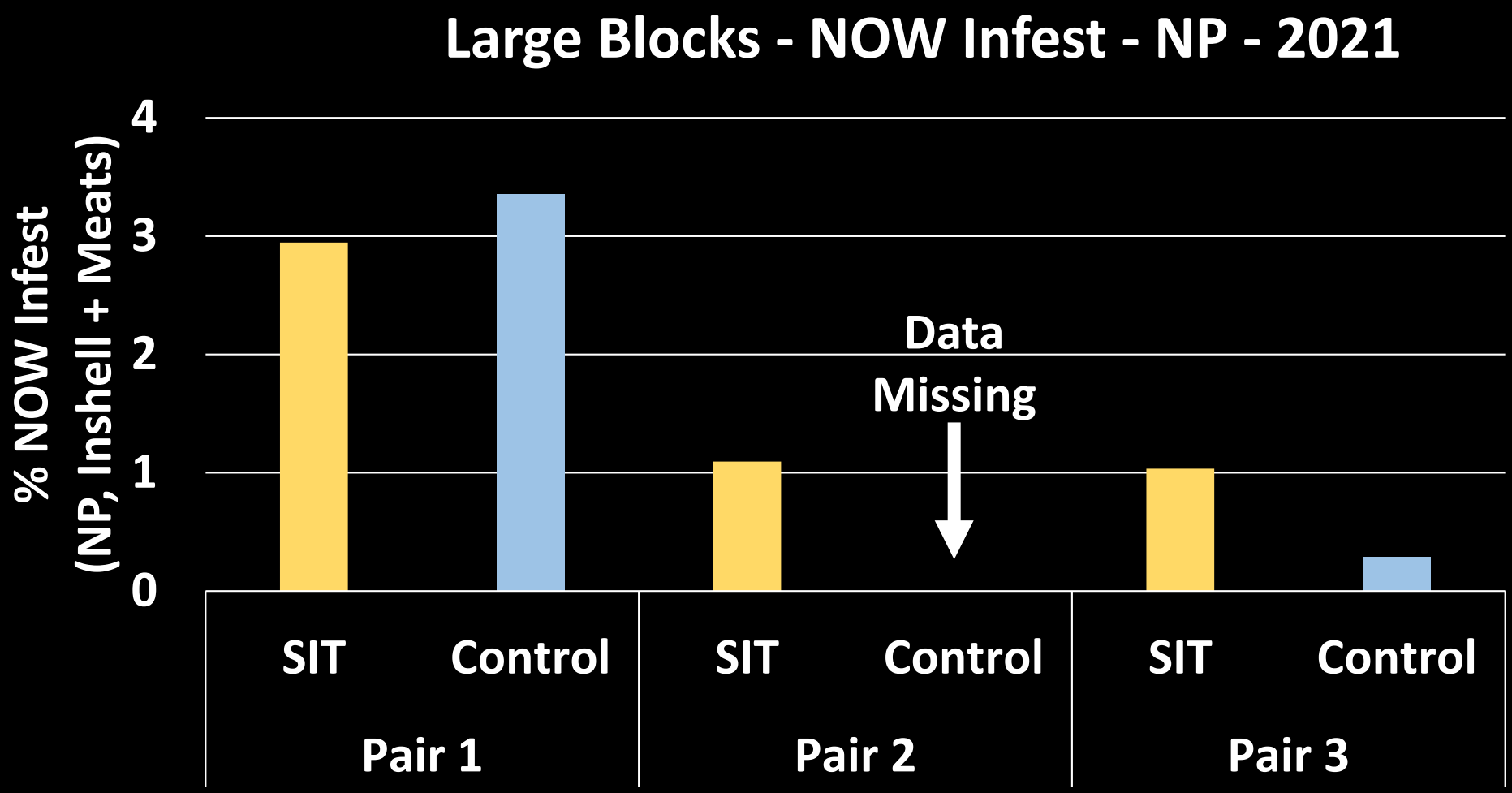
- Traps for weeks with eggs and replicate blocks with a similar number of traps recovered from treatment and control plots
- For all weeks by Aug 18, traps in replicate blocks 1 and 2 showed a consistent pattern of more eggs in the control plots than in the SIT plots
- Pattern was reversed in the week of Aug 18, the only week in which egg traps were recovered from plot 3

Large Block Experiment

Processor Gradesheets

Crop Damage

Mixed Effects, No Clear Differences



Large Block Experiment

Processor Gradesheets

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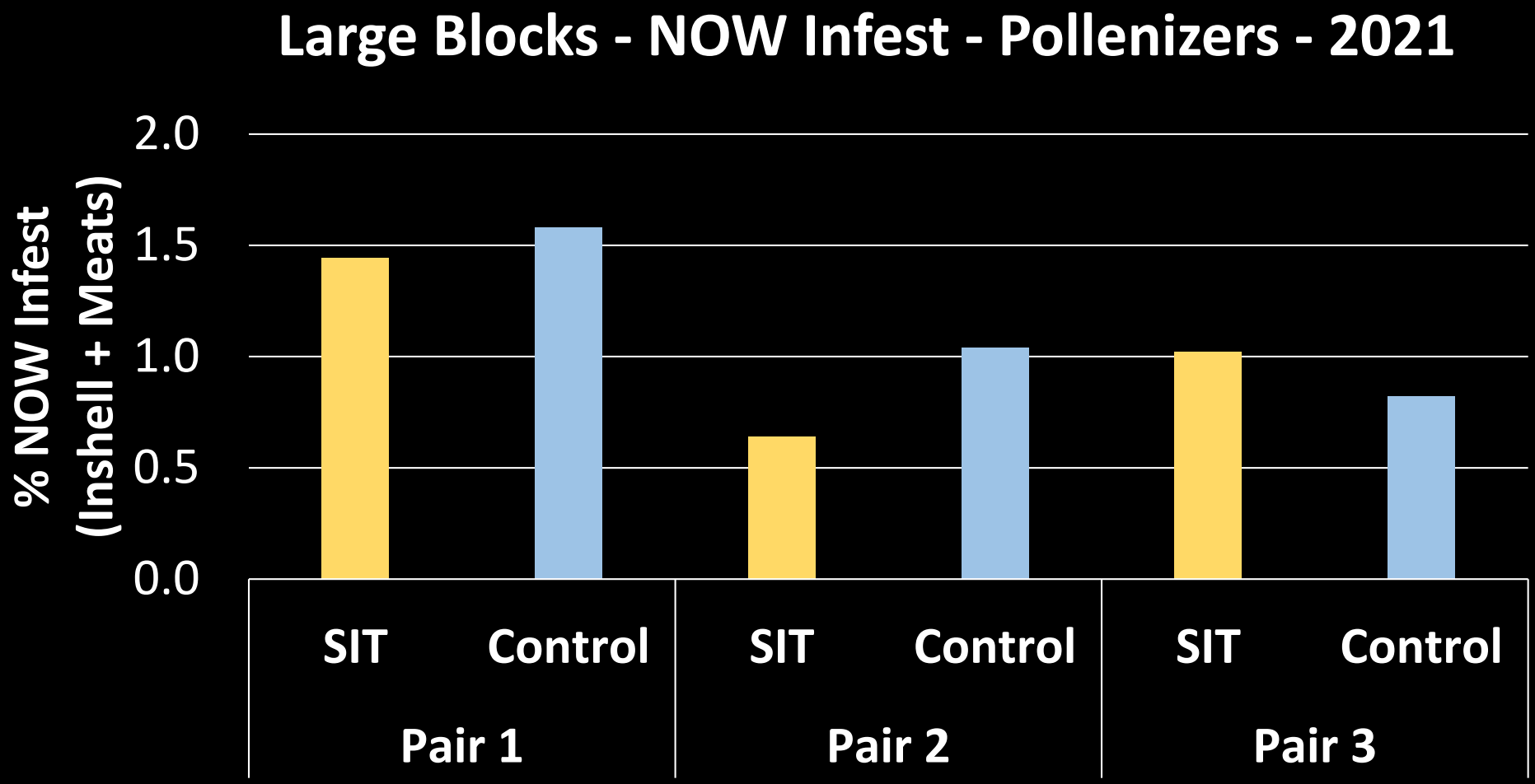


Large Block Experiment
Effects on Wild NOW Populations
Key Points

Recovery of sterile male/females was limited

- Mass-reared moths, released from an airplane…
- Data not shown

Activity of wild male/female seemed to decline in SIT blocks

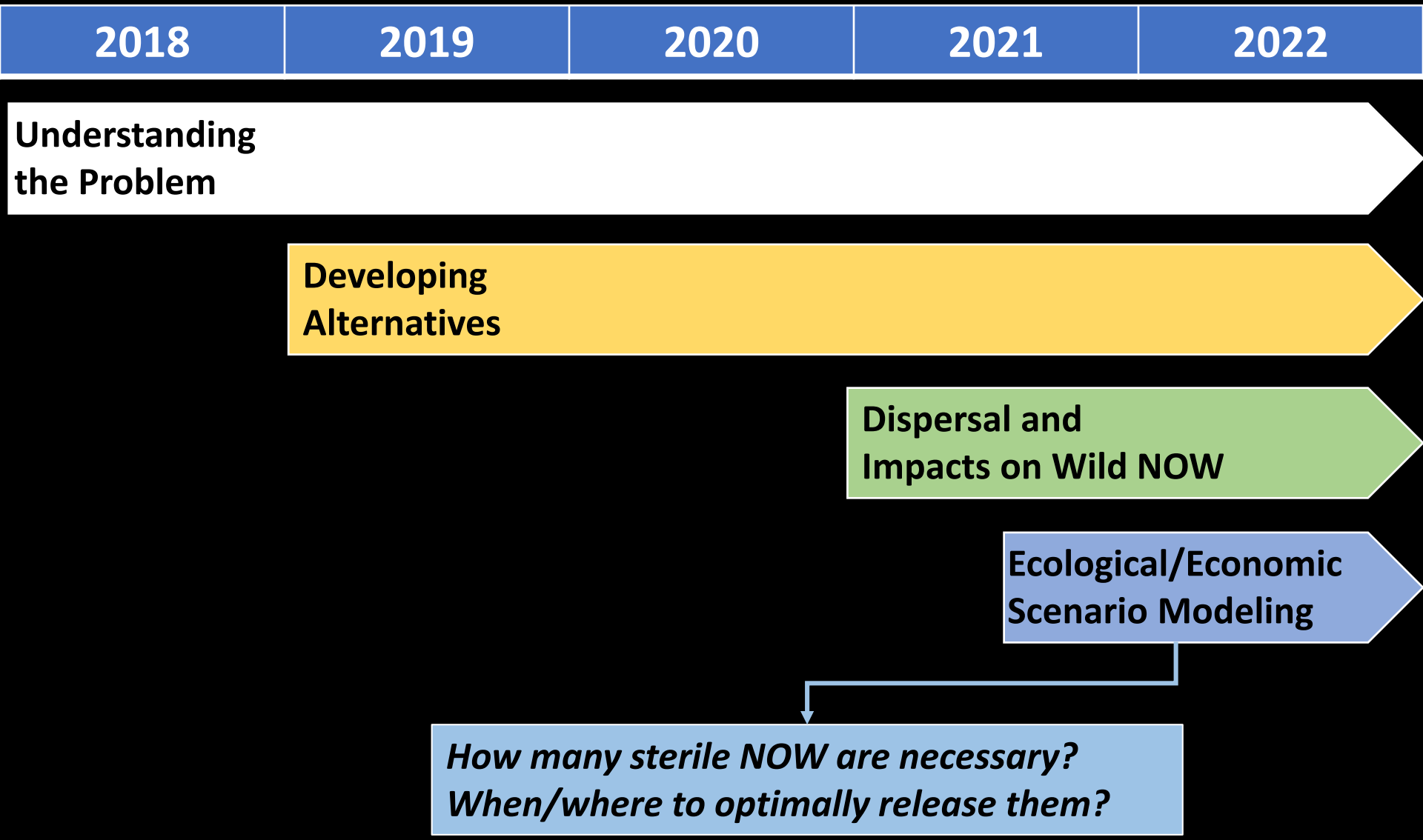
- Lots of variability and logistical constraints
- Need more replication

Effects on crop damage are mixed

- NP data incomplete
- Gradesheets vs. field sampling

Sterile Insect Technique for NOW Project

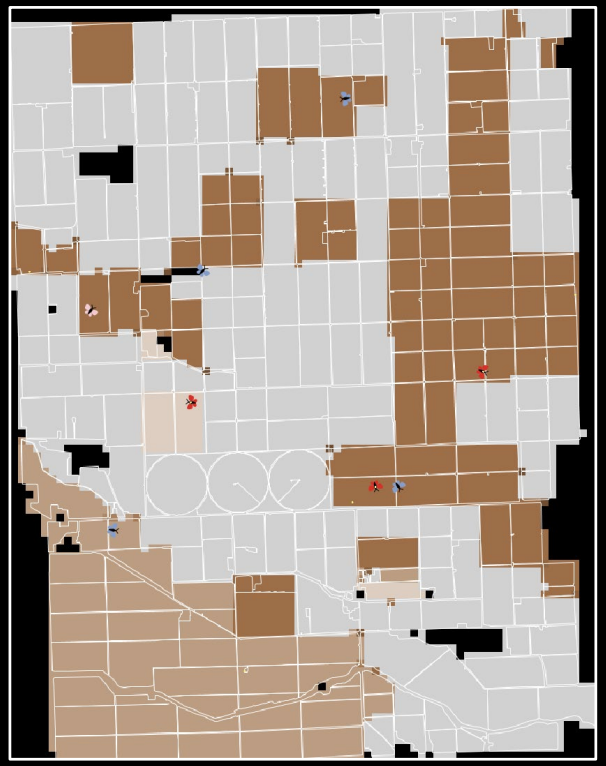
Project Summary 2018-2021

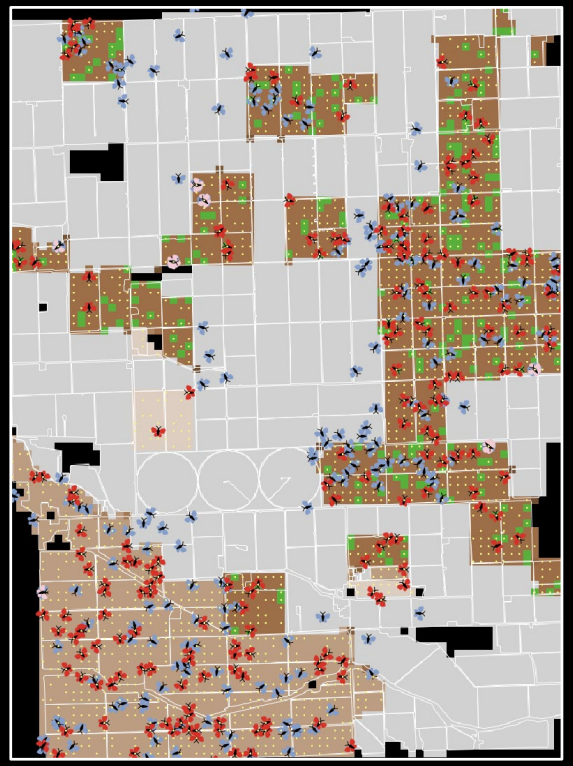


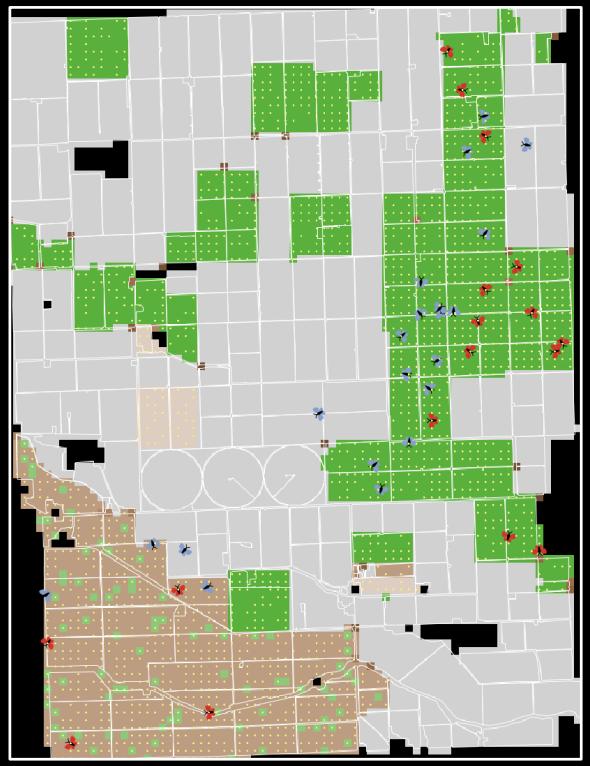
Ecological/Economic Scenario Modeling

When/Where to Release Sterile Moths?

Developing an NOW Population Model



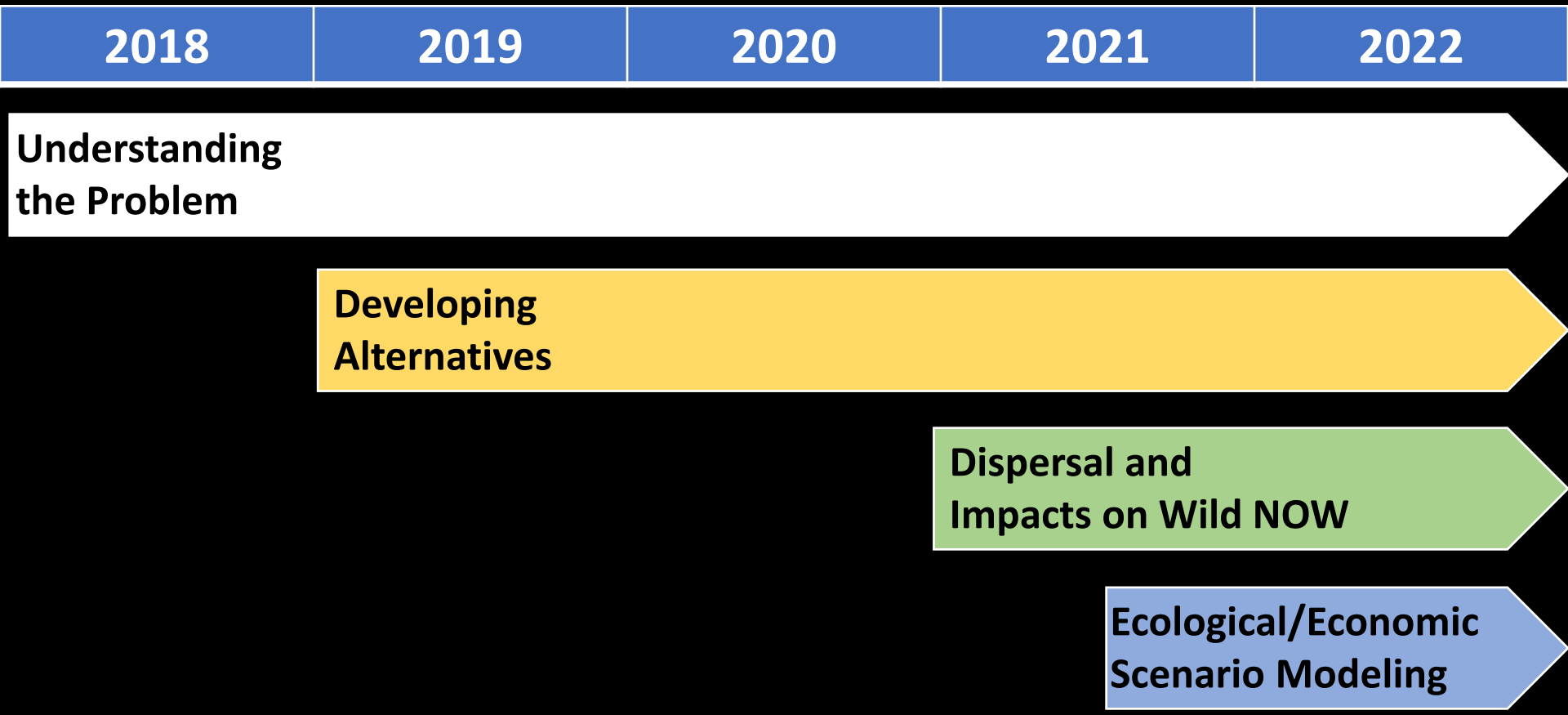




Collaboration with Dr. Ran Wei (UCR), Dr. Yujia Zhang (UCR) and Dr. Brittney Goodrich (UCD)

Sterile Insect Technique for NOW Project

Project Summary 2018-2021



Sterile Insect Technique for NOW Project

Project Summary 2018-2021

2018	2019	2020	2021	2022

Understanding the Problem

Developing Alternatives

Dispersal and Impacts on Wild NOW

Ecological/Economic Scenario Modeling

Pros

- *Strain is fine*
- *Dye uptake is good*
- *Males fly/respond to pheromone*
- *Sterile females attract wild males*
- *Sterile males can locate/mate with sentinel females*

Sterile Insect Technique for NOW Project

Project Summary 2018-2021

2018	2019	2020	2021	2022

Understanding the Problem

Developing Alternatives

Dispersal and Impacts on Wild NOW

Ecological/Economic Scenario Modeling

Cons

- *Collection process damages moths*
- *Still less-than-optimal performance overall*
- *Holding temperatures likely too cold*
- *Poor transportation conditions*
- *Poor release device (airplane)*
- *Female photoperiod issue*

Sterile Insect Technique for NOW Project

Project Summary 2018-2021

2018	2019	2020	2021	2022

Understanding the Problem

Developing Alternatives

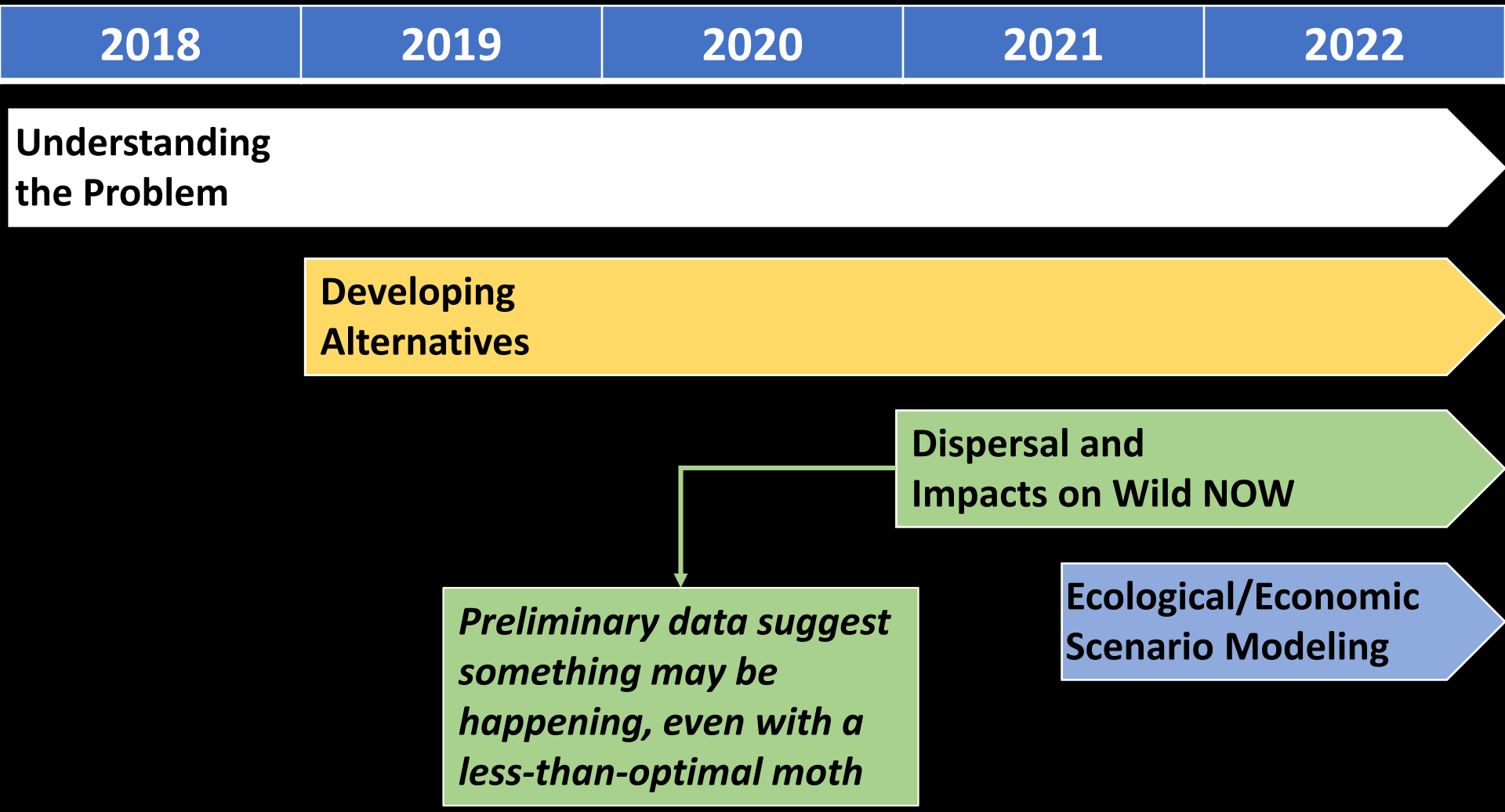
- *Paper bag system*
- *Drone release*
- *Modified transportation processes*
- *New markers to track sterile male spermatophore*

Dispersal and Impacts on Wild NOW

Ecological/Economic Scenario Modeling

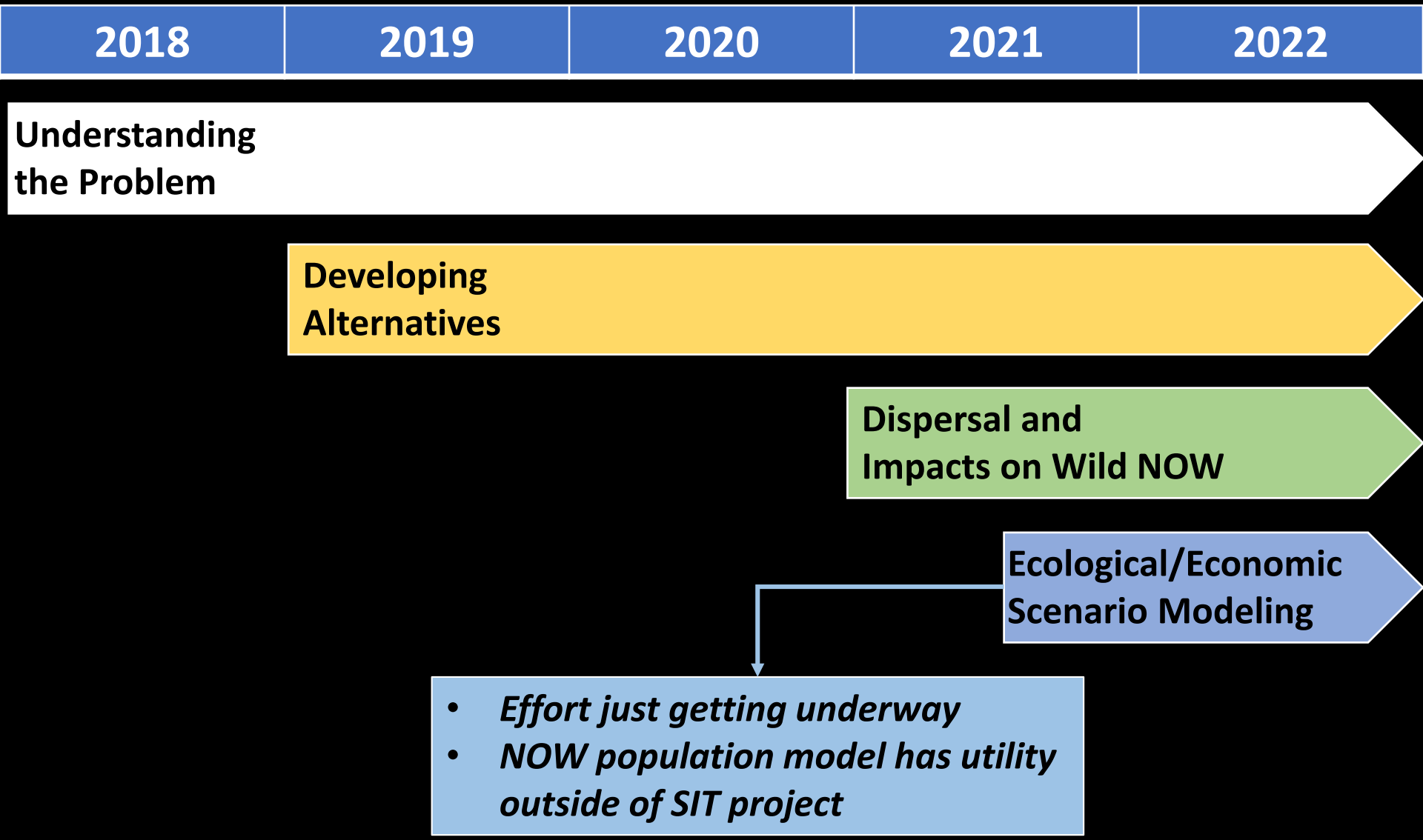
Sterile Insect Technique for NOW Project

Project Summary 2018-2021



Sterile Insect Technique for NOW Project

Project Summary 2018-2021



Sterile Insect Technique for NOW Project

SIT as part of Area-Wide IPM for NOW

How Do We Get There? / What Does This Look Like?

Understanding the Problem

Developing Alternatives

Dispersal and Impacts on Wild NOW

Ecological/Economic Scenario Modeling

Sterile Insect Technique for NOW Project

SIT as part of Area-Wide IPM for NOW

How Do We Get There? / What Does This Look Like?

- **Biological/ecological data will help inform what is possible**
- **Social/economic dimensions of SIT / area-wide IPM need to be better understood**

Alternatives

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SIT as part of Area-Wide IPM for NOW

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- Making progress on a competitive sterile moth now…
- …while also developing a road map for implementation at scale

Ecological/Economic Scenario Modeling

Sterile Insect Technique for NOW Project

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Complexity = various routes to SIT success

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Sterile Insect Technique for NOW Project

SIT as part of Area-Wide IPM for NOW
How Do We Get There? / What Does This Look Like?

- Biological/ecological data will help inform what is possible

Complexity = various routes to SIT success

Regardless of outcome, these efforts generate a lot of new knowledge on NOW

scale

Thank You!

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