Improving and Verifying the Quality of Mass-reared Navel Orangeworm for Sterile Insect Technique

Project No.: **ENTO24A.Wilson/Burks**

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A. Summary *(In laymen's terms – emphasize key findings and recommendations)*

Over the past 2 years quality and performance of irradiated/sterile navel orangeworm (NOW) from the USDA-APHIS Phoenix Mass-Rearing Facility was evaluated on its own and at times relative to two locally-produced strains of NOW, one collected from Mendota in 2010 and the other from the UC Westside Research and Extension Center in 2019. In 2019 experiments also included a locally-produced strain of the moth strain being mass-reared at the Phoeix Facility.

Performance of the mass-reared and irradiated Phoenix strain moths has been mixed overall, with generally very low recovery of males and females in various trap types. Experiments have been conducted to isolate the key variable(s) at play, including radiation levels, moth strain, shipping/handling conditions and release mechanism. Ultimately moth strain and radiation levels appear to have less influence on moth performance relative to shipping/handling conditions and release method. Changes to the release mechanism used in field trials has led to increased recovery of both male and female moths, albeit still inconsistent at times. Mating table assays have demonstrated that irradiated Phoenix-strain females do appear to effectively call and mate with wild moths, while wind tunnel assays have shown that Phoenix-strain males do indeed respond to point-source pheromone. Flight mill studies have demonstrated that moths produced in the Phoenix facility tend to have a poorer dispersal capacity after overnight shipping to California, whereas locally-produced, Phoenix-strain moths appear to fly equivalent with other laboratory strains. Results to date suggest that stress associated with the collection and shipping process is likely the most important factor limiting performance of Phoenix strain navel orangeworm when released following shipment. As such, research efforts in 2020 will focus on the effects of moth handling, shipping and release in order to further improve field performance and competitive abilities of these sterile NOW.

B. Objectives *(300 words max.)*

1. Specify the goal(s) and specific objectives of the proposal – if a collaborative effort, identify who is the lead for each objective

Goal: Develop a mass-rearing, irradiation, shipping and release process that can deliver field competitive sterile NOW for use in an area-wide SIT program.

Objective 1: Identify and modify factors contributing to the poor performance of mass-reared irradiated NOW

2. Identify annual outputs or milestones for each of the objectives

- Identify key factors limiting the performance of mass-reared, irradiated NOW shipped out of the Phoenix Facility
- Adjust mass-rearing and other production/shipping/release processes to improve field competition of sterile NOW
- Recover sterile NOW following release in field settings
- Document sterile NOW males/females mating with wild males/females

C. Annual Results and Discussion *(This is the core function of this report)*

1. Describe activities and outputs for each objective

Research in 2019

Poor recovery of Phoenix-strain males in 2018 could have been due to a variety of variables associated with production, shipping/handling, and/or release conditions. Where to begin? Key factors that were chosen for evaluation in 2019 included irradiation level, rearing/shipping conditions, and moth strain. Furthermore, another key question to address was the expected rate of recovery. While low levels of recovery <0.01% are clearly unacceptable, the expected rate of NOW recovery remained unclear and therefore experiments were designed to address this.

Male Response to Pheromone – Wind Tunnel Experiment

During winter 2018/2019, Phoenix-strain moths reared locally in Parlier (and not irradiated) were flown in a wind tunnel to measure response to pheromone (female gland extracts) relative to males of the Mendota strain males maintained in Parlier. Overall Phoenix-strain moths effectively responded and flew to a point-source pheromone in the wind-tunnel. There was, however, one difference in their pattern of behavior leading up to flight (fanning). The impact of this difference on field performance is unclear, but overall it was important to demonstrate that locally-reared, Phoenix-strain moths did indeed respond to pheromone, at least in laboratory conditions.

Field Recovery in Small Plots

In 2019, Phoenix-strain males and females were released into both a 2 acre pistachio block and a 5 acre almond block. Starting in June, releases were also made in a 2 acre pomegranate block. Initially, a modification of the metal tray release device was used in which two trays were stacked but held apart by a small plastic container. The device was suspended from the canopy and moths were placed inside of the space between the two trays. Releases began in March/April, and initially utilized unirradiated Phoenix-strain moths. Paired wing-traps, one with synthetic pheromone lure and the other with a ground pistachio/almond ovipositional bait, were arranged in a grid to monitor recovery of both moths (pheromone is for the males, bait is for the females).

Recovery of both males and females continued to be very poor, even when using unirradiated moths. In response to this, the release mechanism was again modified. Starting in June, moths were placed into paper bags and suspended from the canopy. Use of this new release method led to increased recovery of moths, and multiple subsequent releases of both unirradiated and irradiated mass-reared Phoenix-strain moths produced fairly consistent recovery data in the range of 0.1-5.2% of total moths released.

In a related effort, and as a result of the increased recovery seen with the paper bags, a single release was carried out using the drone setup currently utilized for sterile codling moth releases in Washington State (hexacopter drone, operated by M3). Here, a large batch of unirradiated mass-reared Phoenix-strain moths was released over the small pistachio block at Kearney. Recovery ranged from 0.1 - 1.1%.

What is the expected recovery rate of NOW? While recovery rates <0.001% in 2018 were considered outright unacceptable, the significance of increased recovery in 2019 associated with the use of the paper bag release system remains unclear. In order to address this in 2019, multiple releases of the local Mendota 2010 strain and Westside 2019 strains were carried out in the small pistachio block. Mendota strain moths were released on two dates by placing out small buckets (1-gallon size) with diet that contained pupae, which allowed moths to eclose directly in the orchard environment. Additionally, Westside strain moths were released as adults using the paper bag system in the same pistachio block. In these experiments, observed recovery ranged from 0.1-15.1%.

Mendota-strain moths were released in the pomegrantes. Recovery ranged from 1 to 15%. Higher recovery was found in June and July. Pomegranates were still in flower then but not later, and other studies have found that pomegrante flowers are attractive to the carob moth *Ectomyelois ceratoniae* (Zeller), a related and ecologically similar moth. In a series of three releases between August 20 and the end of September, half of the locally-produced, non-irradiated moths released were placed in a shipping container at approximately 2-4C and mailed to our own address via overnight express. The other half of this same cohort was left in the colony environmental chamber overnight. The following day each group was marked with a different fluorescent dye color, and they were released. Over the three relaeses, a total of 3 shipped and 15 non-shipped males were recaptured. For females these totals were 0 and 14, respectively. Thus, while based on a small number of captured, significantly fewer navel orangeworm were recpatured following overnight shipment ($P < 0.01$).

Flight Mill Studies

Flight mills were used to measure and compare dispersal capacity of irradiated and unirradiated Phoenix-strain moths after shipping with locally reared and never-shipped moths of the Mendota and Phoenix strains. These studies are still underway at the time of writing, but preliminary summary of the data at this time indicate that dispersal capacity of both irradiated and unirradiated Phoenix-strain after shipping is significantly reduced (4-6 km/night compared to 8-10 km/night). Here, radiation seemed to have no influence on dispersal capacity, but rather any moth that was produced in the Phoenix facility and shipped to California was less robust on the flight mill. Furthermore, there was a slight positive correlation

between moth weight and total dispersal distance.

Large Block Study

As the field season concluded it became apparent that moth strain and irradiation were not as important as production/shipping/handling and release mechanism. While release of locally-produced moths generated recovery rates as high as 15%, there were instances in which recovery was <1% and this was not clearly associated with either of the locally-produced strains, different release mechanisms or nightly temperatures. One explanation could be that because the healthier, locally-produced moths are stronger dispersers, recovery within a 2 acre block may not be entirely relevant. That is, these healthy moths may have a stronger impetus to disperse before attempting to mate, effectively leaving the small block without being drawn into traps.

As such, towards the end of the season (September/October) irradiated and unirradiated Phoenix-strain moths were relased following shipping on two separate dates. The release site was a large 160 acre block of pistachios that had just been harvested at time of the first release, and in which no mating disruption was in use. Release of irradiated Phoenix-strain moths led to recovery that ranged from 0.2 - 2.3%, while unirradiated moths were, surprisingly, never recovered. The significance of these findings is unclear, as these were singular release events that took place towards the end of the season when nightly temperatures were nearing or surpassing known activity thresholds for NOW. Certainly large-block studies of this type will be repeated in 2020 and beyond.

Laboratory Mating Assays

Laboratory assays were conducted to evaluate multiple mating of irradiated and shipped Phoenix-strain moths compared to locally produced Mendota-strain moths. Shipped Phoenix strain moths mated less on the first night, consistent with the results from the mating tables in 2018. They were also more frequently found to be multiply mated. A follow-up experiment compared irradiated and unirradiated Phoenix-strain moths following shipping. No differences seen in the frequency of mating until the third night, at which point unirradiated moths were more frequently found in copula. Likely related to this, dissections revealed that the unirradiated females tended to show higher levels of multiple mating. While both irradiated and unirradiated Phoenix-strain females were commonly found to be multiply mated following shipping, this was more exaggerated in the unirradiated females.

2. Discuss significance of these in terms of progress toward goals, change in approach, next steps or other conclusions based on this year's results

Conclusions

In 2018, multiple lines of evidence indicated that performance of navel orangeworm released in the field were not recaptured by pheromone lures that captured many wild navel orangeworm. We were also unable to capture released males in mating assays using either females from Phoenix or the local strain. Laboratory experiments indicate that flight performance of moths shipped from Phoenix is poorer than that of moths reared locally in Parlier. Females shipped from Phoenix call at different times and mate less frequently compared to wild or locally-produced females. These females shipped from Phoenix may, nonetheless, be more inclined

to mate multiply. Preliminary data indicates a strong effect of shipping conditions, even on moths produced in Parlier and never passing through the Phoenix collection system (which imposes cold). These data are consistent with flight mill data indicating that Phoenix-strain moths reared in Parlier perform significantly better than moths shipped from Phoenix. We therefore consider if likely that conditions associated with collection of moths in Phoenix and shipment to California are the most influential factor in poorer recovery of these moths with pheromone traps in the field.

D. Outreach Activities

1. Please describe outreach activities including the event description (date, location, topic of the presentation, aprox number of participants and type of audience)

Presentations

Wilson, Burks "Performance of Irradiated NOW for Sterile Insect Release" *Orchard Pest and Disease Management Conference*, Jan. 9, 2019, Portland, OR; 60 people, mixed group of growers/PCAs and West Coast university research/extension personnel

Wilson, Burks "Performance of Irradiated NOW for Sterile Insect Release" *UC Statewide Pistachio Day*, Jan. 16, 2019, Visalia, CA; 300 people, mixed group of mostly growers/PCAs and research/extension personnel

Wilson, Burks "Navel Orangeworm Sterile Insect Technology (SIT): Do Irradiated Moths Behave Like Wild Ones?" *American Pistachio Growers Annual Meeting*, Feb. 27, 2019, Palm Desert, CA; 100 people, mostly growers/PCAs

Wilson, Burks "Sterile Insect Technique to Control Navel Orangeworm" *CA Agricultural Aircraft Association District 3 & 5 Annual Meeting*, May 20, 2019, Tulare, CA; 20 people, agricultural aircraft operators

Wilson, Burks "Development of Sterile Insect Technique for Navel Orangeworm in California Orchards" *Okanagan-Kootenay Sterile Insect Release Program*, July 12, 2019, Kelowna, British Columbia; 2 people, program directors.

Wilson, Burks "Development of Sterile Insect Technique for Navel Orangeworm in California Orchards" *UC Pistachio Workgroup*, July 17, 2019, Coalinga, CA; 20 people, mostly UC pistachio researchers.

Wilson, Burks "Update on Sterile Insect Technique for Navel Orangeworm" *JCS Marketing Progressive Crop Consultant Conference*, Sept. 27, 2019, Visalia, CA; 50 people, mostly growers/PCAs.

Wilson, Burks "Update on SIT for Navel Orangeworm" *Almond Board of California – Board of Directors*, Oct. 1, 2019, Modesto, CA (presentation given online); ABC Board of Directors

Wilson, Burks "Development of Sterile Insect Technique for Navel Orangeworm in California Almond and Pistachio Orchards" *United Nations Joint IAEA-FAO Program - Nuclear Techniques in Food and Agriculture*, Oct. 21, 2019, Mendoza, Argentina; 30 people,

international group of researchers representing all active SIT programs for Lepidoptera across the world.

Wilson, Burks "Update on Sterile Insect Program for NOW" *ABC Pest Management Workgroup*, Oct. 24, 2019, Modesto, CA (presentation given online; ABC Pest Management Workgroup Members

Wilson, Burks "Update on Sterile Insect Program for NOW" *Association of Applied Insect Ecologists Annual Meeting*, Nov. 21, 2019, Visalia, CA; 20 people, mostly PCAs

Wilson, Burks "Update on Development of Sterile Insect Technique for Navel Orangeworm" *CA Walnut Board – Pest Research Action Committee*, Dec. 3, 2019, Stockton, CA; 20 people, mixed growers/PCAs and UC researchers/extension

Wilson, Burks "Sterile Insect Panel" *ABC Annual Meeting*, Dec. 11, 2019, Sacramento, CA; 100 people, mostly growers/PCAs.

Wilson, Burks "Development on Sterile Insect Technique for NOW: Field Trials" *Orchard Pest and Disease Management Conference*, Jan. 10, 2020, Portland, OR; 120 people, mix of growers/PCAs and West Coast university researchers/extension

Posters

Reger, Wenger, Burks, Wilson "Flight Mill Performance of Sterile NOW" *Entomology Society of America Annual Meeting,* Nov. 19, 2019, St. Louis, MO; 100+ people, mostly university entomologists from across the US.

Wilson, Burks "Sterile Insect Technique for Navel Orangeworm" *Almond Board of California – Annual Meeting*, Dec. 10, 2019, Sacramento, CA; 100+ people, mostly growers/PCAs and other almond industry stakeholders

E. Materials and Methods *(500 word max.)***:**

1. Outline materials used and methods to conduct experiment(s)

Methods for Research in 2020

1. Characterize chill injury and chill recovery in navel orangeworm

a. Characterize CTmin in a way that facilitates comparison with codling moth, false codling moth, *Lobesia botrana*, and other previous Lepidoptera used in production-scale sterile insect projects

b. Develop a metric to examine impact of various times of cold exposure at various relevant temperatures. Possible measures of impact include time to death and flight measurements (e.g., with a flight cylinder or using fight mills).

2. Continue to characterize a practical and repeatable mark-release-recapture assay in the field.

3. Evaluate and determine the mechanisms that influence the ability of new release methods to improve recovery and competition of Phoenix Facility moths.

a. Evaluate Recapture and Field Competition of Phoenix Facility Males

b. Develop methods to differentiate spermatophores of wild and sterile moths

i. Starting in April, batches of irradiated Phoenix-strain NOW from the Phoenix Facility will be separately released into a 2 acre pistachio orchard and a 5 acre almond orchard. Recovery of moths will be monitored using a grid of paired wing-traps baited with synthetic pheromone lure (to recover males) and host-plant volatile bait bag (to recover females). In addition, at each site, competition of males will be monitoring using a grid of nine paired mating tables (18 total) that will be set out for the first 3 nights following a release event. Each mating table will contain a virgin female <2 days old. Each pair of tables will randomly be assigned to a female from either the locally-produced Mendota strain (or other local strain) or from the Phoenix facility. Mating tables will go out in the late afternoon and be collected the following day. All females will be dissected to determine mating status, and efforts will be made to develop techniques to identify spermatophore source (e.g. chemical marking of Phoenix Facility moths). Releases will take place every 2 weeks, as this is sufficient time for the released insect population to dissipate. A parallel series of releases will also take place in a large (64 ha) pistachio and almond block. Similar methods will be used to monitor recovery and competition. Time permitting, under these large-block conditions it would be useful to compare moth recapture relative to trapping density.

c. Assess the ability of new release mechanisms to improve recapture rates

i. Building upon findings from 2019, additional improvements to the moth release system will be evaluated in the 2 acre pistachio orchard at the Kearney Ag. Center. Using the paper bag method as a control, two cohorts of moths from the Phoenix Facility will be simultaneously released using different release methods. For any given comparison, moths in each release system will be separately marked externally with fluorescent dye. The paper bag release system likely benefits the moths due to the increased vertical surface area for them to rest on while at the same time providing a sheltered environment. As such, novel release methods will be designed to maximize vertical surface area and shelter for the moths. At the same time, it will also be important to begin exploring delivery methods that will work at scale, with emphasis on an aerial release device such as a drone or modified fixed-wing aircraft.

d. Generate laboratory assays to identify key mechanisms responsible for improved moth recapture

i. In order to further improve the release process, laboratory assays will be developed to identify key mechanisms that influence moth dispersal from the release device. Here the goal is to characterize the specific aspects of the paper bag release system that are leading to improved moth performance.

2. Note any challenges or unforeseen developments that were encountered resulting in change of methodogy, timeline, or scope of project

F. Publications that emerged from this work

1. List peer review publications in preparation, accepted or published
   None to report

2. Other publications (e.g. outreach materials)

Media/Interviews

Wilson “Navel Orangeworm Control” *California Tree Nut Update*, Nov. 2019
https://www.aginfo.net/report/44190/California-Tree-Nut-Report/Navel-Orange-Worm-Control

Wilson “Update on SIT Program” *West Coast Nut Newsletter* July 2019
https://www.wcngg.com/2019/07/01/sit-update/

Wilson “Sterile Insect Program in Pistachios” *UC ANR Growing the Valley Podcast*, Jan. 2019
https://www.growingthevalleypodcast.com/podcastfeed/2019/1/28/sterile-insect-program-in-pistachios-with-houston-wilson

Wilson “Sterile NOW Project Tweaks Expected” *AgNetWest* Jan. 7, 2019
http://agnetwest.com/sterile-navel-orangeworm-tweaks-expected/

Wilson “Female Sterile NOW Moths Did Perform in Trial” Jan. 2, 2019. AgNetWest
http://agnetwest.com/female-sterile-now-moths-performing-well/

Please provide copies of publications

See links above.