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

Project No.: ENTO24A

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A. Summary

Navel orangeworm (*Amyelois transitella*) (NOW) is the primary pest of almonds. Adults oviposit onto the nuts and larvae feed on the nutmeats, which reduces crop yield and quality, as well as increase processing costs. NOW infestation is also correlated with incidence of aflatoxin, a known human carcinogen that is heavily regulated in almond markets. As such, growers have a low tolerance for NOW (goal <2% infestation). Management of NOW consists of winter sanitation, mating disruption, well-timed insecticide sprays and timely harvest.

Recently, the Almond Board of California (ABC) made a significant investment in the development of sterile insect technique (SIT) for NOW that leverages the availability of a preexisting mass-rearing and irradiation facility operated by USDA APHIS in Phoenix, AZ. This facility was originally developed for a sterile pink bollworm (*Pectinophora gossypiella*) (PBW) program and is now being retrofit for production of sterile NOW. The goal is to develop the use of SIT as a complementary strategy to augment the existing IPM toolkit for NOW.

Over the past 3 years, with support from ABC, co-PIs Wilson and Burks have led scientific efforts to evaluate the quality and performance of sterile NOW produced by this Phoenix facility. Findings to date have yielded new information on ways to improve the production, handling, transport and release of sterile NOW, as well as verify the field competitiveness of sterile moths with wild moths in orchards. These studies have documented that, when handled and released properly, sterile male and female NOW disperse throughout the orchard and are able to mate with wild NOW – this includes both sterile females attracting/mating with wild males and sterile males locating/mating with sentinel wild-type females. While irradiation can influence NOW performance, the mass-rearing process itself seems to have the strongest negative impacts on moth quality, and findings to date have implicated the chilling and storage process currently being used in the Phoenix facility (they are kept too cold for too long).

While these results are promising, a lot of additional work is still necessary to know exactly (or even approximately) the quantity and timing of sterile NOW needed to lower crop damage, much less how their ability to do so might be influenced by covariables such as orchard size, proximity to other tree nuts, and background NOW populations. The answers to these questions will determine the feasibility of SIT and our ability to integrate it with competing technologies such as mating disruption and insecticide sprays. In response, co-PIs Wilson and Burks have developed a new project to model various ecological and economic scenarios for

the use of SIT in California (Wilson, Burks, Wei, Goodrich “Spatiotemporal Models to Evaluate the Potential Value of Sterile Insect Technique for Control of Navel Orangeworm”). At the same time, Wilson and Burks are also beginning to explore an alternative approach to SIT based on the use of x-ray sterilization, and to that end have acquired an x-ray irradiator at the Kearney Ag. Center. As such, research on SIT for NOW is entering a new phase in California. Over the next two years our goal is to evaluate the potential of x-ray sterilization of NOW, improve the chilling and cold storage process, refine our understanding of sterile NOW dispersal in large orchards (both x-ray and Phoenix facility moths) and explore the use of SIT in a mating disruption environment.

Findings from these efforts will not only contribute to the development of SIT for NOW as a complimentary IPM strategy, but also more generally improve our understanding of NOW biology, behavior and movement in orchards, which benefits many other areas of NOW management. Were a large-scale SIT program to prove economically unfeasible, knowledge about how to sterilize NOW in a way that minimizes negative impacts on their field performance would provide an incredibly useful research tool. For instance, sterile NOW could be used for mark-release-recapture studies that would allow us to better understand different aspects of NOW ecology, such as dispersal between orchards or movement within orchards under mating disruption. In this way, this NOW SIT research program not only represents an opportunity to develop a new IPM strategy, but also provides a new research tool to improve our current management strategies.

B. Objectives

Goals and Objectives

Goal: Identify key limiting factors that negatively impact the performance of mass-reared and irradiated NOW from the USDA APHIS Phoenix Facility.

Objective 1 – Characterize Chill Injury and Chill Recovery

Objective 2 – Evaluate New Release Mechanisms

C. Annual Results and Discussion

Effect of chill injury on flight cylinder performance

Two experiments used flight cylinders to examine whether we could replicate, in Parlier CA, the reduced performance observed in moths arriving in California from the Phoenix AZ mass production facility. These laboratory studies used 6.5 x 6.5 inch (height x diameter) polyethylene pipes to determine whether NOW males flew out in a single night. This test is widely used in lepidopteran SIT, and previous such experiment found that 90% of locally-produced NOW males fly out of these cylinders, but only around 50% of the NOW received from Phoenix. In one such test males from the Parlier ‘Mendota 2011’ line, 0-2 days after adult emergence, were either tested immediately in flight cylinders, or placed cooled to 4C, packed in the same way as nights shipped overnight from Phoenix, and sent overnight with UPS from Parlier CA to Parlier CA. This overnight shipment reduced flight cylinder performance from 95 to 78% (Table 1). This reduction was statistically significant, but not of the magnitude of the difference in performance between locally-produced moths and moths received from the mass rearing facility. A second experiment compared 0-2 days adult males from the Parlier ‘Mendota’ line, that were either tested in the flight cylinder immediately, or after being held 48, 72, or 96 hours in an environmental chamber at 4C. Most replicates were either

no cold treatment or 27 hours at 4C. The 0 hour and 48 hour treatments showed relatively good flight cylinder performance (80-90% fliers), while the 72 and 96 hour treatments had poorer performance (Ca. 65% fliers) (Table 2). These data indicate that at least three days are required to significantly reduce flight performance as assessed by the flight cylinder, but that shipping an cold might reduce flight performance more than chilling injury alone. Also, while not conclusive, these data are consistent with the hypothesis that substantial reduction in performance comes from an early (pre-radiation) step in the mass production process.

Table 1. Effect of shipping on the percent of NOW flying from a flight cylinder in 24 hours

Treatment	n	Percent fliers
Not shipped	12	95 ± 5a
Shipped	12	78 ± 11b

n = number of flight cylinder trials with 10 males. Means followed by different letters are significantly different.

Table 2. Effect of time at 4C on the percent of NOW flying from a flight cylinder in 24 hours

Time at 4C (hr)	N	Percent fliers
0	48	83 ± 5a
48	3	93
72	39	62 ± 8b
96	3	67

n = number of flight cylinder trials with 10 males. Means followed by different letters are significantly different. The 48 and 96 hr treatments illustrate trends, but are not sufficiently replicated to test statistically.

Recovery of Sterile NOW in Flight Traps and Mating Tables

Overall, in 2020 sterile NOW were consistently recovered under field conditions in both the flight traps and mating table assays, but the overall proportion of moths recaptured from a given release event varied significantly (average 2% recapture, range 0.001 - 14%). Data analyses indicate that this variability in recapture rate was influenced by both transportation and release methods (Fig. 1), but surprisingly the strongest predictor of sterile NOW recapture was background wild NOW population (Fig. 2). Upon closer inspection, it appears that recovery rate of sterile NOW was positively correlated with trap catch of wild NOW, indicating that increased activity of wild NOW may somehow lead to increased activity of sterile NOW (or vice versa). This is a unique new dimension to sterile moth performance that will be more closely evaluated in 2021/2022. The total number of sterile moths in a given release event (mean = 5736 ± 168 moths) did not have any influence on recovery rate.

As seen in 2019, release method does have an impact on moth performance in field plots. In 2020, the paper bag method that was developed in 2019 continued to produce consistent recovery, which was enhanced further through the addition of tightly packed cardboard tubes into the paper bags (Fig. 3). Moths were also consistently recovered when released by unmanned aerial vehicles (UAVs, i.e. drones) as well as from piloted fixed-wing aircraft (Fig. 1). While the paper bag system works well for experimental purposes, it is critical

to continue evaluating different aerial and ground release systems that could potentially work at scale.

Finally, while efforts in 2018 demonstrated that sterile female NOW were able to effectively call and mate with wild males, mating table assays from 2020 are the first to demonstrate that sterile male NOW are able to locate and successfully mate with wild-type sentinel females (Fig. 4). This is a major achievement, and when paired with recovery data indicate that indeed the sterile NOW from the Phoenix facility can successfully disperse in an orchard and both sexes can locate and mate with wild moths.

Conclusions and Future Directions

While preliminary findings are promising and many key limiting factors have been identified (and overcome) over the past three years, there are still many challenges ahead to the development of SIT for NOW. For instance, NOW dispersal and mate-finding in a small block setting is likely quite different from how NOW would distribute themselves across a much larger area. Trapping density and release rates also certainly have an impact on overall recovery rate. On the production side, more information is currently needed about the impacts of chilling and cold storage for NOW aggregation, irradiation and transport, as well as how vibrations and motion impact the quality of NOW while in transit. Furthermore, the potential to combine SIT with mating disruption holds some promise, and additional information is needed on sterile NOW performance in mating disruption blocks. Finally, x-rays offer a safer and potentially more cost-effective opportunity to produce sterile NOW using a non-nuclear irradiation source. As such, efforts in 2021-2022 will focus on these and other related questions. In addition, co-PIs Wilson and Burks have developed a parallel proposal to model the ecological and economic potential for SIT in California (Wilson, Burks, Wei, Goodrich “Spatiotemporal Models to Evaluate the Potential Value of Sterile Insect Technique for Control of Navel Orangeworm”). Even in the absence of an economically viable SIT program for NOW, understanding how to produce a sterile moth that can perform on par with wild NOW will allow open many additional avenues for ecological research on NOW, which can contribute to the refinement of current management strategies.

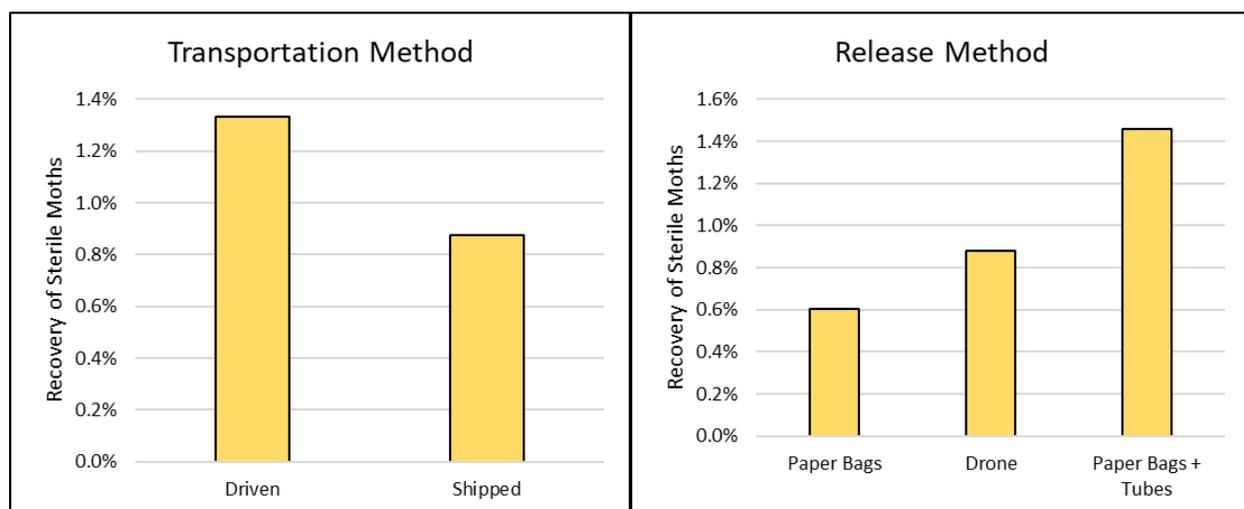


Figure 1. Recovery of sterile NOW increased when moths were driven in a controlled climate (left) and when released using the paper bag with cardboard tubes or a UAV/drone (right).

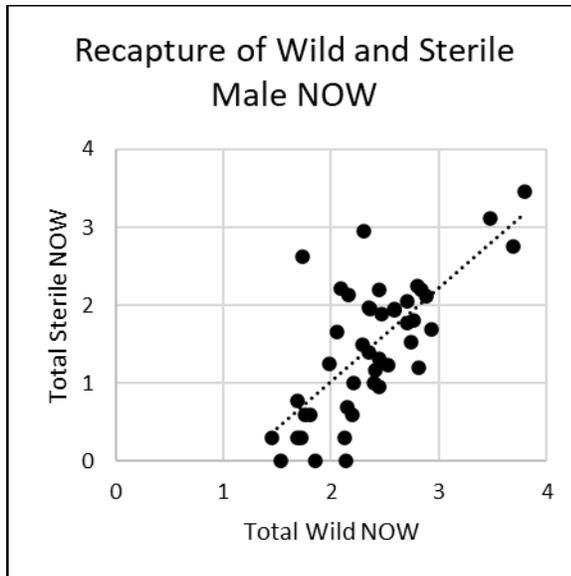


Figure 2. Recapture of sterile NOW was positively correlated ($R^2 = 0.51$) with capture of wild NOW. Data are summed across the 2-week period following each release event and $\log(x+1)$ transformed for scale.



Figure 3. Recovery of sterile NOW was consistent when using paper grocery bags (left), but was further improved when moths were released from a UAV/drone (center) or when the paper grocery bag was filled with tightly packed cardboard tubes (right).

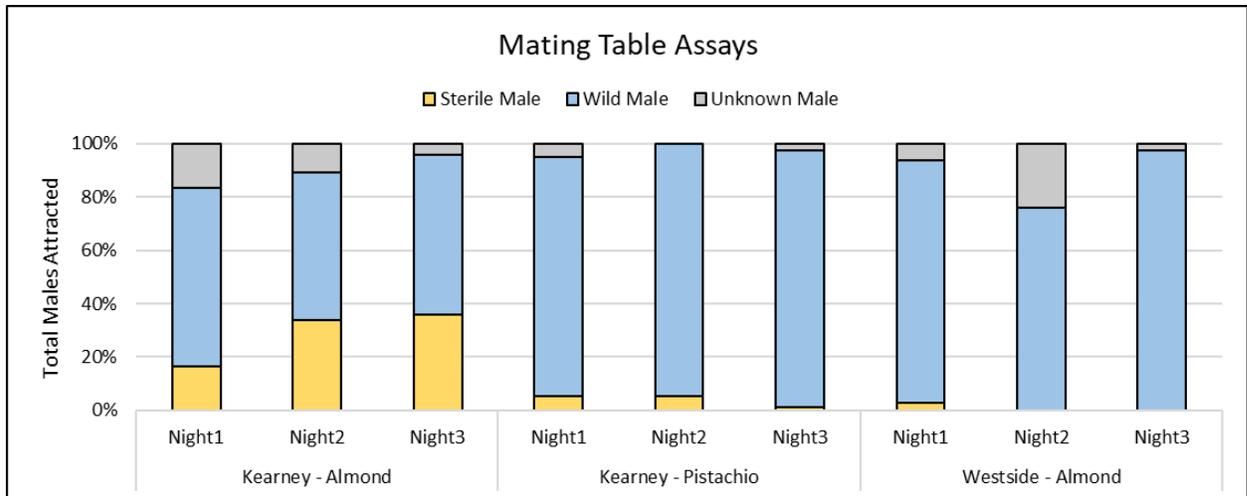


Figure 4. Mating table assays demonstrated that sterile NOW could indeed locate and successfully mate with wild-type sentinel females.

D. Outreach Activities

- **Wilson, H., and C. Burks.** “Understanding Navel Orangeworm Mating Disruption and the Sterile Moth Release Program” American Pistachio Growers Annual Meeting, Monterey, CA. Mar. 2020, 80-100 pistachio/almond growers, PCAs and related industry personnel.
- Reger, J., **H. Wilson, C. Burks** and J. Wenger. “Relative Impact of Strain, Irradiation and Handling on Flight Performance of Navel Orangeworm (Lepidoptera: Pyralidae). Fresno, CA. American Society of Agronomy - California Chapter (CalASA) Annual Plant and Soil Conference, Feb. 2020, 80-100 faculty and graduate students from across California.
- **Wilson, H., and C. Burks.** “Development of Sterile Insect Technique for NOW: Field Trials” Orchard Pest and Disease Management Conference, Portland, OR. Jan. 2020, 50-60 academics and cooperative extension personnel from across the West Coast.
- **Wilson, H., and C. Burks.** "Update on Sterile Insect Program for Navel Orangeworm", UC Statewide Pistachio Day, Jan. 2020, 150-200 growers, PCAs and other industry stakeholders.
<http://lecture.ucanr.edu/Mediasite/Play/e0ff33a623ae468490ecec7888e89cbe1d>

E. Materials and Methods:

Experimental Designs

Flight cylinder experiments were conducted using locally-reared moths as described in Reger et al. (2020). NOW were separated by sex as larvae, allowed to emerge in gallon jars, and removed at 3-day intervals. Flight cylinder prepared from used 6.5 x 6.5 inch (height x diameter) polyethylene pipes were placed in 1 foot cube aluminum wire cages. . The upper portion of the pipe was coated with fluon so the moths could not leave by

crawling up the tube. Six such units were held in a walk-in environmental chamber maintained at 14:10 L:D, 26C, and 65% r.h. Adults were placed on the floor in the center of the cylinder mid day, and the results were assessed soon after lights-on the following day.

While field experiments in 2018 and 2020 did include large blocks (640 acre), most recapture data of sterile NOW have been collected in small orchard blocks (<7 acres). Initially low recapture rates in 2018 were potentially attributed to a lack of pheromone response by sterile males. This was subsequently disproven through wind-tunnel assays (Reger et al. 2020) and instead changes to the release method in 2019 led to significantly increased recovery of sterile NOW (Wilson and Burks 2019).

In 2020, recovery of sterile NOW was compared in 3 small blocks (2 almond, 1 pistachio, 2-5 ac.) and 1 large block (pistachio, 640 ac.). Each block contained a grid of trapping points with paired flight traps, one baited with pheromone lure (captures males) and the other with an ovipositional bait (captures females). The trap grid was 4 x 4 (16 trapping points total) in the small block and 8 x 8 in the large block (64 trapping points total). Sterile moths were released every 2-weeks between May – October using a variety of transportation and release methods. In the small blocks, a grid of 12 mating tables were set out for the first 3 nights following each sterile moth release. Each mating table contained a tethered virgin female NOW that was replaced daily. These sentinel female NOW were collected and dissected the following morning to determine mating success. When a male moth was present, it was also dissected to determine if it was sterile or wild.

F. Publications that emerged from this work

Peer-Reviewed Publications

- Reger, J., J. Wenger, G. Brar, **C. Burks, H. Wilson**. 2020 "Evaluating response of mass-reared and irradiated Navel orangeworm, *Amyelois transitella* (Lepidoptera: Pyralidae), to crude female pheromone extract" *Insects* 11(10), 703; doi.org/10.3390/insects11100703

Industry Publications

- **Wilson, H.**, M. Zwieniecki, and L. Ferguson. "New Research Includes Focus on NOW and Tree Physiology in Pistachio", *West Coast Nut*, Apr. 2020, <http://www.wcngg.com/2020/04/07/new-research-includes-focus-on-now-and-tree-physiology-in-pistachio/>