
Reducing Cost of Mating Disruption for Navel Orangeworm (NOW)

Project No.: 14-ENTO2-Burks

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Objectives:

Conduct research on the effect of emission parameters on efficacy of aerosol mating disruption for the navel orangeworm (NOW). Specific objectives are to examine suppression of sexual communication (i.e., trap suppression) by: 1) varying emission frequency; and 2) varying the total time of nightly treatment.

Interpretive Summary:

Current navel orangeworm mating disruption formulations use a prominent component of the female sex pheromone blend (Z11,Z13-16:Ald) emitted from aerosol dispensers. This conjugated diene is more expensive to manufacture than the pheromones used for control of other species by mating disruption. We therefore examined effects of variation in emission frequency and nightly treatment time. Treatment for six hours per night instead of the current 12 was equally effective in suppression of sexual communication. Suppression of sexual communication improved with increased hourly emission frequency up to at least three times the hourly emissions currently used. These findings offer the possibility of increased cost-effectiveness of mating disruption for the navel orangeworm.

Materials and Methods:

In 2014, the effect of time of emission was tested using Checkmate Puffer NOW (Suterra, Bend OR). In 2015, the effect of emission frequency was tested using navel orangeworm pheromone emitted from MIST dispensers (Pacific Biocontrol, Vancouver WA).

Time of emission, 2014. The rudimentary programming capability of the current Suterra Puffer dispensers was used to compare 12 hours of treatment starting at 6 PM or midnight Pacific Daylight Time. Puffers cabinets emit a fixed volume of formulated product (40 µl per emission, resulting in 1.92 ml per day) is emitted, either every 15

minutes for 12 hours starting at 6PM, or every 30 minutes for 24 hours. In this experiment, cabinets were set in 12 hour mode. The 6PM start time specified by the label is obtained by setting the internal clock on a 1-hour delay, so that the clock reads 5PM when actual local time is 6PM. Similarly, a midnight start time is obtained by setting the internal clock on a 7-hour delay. Since astronomical sunrise in Fresno County occurs between 5:40 and 6:10AM between June and August, the midnight start time results in roughly 6 hours of pheromone emission prior to dusk. Release of sex pheromone by navel orangeworm females, and response by males, occurs over the last few hours prior to dusk in typical summer conditions, and begins earlier in early and late season (before May 15 or after September 15) or under unseasonably cool conditions (Landolt and Curtis 1982). It was presumed, for this experiment, that pheromone released during the daytime has no effect.

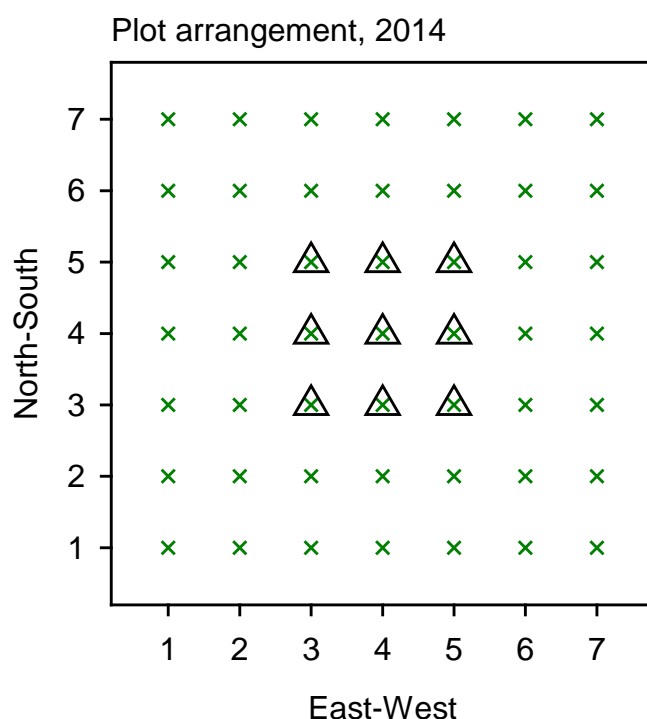


Figure 1. Plot arrangement, 2014 emission time experiment. Individual trees are indicated by x. Mating disruption dispensers were placed in the corner positions, ~148 feet apart, equivalent to a density of 2 dispensers per acre. Wing traps containing pheromone lures were placed in a 3x3 pattern in the middle 9 trees in the plot.

This experiment was conducted in pistachios because previous research indicated that pistachios often support higher navel orangeworm abundance over a greater part of the year compared to almonds (Burks et al. 2008). The research site comprised of three square pistachio fields of 160 acres (65ha) each, located at 36°22'52.51"N, 120°22'9.83"W (17 miles north of Coalinga, CA) and set diagonally along a northwest-to-southeast line. The pistachios were over 30 years old, 4.6 to 6.1 m (15 to 20 ft) high, and planted in north-south rows with 5.8 m (19 ft) row spacing and 5.2 m (17 ft) between trees within rows. The northern- and southern-most of these blocks were treated as separate replicate blocks. Plots comprised grids of nine orange wing traps (Suterra, Bend OR) surrounded by four CheckMate NOW Puffer dispensers (Suterra,

Bend OR) aerosol dispensers (**Figure 1**). The traps baited with NOW Biolure (Suterra, Bend OR); with traps 1.5 m from the ground in three sequential trees in each of three sequential rows. These plots (four per 160 acre field) were placed near the corners of the 160-acre fields, ≥ 170 feet from the corners. The dispensers described as part of the treatment plots were the only navel orangeworm mating disruption dispensers in or near the experimental site.

In addition to the 8 replicate blocks, the experiment was replicated in time over 8 iterations from June to August. In the first and third iterations, dispensers were turned off and no mating disruption treatment was applied to any block. In the second iteration, dispensers were activated the 12- or 6-hr treatments were randomly assigned to two positions each within replicate blocks, and in the fourth iteration the treatment plots were reversed. The procedure for the first four iterations were repeated for iterations 5-8. New trap liners were placed at the beginning of each of these iterations, and liners were removed and data collected 7 days later. Beginning dates for these iterations were June 12 and 24, July 1, 8, 15, 22, and 29, and August 5. The insecticide λ cyhalothrin was applied to this site control shortly after the beginning of July, and trap totals for the third iteration were distinctly less compared to other periods where dispensers were turned off.

The intended analysis for these fields was a cross-over design, which would allow trap counts for the non-mating disruption iterations to serve as a factor to control for position effects. However, when initial analysis showed no correlation between plot counts across iterations, the data were analyzed with a simple generalized linear model. The binomial distribution because it provided a good fit (as determined by the deviance) of these count data, which included low counts in some treatments as well as wide heterogeneity between treatments.

Emission frequency, 2015. This experiment tested a null hypothesis of no difference in number of males captured between five treatments: untreated plots, or plots with dispensers emitting every 5, 10, 15 or 30 minutes between 18:00 and 06:00 hours Pacific Daylight Time.

The research site was the same as that described for the 2014 experiment, but plots were located in the center of the 40 acre quarters of the 160 acre fields. The northwest and southeast quarter of the intervening 160 acre field provided a fifth plot for the northern and southern replicate blocks. Plots contained 19 trees of 17 rows and contained a grid of nine wing traps baited with pheromone lures in the center, as described for the 2014 experiment. The dispensers in the plot corners were thus 98 m apart, the plot size was 0.97 ha (2.4 acres), and the effective dispenser density was 1/ha (0.4 dispensers/acre). This plot size was used to avoid complete suppression of males captured in traps, and thereby increase statistical resolution of treatment effects.

Dispensers (MIST, Pacific Biocontrol, Vancouver WA) emitted 0.38 mg of Z11,Z13-16:Ald in 40 μ l every 15 minutes between 6PM and 6AM Pacific Daylight time (i.e., the current standard treatment). In addition to the two replicate blocks, there were three

replicates in time. Treatments were re-randomized among plots within blocks between these replicates in time. The length of these replicate interval (number of days) was variable, depending on navel orangeworm activity and insecticide application schedules. The dates for these replicates in time were: 1) 31 March to 8 April; 2) 8 April 8 to 22 April 22, and 3) 22 April 22 to 12 May, 2015. The treatment site received an unusually high number of treatments with the insecticide λ cyhalothrin due to atypical infestation with *Lygus hesperus* (Knight) (Hemiptera: Miridae); applications were made on March 24, April 8, and May 1. The data were analyzed using a generalized linear mixed model with a binomial distribution. The treatment was a fixed effect, and blocks and iterations were random effects.

Results and Discussion:

Time of emission, 2014. Both the standard mating disruption treatment and the treatment starting at midnight resulted in similar and substantial trap suppression compared to the untreated control (**Table 1**). These data suggest that, under summer conditions, treatment time and therefore nightly pheromone use could be cut in half. This is consistent with previous observations of navel orangeworm sexual behavior, which revealed that most mating occurred within 4 hours before sunset under warmer conditions (Landolt and Curtis 1982). This finding suggests that, under summer conditions, pheromone use might even be cut by two-thirds without impacting efficacy. However, previous data also indicate that sexual activity extends to as much as 9 hours before sunset under cooler conditions (Landolt and Curtis 1982). Therefore, if the nightly treatment time is fixed, a shorter treatment time will result in a tradeoff between greater cost-effectiveness for much of the season at the expense of reduced efficacy in early and late season. Currently some manufactures of aerosol mating disruption dispensers registered for control of other insects have a temperature cut-off, so that pheromone is not dispensed if the temperature falls below a set point. For the navel orangeworm, it might be more useful if a temperature threshold could be used to toggle between an earlier and later start time for treatment. These results need to be verified using equipment that truly supports variable treatment time, as opposed to relying on presumed irrelevance of material dispensed during daylight hours. Empirical data on the effect of hours of treatment also needs to be gathered in early and/or late season, when evidence indicates sexual activity starts earlier. Such experiments are currently in progress.

Table 1. Navel orangeworm males per plot (mean and SE) in untreated control plots, or plots treated with Sutterra Puffers starting at 6PM or midnight, in mid-summer conditions

Treatment	Replicates	Males per plot	Trap suppression
No mating disruption	32	88 ± 10.7a	-
Mating disruption beginning midnight	16	1.8 ± 0.36b	98
Mating disruption beginning 6PM	16	1.5 ± 0.71b	98.3

Means followed by different letters are significantly different ($P < 0.05$, GLM with negative binomial distribution).

Emission frequency, 2015. Data from the 2015 experiment examining emission frequency revealed that, compared to the untreated control, all emission frequencies times suppressed the ability of males to locate a pheromone lure (**Figure 2**). However, there was also a dose-dependent increase in trap suppression with increased emission frequency, and significantly fewer males were captured in plots treated with dispensers emitting every 5 minutes compared to those emitting every 30 minutes. In the current experiment, it is not clear whether increased suppression at higher emission frequencies is due to the emission frequency itself, or is a result of a greater total amount of pheromone dispensed. This can be clarified by using offsetting concentrations and emission frequencies. Such an experiment is underway, and initial indications are that emission frequency is more important than the amount of pheromone emitted.

For the navel orangeworm, it is particularly important to consider mode of action when attempting to refine mating disruption treatments. All commercial mating disruption treatments for the navel orangeworm to date have used Z11,Z13-16:Ald (Higbee and Burks 2008). This is the earliest known of the four components comprising an attractive pheromone blend for the navel orangeworm (Coffelt et al. 1979, Kuenen et al. 2010). In the wind tunnel, Z11,Z13-16:Ald is not, by itself, attractive to the navel orangeworm (Kanno et al. 2010). In contrast, most commercial mating disruption formulations are attractive to the target species (Miller et al. 2006, Miller and Gut 2015). The citrus leafminer is a notable exception (Stelinski et al. 2008, Lapointe et al. 2009), and the oriental fruitworm provides an example in which an attractive formulation results in mating disruption by a non-competitive mechanism (Reinke et al. 2014). Nonetheless, it seems that competitive mechanisms for mating disruption are more common (Miller et al. 2006, Miller and Gut 2015). Mating by non-competitive mechanisms can be advantageous because the target male is less likely to be diverted by the plume of a calling female before arriving at a dispenser (Miller and Gut 2015). In the case of the navel orangeworm, using the unattractive formulation has the additional advantage of avoiding the difficulty of maintaining a stable attractive formulation in the field (Higbee et al. 2014, Burks and Higbee 2015). It also avoids the additional regulatory requirements (Bailey et al. 2010) that will be required for registration of attractive formulations necessarily including the C23 pentane, which is not a straight-chain lepidopteran pheromone. It seems likely that the mechanism for present mating disruption formulations for the navel orangeworm involves camouflage, although more stringent testing is required to demonstrate this (Miller and Gut 2015). If that is the mechanism, then this implies that it is particularly important to obtain even distribution of the pheromone in the canopy while males are receptive to sex pheromone. That would explain why increased emission frequency is beneficial for this species, and explain while this finding differs from unpublished observations concerning the relationship between emission frequency and efficacy of aerosol mating disruption for the codling moth.

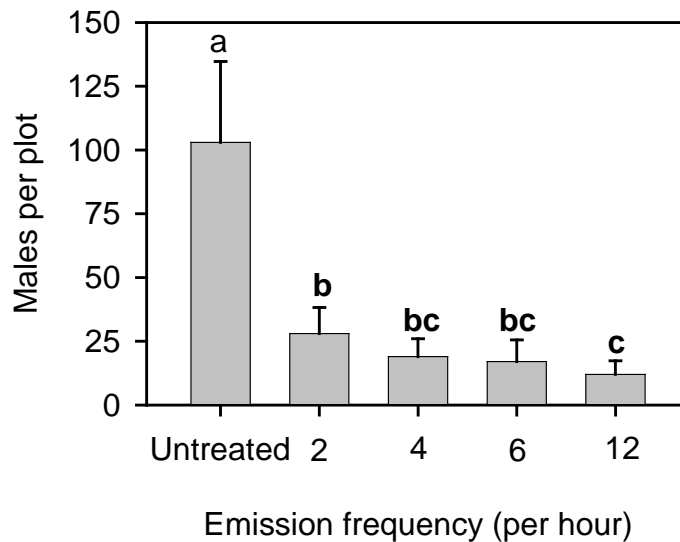


Figure 2. Navel orangeworm males per plot (mean and SE, n = 6) in untreated control plots, or plots treated with mating disruption from MIST dispensers every 30, 15, 20, or 5 minutes. Means with different superscripts are significantly different (GLMM with nb distribution, $P < 0.05$). The current standard treatment is emission at 15 minute intervals (4 emissions per hour).

For aerosol mating disruption systems, parameters that can be varied to improve cost-effectiveness of mating disruption include: 1) the emission frequency; 2) the number of hours of treatment per day; 3) the amount of active ingredient per emission; and 4) the density of dispensers per acre. Previous research suggests that the ideal dispenser density is higher for navel orangeworm compared to other species controlled with mating disruption. For aerosol mating disruption, the dispenser themselves are a form a significant proportion of the cost of the treatment. This might, however, be less true for the navel orangeworm, since the conjugated dienes are much more expensive to produce in pure form compared to other pheromones commonly used for mating disruption. The results reported here indicate that treatment cost can be cut by reducing the nightly treatment time, and suggest that the pheromone might be used more effectively by more using more frequent emissions containing less active ingredient per emission. Future research needs includes testing further testing, combining the two variables tested here with the other two mentioned above to optimize suppression of sexual communication. Tests are also needed to compare damage reduction between such optimized treatments and the *status quo*.

Research Effort Recent Publications:

- Burks, C. S. 2014. Effects of delayed mating and access to water on oviposition and longevity in female *Amyelois transitella*. International Journal of Insect Science 2014: 89-98.
- Burks, C. S., and B. S. Higbee. 2013. Effect of abundance of the navel orangeworm on sampling range and interference between pheromone traps. Environ. Entomol. 42: 143-149.
- Burks, C. S., and B. S. Higbee. 2015. Impact of trap design and density on effectiveness of a commercial pheromone lure for monitoring navel orangeworm (Lepidoptera: Pyralidae). J. Econ. Entomol. 108: 600-610.
- Higbee, B. S., C. S. Burks, and T. E. Larsen. 2014. Demonstration and characterization of a persistent pheromone lure for the navel orangeworm, *Amyelois transitella* (Lepidoptera: Pyralidae). Insects 5: 596-608.
- Sappington, T. W., and C. S. Burks. 2014. Patterns of flight behavior and capacity of unmated navel orangeworm adults (Lepidoptera: Pyralidae) related to age, gender, and wing size. Environ. Entomol. 43: 696-705.

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- Miller, J. R., and L. J. Gut. 2015. Mating Disruption for the 21st Century: Matching Technology With Mechanism. *Environ. Entomol.* 44: 427-453.
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