# Monitoring the Adult Navel Orangeworm (NOW) Moth with Pheromone and Hostplant Volatiles

**Project No.:** **14-ENTO9-Cardé**

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**Objectives:**

1. An overarching goal has been the development of a long-lasting pheromone lure to replace the use of caged females in monitoring traps. In support of this goal, we used our wind-tunnel assay to define the 4-component pheromone blend and to establish that the expected contaminants of the synthetic pheromone arising during synthesis or as breakdown products are not antagonists of attraction. We have a new lead for a missing pheromone component. We will continue to assist field tests by providing candidate lures. There remain unanswered questions, as the new commercial field lure is not as attractive as females, especially when deployed in pistachio orchards.
2. Use a large, still-air flight chamber to screen for compounds and combinations of compounds that mediate mated-female location of oviposition sites. Such compounds could serve as field lures for monitoring female density.
3. Use wind-tunnel assays to increase the potency of the male lure by adding host-plant volatiles to the 4-component pheromone blend. We also are testing key host volatiles alone to see if they have attractive properties individually or in blends. Both males and females will be tested.

**Interpretive Summary:**

Based in part on our work and recommendations, Suterra has developed a plastic membrane formulation of pheromone that sustains attraction of male navel orange worm moths (NOW) for approximately one month. This recent breakthrough (Higbee et al. 2014) is enabling pest managers to monitor populations more effectively and cheaply than by using traps baited with virgin females. After several years of field use, information from temporal and spatial patterns of trap capture ought to be useful for modeling seasonal development and for estimating density, and therefore for guiding decisions on the need for control measures.

As promising as this new commercial lure is, our wind-tunnel studies have shown that female extracts outperform synthetic blends in terms of proportion of released moths finding the lure. Moreover, while the new Suterra lure clearly is efficacious for trapping males over several weeks, its attractiveness relative to female-baited traps varies markedly in almond versus

pistachio orchards (Higbee et al. 2014). In almond orchards, the Suterra lures outperformed females by a factor of roughly 1.5X; however, in pistachios, these lures were less than a tenth as attractive as females. Clearly there are aspects of this mate-finding system that we have yet to understand.

A second strategy under investigation is the use of host volatiles to either serve as a “stand-alone” monitoring lure for females (and possibly males) or in combination with pheromone for males. This would be comparable to the pear ester that has proved promising for monitoring codling moth. John Beck and colleagues (Beck and Higbee 2013; Beck et al. 2009, 2011, 2014a,b) has identified a large number of volatiles released from almonds, almond mummies (and also from pistachios). We are seeing if any of these alone or in combination are efficacious lures. At present, the only system in use for monitoring females relies on a bait of almond meal to induce egg laying. These volatiles are being evaluated in a wind-tunnel assay.

**Materials and Methods:**

Our host-plant volatile bioassay uses a large screened cage set on a base that completes a 360° rotation every 27 minutes. This set-up is housed in a controlled environment room with an 8-hour dark period. During scotphase there is a low light level simulating natural nighttime conditions in an orchard and the slow rotation of the cage is intended to obviate any preferential orientation issues arising from any uneven distribution of light. Capture jars loaded with natural bait such as almond expeller press cake (positive control) are compared to jars baited with candidate lures (e.g., synthetic chemicals supplied by John Beck) or empty control jars (negative control). Moths enter jars via a funnel through 1-cm-diameter port. To be captured a moth needs to land on the jar and walk downward to enter the port. On the first assay day, equal numbers of 1- to 2-day-old males and females (usually 150 of each) are released into the cage just before the start of the dark scotophase. Most females mate during the first night and so are available to respond to host volatiles, which are setout during the next night. We collect captured moths after the end of the 2$^{nd}$ and 3$^{rd}$ scotophases. All captured moths are placed in 10% KOH solution for clearing. This enables ready determination of sex and the mating status of females (spermatophore present or not). Air is vented out of the bioassay room between assays. Generally the negative control (an empty jar) captures 0-1 females over two nights. The positive control, expeller press cake, captures 20-50 females, provided there is no other attractive source. We test either single compounds or blends of compounds (for a listing of chemicals identified see Beck and Higbee 2014, Beck et al. 2009, 2011, 2014a, 2014b, Mahoney et al. 2014).

A second assay uses a wind tunnel. Males or females are released individually at the downwind end of the tunnel and their reactions monitored by direct observation and by recording via video with infrared illumination. Lures are prepared by using serial dilutions of host plant volatiles. These lures are tested alone and paired with pheromone lures. Successful orientation is defined as lure contact, which we expect would translate into a lure that would be field efficacious.

## Results and Discussion:

**Development of a host-plant volatile bioassay.** As noted above, we have a diagnostic laboratory capture jar assay to test attraction of NOW adults to host-plant volatiles. Beck and Higbee (2014) and Beck et al. (2009, 2011, 2014a, 2014b) and Mahoney et al. (2014) have identified many volatile compounds from foliage, nuts and mummies. We have a surfeit of candidate compounds (well over 100 to consider), although we can make some assumptions about which are most likely to be behaviorally active, based on their relative abundance in airborne collections and especially whether a compound evokes a good response in an electroantennogram (EAG) assay. So far our work has found that in this assay the only responding class of NOW adults is mated females. In the case of other moths, however, host volatiles may be attractive to females and males and, in the case of the codling moth, the pear ester usually adds to male attraction to the female's pheromone (Light et al. 2005). These findings with the codling moth and studies with other orchard pests such as the oriental fruit moth (Pinero and Dorn 2009) indicate that the interactions between host plant cues and mate-finding signals are complex. A recent review of this topic by Deisig et al. (2014) documented many cases of host-plant volatiles "synergizing" male attraction to female-emitted pheromones, suggesting that this phenomenon is widespread among moths. These findings in aggregate suggest that odor cues released by host plants can be powerful lures that will be useful for pest management decisions.

Our wind-tunnel tests to date have tested the most likely of these host volatiles to be attractive, based on two criteria from the Beck et al. studies: the compounds identified are major constituents of the blend and they are active in an electroannogram assay. We test volatiles at 3 doses (10, 100 and 100 micrograms per dispenser) with and without a pheromone lure for males and 3 doses (without pheromone) for females. Among the compounds tested so far are octanal plus nonanal, sabinene, (*Z*)-3-hexenal, (*Z*)-3-hexenal plus (*Z*)-3-hexenol, 3-octen-2-one, methyl salicylate, sabinene hydrate, linalool, limonene, a pistachio blend and other compounds. Typically tests are run with 20 insects per treatment or a total of 100 male moths per volatile or volatile blend. So far none of these has proved attractive alone or in combination with pheromone. One issue that we are considering is that it may not be a single active compound but rather a blend that evokes attraction. As was demonstrated with the black bean aphid (Webster et al. 2010), a blend of host volatiles is required for attraction and surprisingly these compounds presented individually are either inactive or even repellent. So besides testing compounds alone or paired with pheromone, we will prepare blends to see if these are attractive. John Beck will continue to provide guidance as to the best candidate compounds or blends for us to evaluate.

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