Monitoring the Adult Navel Orangeworm (NOW) Moth with Pheromone and Host-Plant Volatiles

Project No.: **13-ENTO9-Cardé**

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Objectives:

1. One 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 have 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 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.

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 orangeworm moths (NOW) for approximately one month. This recent breakthrough (Higbee et al. 2014) will enable 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.

Our collaboration with John Beck on identification of host-plant volatiles that cause males and females to orient to almond foliage or almonds uses a laboratory-based, rapid put-through behavioral bioassay. We use a large flight chamber that permits male and female moths to enter baited jars though a small (1-cm-diameter) port and it permits the simultaneous evaluation of up to four treatments. This method is effective in establishing which treatments are attractive and also is advantageous in that it can be conducted year-round.

Materials and Methods:

Development of effective pheromone lures**.** Field tests were conducted by Brad Higbee using standard methods (sticky wing traps, weekly replacement of females, see Higbee et al. 2014). In parallel, we have continued to pursue the outstanding question of why in wind-tunnel trials a female extract consistently outperforms (by a factor of 30-40%) our best synthetic lures. We have found that potential contaminants of the aldehyde and two alcohol components (such as their geometrical isomers) are not inhibitory to attraction.

We have new evidence to pursue a missing component with standard techniques of compound isolation and bioassays in a wind tunnel (Kanno et al. 2010; Kuenen et al. 2010). What has been surprising is the extraordinary disparity in the attractiveness of the Suterra synthetic lure versus virgin females in almond and pistachio orchards (Higbee et al. 2014). This points to 1) either background volatiles from these two host plant volatiles playing as unexpectedly large and differential role in female attraction of males or 2) to an effect of differential capture wherein males are attracted from a longer distance by synthetic lures but are not as apt to be captured in traps as with female baits, the "authentic pheromone source" (Higbee et al. 2104). This explanation also appears consistent with the changes in ratio of captures with females vs. lure when traps spaced 37 vs. 60 m apart (see discussion in Higbee et al. 2014).

Host-plant volatile bioassay**.** 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 the dark scotophase 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 scotophase. Most females mate during the first night and so are available to respond to host volatiles, which are set-out 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).

Results and Discussion:

Development of effective pheromone lures. Higbee et al. (2014) confirmed that the Suterra membrane formulation is useful as bait in monitoring traps. What is clear from this trial and another reported in Beck and Higbee (2013) is that fresh lures are evidently comparable in attractiveness to females. It should be noted, however, that sample dates were 7 days apart and consequently some females would not have been alive toward the end of the 7-day period. This means that even fresh lures may not fully match the attractiveness of females.

We had suggested that the decline in potency with time of exposure of the lure in the field could be due to either a decline in release rate or the buildup of inhibitory compounds (i.e., breakdown products of one or more of the four pheromone components). Our wind-wind tunnel trials, however, did not find that any of the tested compounds (3 isomers of the aldehyde component, the 2 isomers of the 2 alcohol components) and other candidate inhibitors influenced attractiveness of the pheromone blend. While the commercial lure is now field-ready, we believe that improvements in its potency are attainable. This also has implications for mating disruption, as generally the more complete the blend, the more efficacious it is in mating disruption (Minks and Cardé 1988, Cardé and Minks 1995).

Development of a host-plant volatile bioassay. We have a diagnostic laboratory 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 classes of NOW adults are 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.

Research Effort Recent Publications:

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