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

Project No.: **12-ENTO9-Cardé**

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Objectives:

1. The overarching aim is to develop a **long-lasting pheromone lure** to replace caged females currently used in monitoring traps. In support of this goal, we will use our wind-tunnel assay to verify which contaminants of the synthetic pheromone arising during synthesis or as breakdown products cause variation in attractiveness and loss of potency after deployment in the field. We will assist field testing by providing candidate field lures.
2. Testing if conventional wind-tunnel assays are effective in helping refine the most active blend for a **female lure** based on **host-plant volatiles**. As one alternative assay method, we will use a two-choice assay in which **mated** females are confined in a 4 L glass chamber with access to two ports that contain either of the candidate lures: a positive control ("almond meal") or a negative (unbaited) control. We also will evaluate a conventional Y-tube choice assay system. As these assays will be run mainly off-season (when moths are not flying), their purpose is to speed up defining the most useful blends for field testing.

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 (NOW) moths for approximately one month. This recent breakthrough permits pest managers to monitor populations more effectively and economically 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, therefore guiding decisions on the need for control measures.

Our collaborative work on the identification of host-plant volatile that causes males and females to orient to almond foliage or almonds has focused on devising a laboratory-based, rapid put-through behavioral bioassay. Our new method uses a large flight chamber that permits male and female moths to enter baited jars though a small (1-cm diameter) port and permits the simultaneous evaluation of up to four treatments. This method is effective in

establishing which treatments are attractive and therefore worthy of further refinement. This method is advantageous in that it is not reliant on field populations and 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 or delta traps, weekly replacement of females, 3 per trap). 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 (for details on methods see Kanno et al. 2010). We will turn our focus away from the potential inhibitory effects of contaminants of the aldehyde component (such as its geometrical isomers) to possible contaminants in the two alcohol constituents.

Development of a host-plant volatile bioassay. Our host-plant volatile bioassay uses a large screened cage (**Figure 1**) 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 scotophase (the dark phase in a cycle of light and darkness) 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 (**Figure 1**, seeinserts) loaded with natural bait such as almond press (positive control) are compared to jars baited with candidate lures (e.g., almond mummies or synthetic chemicals supplied by John Beck) or empty control jars (negative control). Moths enter jars via a funnel through a 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-day 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. We collect captured moths after the end of the 2nd scotophase. All captured moths are placed in a 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.

Results and Discussion:

Development of effective pheromone lures. Brad Higbee's field studies confirm that the Suterra membrane formulation is useful as bait in monitoring traps. **Figure 2** shows the relative performance in traps of females versus fresh lures versus lures aged 2, 4 or 6 weeks. 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 also suggest that the decline in potency with time of exposure of the lure in the field is 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). This needs to be determined, although at this time we not have identified any inhibitors (several logical candidates have been tested in our wind tunnel but they have no effect on 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 when used in mating disruption (Minks and Cardé 1988, Cardé and Minks 1995).

Development of a host-plant volatile bioassay. We have developed a diagnostic laboratory method to test attraction of NOW adults to host-plant volatiles (from natural substrates and or synthetics of candidate attractants identified by John Beck). An example of the bioassay results is provided in **Figure 3**. So far our work has found that in this assay the only responding class of NOW adults are mated females. However, in the case of other moths, host volatiles may be attractive to females and males. In the case of the codling moth, the pear ester usually adds to the attractivity of 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. They also demonstrate that odor cues released by host plants can be powerful lures useful for pest management decisions.

Research Effort Recent Publications:

Girling, R.D., B.S. Higbee and R.T. Cardé. 2013. The plume also rises: trajectories of pheromone plumes issuing from point sources in an orchard canopy at night. J. Chem. Ecol. (in press)

References Cited:

Beck, J.J., and B.S. Higbee. 2013. Volatile natural products for monitoring a California tree nut insect pest. In *Pest Management with Natural Products*. (Eds. Beck, J.J., Coats, J.C., Duke, S.O., Koivunen, M.J.). American Chemical Society Publishing. Washington, D.C. (in press)

Cardé, R.T. and A.K. Minks. 1995. Control of moth pests by mating disruption: successes and constraints. Annu. Rev. Entomol. 40:559-585.

Kanno, H., L.P.S. Kuenen, J.G. Millar and R.T. Cardé. 2010. Attractiveness of a four-component pheromone blend to male navel orangeworm moths. J. Chem. Ecol. 36:584-591.

Knight, A.L. and D.M. Light. 2005. Seasonal flight pattern of codling moth (Lepidoptera: Tortricidae) monitored with pear ester and codlemone-baited traps in sex pheromone-treated apple orchards. Environ. Entomol. 34:10-281035.

Minks, A.K. and R.T. Cardé. 1988. Disruption of pheromone communication in moths: is the natural blend really most efficacious? Entomol. Exp. Appl. 49:25-36.

Pinero, J.C. and S. Dorn. 2009. Response of female oriental fruit moth to volatiles from apple and peach trees at three phenological stages. Entomol. Exp. Appl. 131:67-74.

Figure 1. Bioassay cage with insets pictures of the capture jars. The cage is 1.5 m high and 1.5 m across. Virtually no males are captured in any treatment that we have tested to date and empty jars (negative controls) captured ca. 1% of the released moths (0-2 moths per replicate).

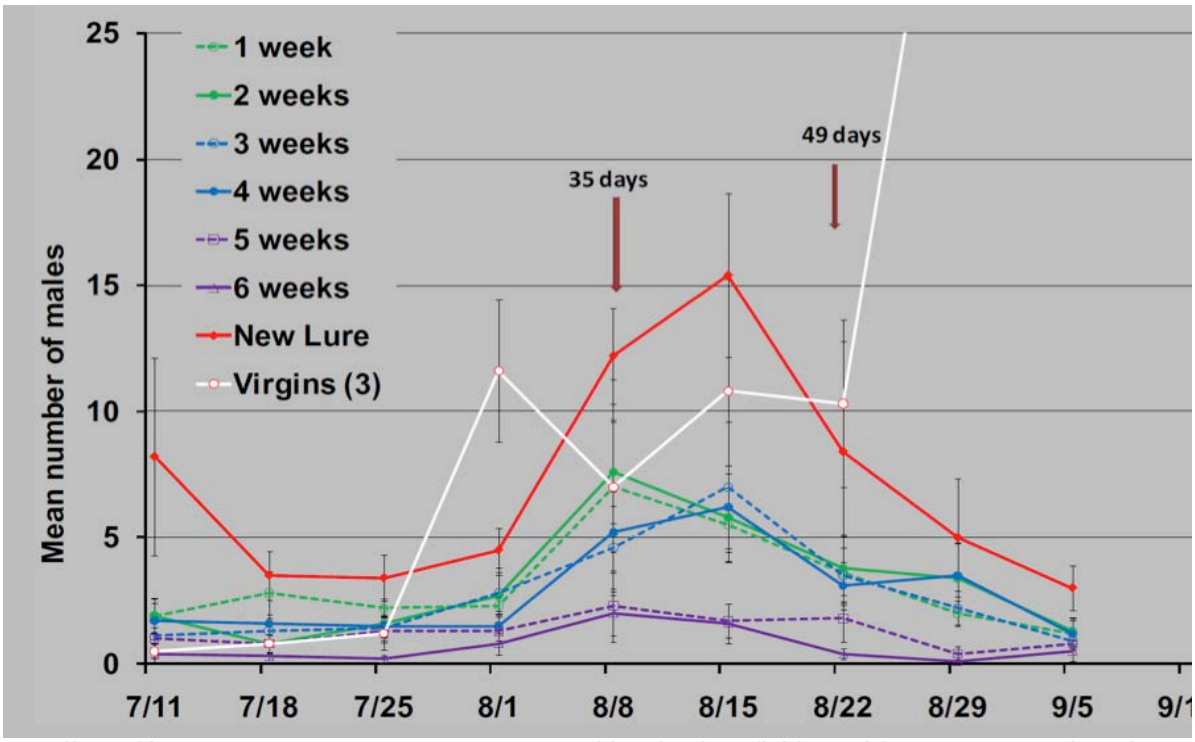

Figure 2. Effect of lure age on attractancy. Lures were either fresh or field-aged for 2, 4 or 6 weeks prior to the start of the test. Traps with females held 3 virgins. Fresh lures out performed older lures and were comparable to females (see text). Error bars are standard errors of the means. Data from Beck and Higbee (2013).

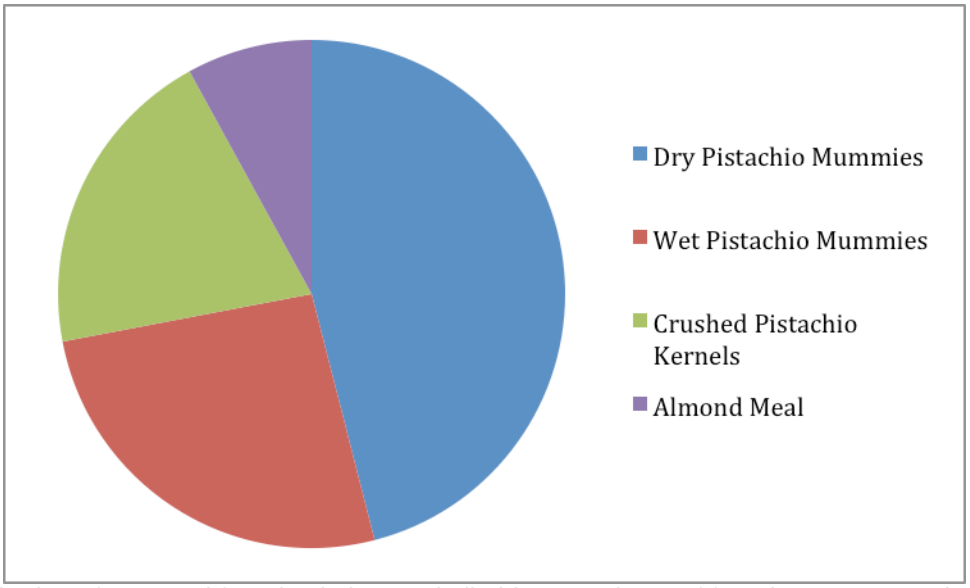

Figure 3. Distribution of captured females in host-volatile bioassay (99% of females were mated; only ca. 1% of the males were captured). The test was replicated 10 times with 150 females and 150 males released per replicate. All distributions differ at $P<0.0001$ by ANOVA.