Improving Mating Disruption and Monitoring Lures of the Navel Orangeworm (NOW)

Project No.: **11-ENTO9-Cardé**

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Objectives:

1. Compare in a wind tunnel assay the in-flight and close-range male orientation and courtship behaviors evoked by point-source formulations of pheromone, including dispensers prepared for evaluation in the field. Determine which blend of components evokes the highest proportion of source finding.
2. Use the wind tunnel-assay to determine why some batches of synthetic pheromone and formulated lures are more attractive than others. In collaboration with Brad Higbee, field test formulated dispensers of pheromone for efficacy as lures in traps.
3. Determine the role of sound in courtship.
4. Determine the pattern of pheromone dispersal, with particular attention to vertical movement of odor plumes, using the almond orchard as a model habitat. These measurements will aid in determining the optimal height in the canopy for placement of monitoring traps and puffers used for mating disruption.

Interpretive Summary:

We have two overarching goals: 1) to improve the efficacy of mating disruption in the navel orangeworm moth, *Amyelois transitella*, referred to as “NOW.” 2) To aid in the development of a highly attractive lure that would be useful as a monitoring tool in pest management programs. To these ends we have: 1) Optimized the composition and ratio of components for maximal male attraction in a wind tunnel using a simple, filter-paper dispenser. 2) Shown that four expected breakdown products of the main aldehyde pheromone component do not affect attraction. 3) Tested in the wind tunnel a number of lures designed to provide longevity in the field and competitiveness with female-baits. 4) Documented the courtship behaviors and sensory inputs (pheromone, sound and tactile) that occur once the male and female are in proximity. 5) Established the patterns of pheromone dispersal in almond orchards using visual tracers and measured wind flow and turbulence patterns with a sonic 3-D anemometer.

There are a number of potential components of the pheromone (Leal et al. 2005). One of our goals has been to verify which of the 9 published compounds from this and other published work by Millar, Kuenen and others (as well as others newly discovered but unpublished) mediate attraction and courtship. We have now defined an optimal blend of 4 components (Kanno et al. 2010; Kuenen et al. 2010). Such information is a crucial to devising highly attractive lures for monitoring traps. Furthermore, it is widely thought that the complete pheromone blend should be the most efficacious mixture for mating disruption (Minks and Cardé 1988, Cardé and Minks 1995; Cardé 2007). Our “best” 4-component blend, however, does not match in a wind-tunnel comparison the attractiveness of female extract. This tells us that we are still missing a pheromone component and/or the synthetic blend contains a contaminant that is inhibitory to attraction.

Materials and Methods:

1. **Blend Optimization**. To characterize the blend we have used a large (3-m-long) wind tunnel and monitored the behavior of individual male NOW moths from take off until landing on a candidate lure, including time on the lure. When there are 4 or more potential components, there are many possible combinations, ratios and dosages to evaluate. For example, does the presence of a compound in addition to the main component (*Z,Z*)-11,13-hexadecandienal, affect attraction and, if so, is its ratio to other components crucial to efficacy? Because of the large number of possible combinations using an additive approach to testing blends, we instead used a subtractive paradigm to define the best blend. This blend is now published (Kanno et al. 2010) and is one standard used when we examine the possible suppressive effects of breakdown products of the aldehyde component or possible new components.
2. **Courtship and sound.** We previously reported the sonographs produced by male wing fanning (with sound production extending into the ultrasound range). To determine the possible influence of sound produced by the male on the behavior of a calling (pheromone-releasing) female, we monitored in our wind tunnel with video the frequency and rapidity of mating in males that were sound-muted (i. e., their tegulae were removed). Their success in mating was compared to two control groups, males that were sham-operated and males that received no experimental manipulation.
3. **Field observations pheromone dispersion.** To document the patterns of pheromone dispersion we have photographed the “smoke” issuing from point sources of titanium tetrachloride at various heights in the almond canopy. Our efforts are aimed at documenting thorough field observation how males might have to navigate along pheromone plume to locate females within a tree canopy and further to suggest an optimal height for deployment of pheromone puffers used in mating disruption. We have extended our observations by recording in an almond orchard wind flow in 3-D with a sonic anemometer system that is sensitive to wind movements >2 mm per second in all directions. We collected continuous 5-minute-samples at 60 readings per second every 3 hours over 4 consecutive days. Samples were taken at 6 heights ranging from 2.08 to 6.65 m (within and above the canopy). These measurements created a huge data base with multiple possibilities for analysis.

Results and Discussion:

1. Upwind flight along the pheromone plume and landing on the odor source required the simultaneous presence of two components, (11*Z*,13*Z*)-hexadecadienal, (3*Z*,6*Z*,9*Z*,12*Z*,15*Z*)-tricosapentaene, and the addition of either (11*Z*,13*Z*)-hexadecadien-1-ol or (11*Z*,13*E*)-hexadecadien-1-ol. A mixture of all four components produced the highest levels of rapid source location and source contact. In wind-tunnel assays, males did not seem to distinguish among a wide range of ratios of any of the three components added to (11*Z*,13*Z*)-hexadecadienal. Dosages of 10 and 100 ng of the 4-component blend produced higher levels of source location than dosages of 1 and 1000 ng (Kanno et al. 2010; Kuenen et al. 2010). The broad range of component ratios that evoke attraction will simplify some aspects of development of a field lure.
2. We are working with Walter Leal and Jocelyn Millar is trying to identify contaminants and breakdown products that may compromise the efficacy of lures in the field. So far 4 possible contaminants have been evaluated for their possible suppressive effect on attraction. These compounds were prepared and supplied courtesy of Bedoukian Research. Briefly, adding any of the three geometrical isomers of the aldehyde pheromone component (the 11Z, 13E, the 11E, 13Z, and the 11E, 13E) pheromone component to the complete blend did not diminish attractiveness. Work with many other moth species often has found that geometrical isomers of monounsaturated or doubly unsaturated pheromones are inhibitory to attraction, but this is not the case with the NOW. Similarly, the adding the acid of the aldehyde to the 4-component blend also did not affect attractiveness. This is very helpful to lure formulation, in that such breakdown products would be expected in field-exposed lures.

   A second approach has been to compare, for example, lures using the principal pheromone component (the aldehyde) from different sources, or following purification, while holding the other 3 components constant. Clearly batches have differed somewhat in their attractively, but the chemical identity(s) of the antagonist(s) remains unclear. We also have evaluated synthetics of the aldehyde, alcohol and hydrocarbon components with capillary gas chromatography linked to an electroantennographic detector (GC-EAD) in Jocelyn Millar's lab. As expected, the pheromone components evoked a strong EAG response, but there was no evidence of any other components (contaminants) evoking an EAG response. These GC-EAD runs thus have offered no evidence of unknown inhibitory contaminants.

   In Christer Lofstëdt's group at Lund University, 4 new components of the NOW pheromone gland have been discovered and definitively characterized. These have been tested in wind-tunnel trials at UCR, but to date no boost in attractivity can be ascribed to these compounds, even though two of these are EAG active. Work on various combinations and ratios of these added to the established 4-compoent blend continues.

   Work on lures for the field has relied on membrane dispensers prepared by Suterra. These have been evaluated in the field by Brad Higbee and in our wind tunnel. Our tests used dispensers aged outside in traps between wind-tunnel evaluations. A few dispenser and synthetic combinations have proved encouraging. For example, two of these were evaluated with 360 males each over 50 days. One formulation evoked 31.1% lure contact and another evoked 35.8% lure contact; both formulations appeared to be as attractive at the end of the test as at the beginning. As heartening as these findings were, we note that an experimental control treatment of 3 female equivalents (pheromone gland extract) evoked a source finding rate of 81.1%.

3. Courting males produce ultrasonic "clicks" during wing fanning. This signal inhibits calling (pheromone-emitting) females from moving away when contacted by a male and increases the likelihood of mating. This study is complete and is being prepared for publication.
4. Nighttime observations by Brad Higbee in almond orchards found that pheromone plumes might have considerable vertical movement, sometimes exceeding their rate of horizontal flow. Working with Brad Higbee, we documented this dispersal pattern, initially using titanium tetrachloride sources positioned from a meter above ground level to the top of the canopy. These sources produce a white "smoke." The photographic records verified considerable vertical displacement of the tracer plume. In 2010 we measured wind flow in 3-D with a sonic anemometer at 6 heights from 2.08 to 6.65 meters above the ground level, monitoring the speed, turbulence and direction of airflow for 5 minutes at each height every 3 hours over 4 days. We have now analyzed these recordings and are preparing a publication, but we provide some new comparative data (**Figures 1 & 2**). These records show that a marked net flow of air during the time when NOW mate (late night to dawn) is upward and not simply planar as has been generally assumed. Air flow in daytime also is upward. The rate of upward flow is approximately one-tenth of planar flow (**Figure 2**), but that would be sufficient to push pheromone through and out of the canopy.

   One practical implication of these observations for mating disruption is that when pheromone puffers are deployed at height of the top of the orchard canopy, as is current practice, much of their output will be propelled to above the canopy. This suggests that much of the output of puffers so deployed would be unavailable to disrupt NOW mate finding. This suggests that puffers would be just as (or more) efficacious if deployed and mid or lower canopy levels. As a result of these observations and his own work, Brad Higbee has evaluated male capture in female-baited traps positioned at several canopy heights and the effect of the height of puffers on the efficacy of mating disruption.

Research Effort Recent Publications:

Girling, R.D. and R.T Cardé. 2006. Analysis of the courtship behavior of the navel orangeworm, *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae), with a commentary on methods for the analysis of sequences of behavioral transitions. J. Insect Behav. 19:497-520.

Kanno, H., L.P.S. Kuenen, K.A. Klingler, 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.

Wang, H.-L., C.-H. Zhao, J. G. Millar, R.T. Cardé and C. Löfstedt. 2010. Biosynthesis of unusual moth pheromone components involves two distinctly different pathways in the navel orangeworm, *Amyelois transitella*. J. Chem. Ecol. 36:535-547.

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Cardé, R.T. 2007. Using pheromones to disrupt mating of moth pests. In: *Perspectives in Ecological Theory and Integrated Pest Management*, M. Kogan and P. Jepson (eds.) Cambridge University Press, Cambridge, pp. 122-169.

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, K.A. Klingler, 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.

Kuenen, L.P.S., J.S. McElfresh and J.G. Millar. 2010. Identification of critical secondary components of the sex pheromone of the navel orangeworm, (Lepidoptera: Pyralidae). J. Econ. Entomol. 103:314-330.

Leal, W.S., A.L. Parra-Pedrazzoli, K.-E. Kaissling, T.L. Morgan, F.G. Zalom, D.J. Pesak, E.A. Dundulis, C.S. Burks and B.S. Higbee. 2005. Unusual pheromone chemistry in the navel orangeworm: novel sex attractants and a behavioral antagonist. Naturwissenschaften 92: 139-146.

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.

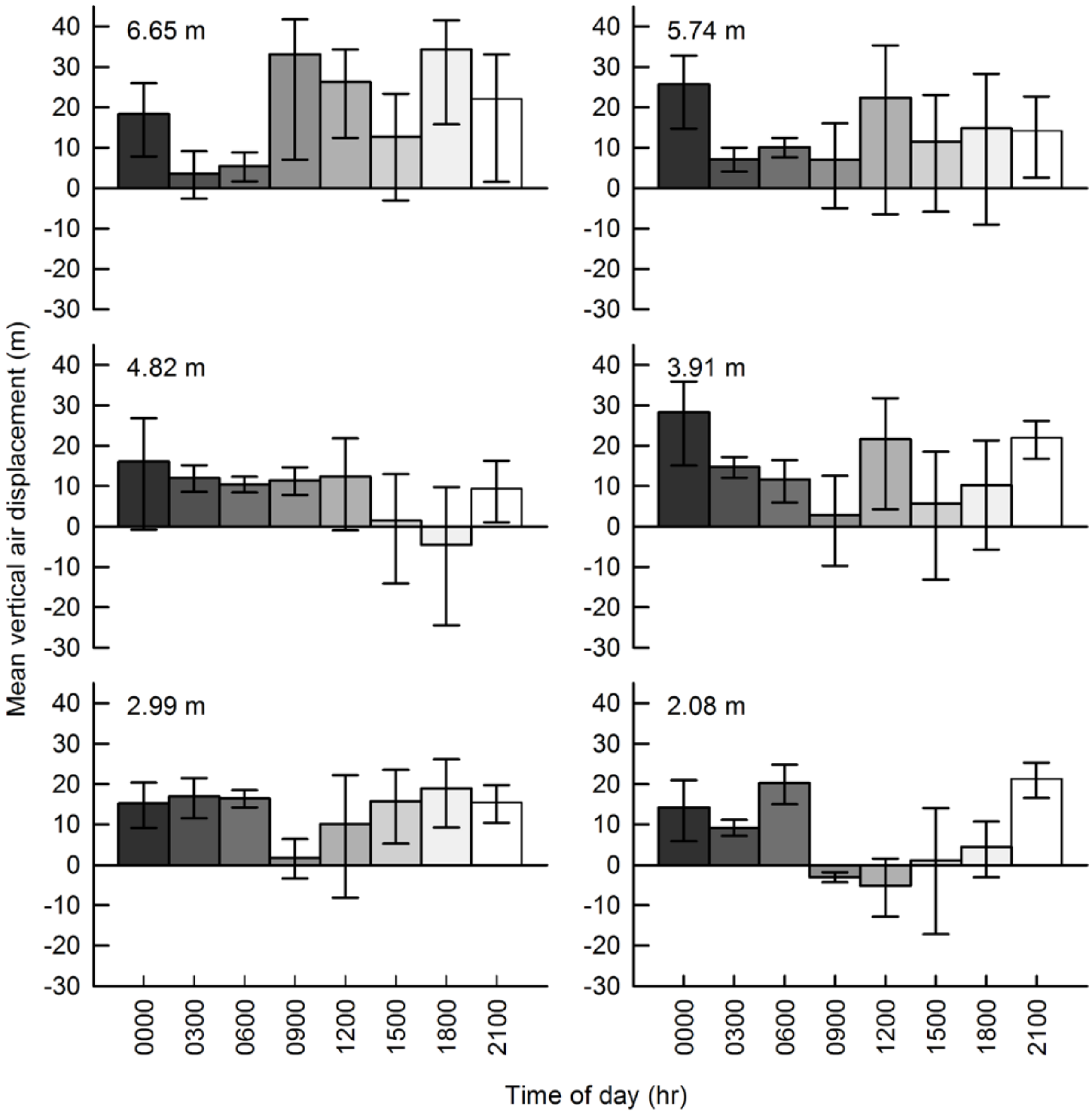

Figure 1. Mean vertical air displacement in meters over a five minute period (±SE) recorded within (<5 m) and above (>5 m) the canopy of an almond orchard. A positive value equates to a net rise in air movement and a negative value a net fall. Measurements were taken at six different heights above ground level every 3 h between July 13 and 17, 2010.

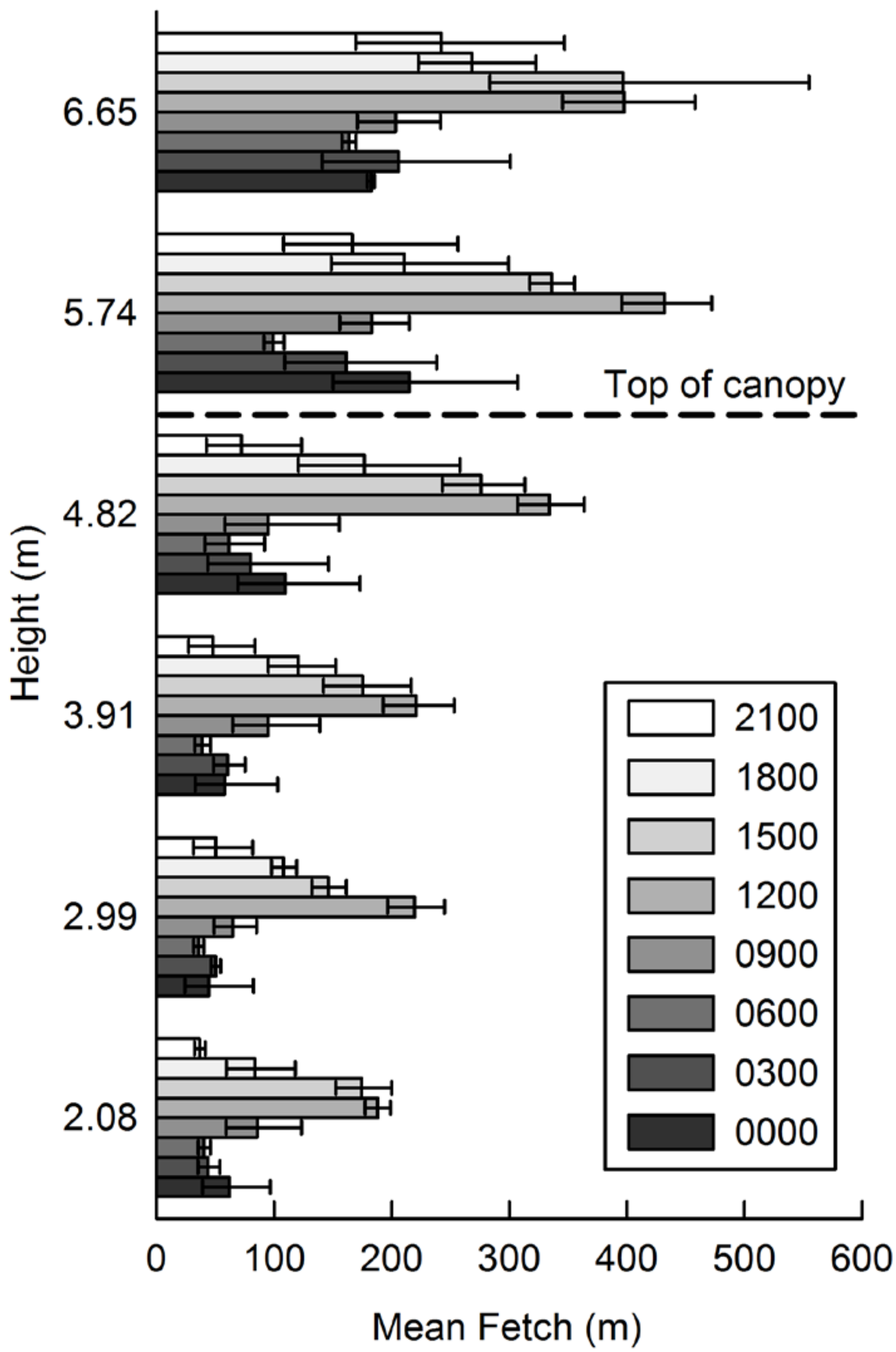

Figure 2. Mean fetch in meters over a five minute period (±SE) recorded within (<5 m) and above (>5 m) the canopy of an almond orchard. Measurements were taken at six different heights above ground level every 3 h between July 13 and 17, 2010. Results shown in bars above the dotted line were recorded above the canopy of the orchard.