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

Project No.: **09-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. Determine which blend of components evokes the highest proportion of source finding.
2. Use the wind tunnel-assay to find out why some batches of synthetic pheromone are more attractive than others. Field test formulations of pheromone for efficacy as lures in traps.
3. Determine the role of sound in courtship.
4. Determine the pattern of pheromone dispersal using the almond orchard as a model habitat. This may aid in optimal trap and puffer placement within the canopy.

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. 2) Documented the courtship behaviors and sensory inputs that occur once the male and female are in proximity. 3) Documented 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 One of our goals has been to verify which of the 9 published compounds (as well as others unpublished) mediate attraction and courtship.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 step in 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).

Materials and Methods:

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.

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.

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 navigate a pheromone plume to locate females within a tree canopy. Recently we have extended our observations by recording the wind flow in 3-D with a sonic anemometer system.

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 (See Project No 09-ENTO2-Leal) and Jocelyn Millar is trying to identify contaminants and breakdown products that may compromise the efficacy of lures in the field. The 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 differ in their attractively, but the chemical identity (s) of the antagonist(s) remains unclear.

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 indicated that pheromone plumes might have considerable vertical movement, often exceeding their rate of horizontal flow. Working with Brad Higbee, we confirmed this using titanium tetrachloride sources positioned from a meter above ground level to the top of the canopy. The photographic record indeed verified this phenomenon. This field season we documented wind flow in 3-D with a sonic anemometer at 6 positions from 2 to 6 meters above the ground level, monitoring flow for 5 minutes at each height every 3 hours over 4 days. We are now analyzing these recordings. These wind flow patterns will be compared with the findings of Brad Higbee on male capture in female-baited traps positioned at several canopy heights.

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.

References Cited:

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, E.A. Dundulis and C.S. Burks. 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.