Mating Disruption of the Navel Orangeworm

Project No.: 08-ENTO9-Carde

Project Leader: Ring T. Cardé
Department of Entomology
UC Riverside
Riverside, CA 92521
(951) 827-4492
e-mail: ring.carde@ucr.edu

Project Cooperators and Personnel:
Brad Higbee, Paramount Farms
Jocelyn Millar, Entomology, UC Riverside

Objectives:

1. Compare in a wind tunnel assay the in-flight and close-range male orientation and courtship behaviors evoked by point-source formulations. Determine which blend of components evokes the highest level of source finding.
2. Determine the role of sound in courtship.
3. Use the large-cage method described by Koch et al. (2008) to compare disruptant formulation types, rates and blend combinations.

Interpretive Summary:

We have two 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) Explored whether the large field-cage assay method successfully used by Koch et al. (2008) in Europe to compare disruptant formulations in vineyards and trellised dwarf fruit tree orchards can be used in California in almond orchards with their larger trees.

There are a number of potential components of the pheromone, based on two studies of the constituents of the NOW pheromone gland (Leal et al. 2005; Millar 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 One of our goals has been to verify which of the 9 published compounds (as well as others unpublished) mediate attraction and courtship. Such information should aid 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:

1. Blend Optimization

To characterize the blend we have used a large (3-meter-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 as in this case 4 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 have instead used a subtractive paradigm to define the best blend. We look for likely complete blends by dropping out individual components. If a component proves important to attraction, we then vary its ratio to determine the optimum.

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 a calling (pheromone-releasing) female, we monitored with video in a wind tunnel the frequency and rapidity of mating in males that were sound muted (tegulae removed), compared to two control groups, males that were sham operated and males that received no experimental manipulation.

3. Field observations of mating and its disruption

Our efforts are aimed at documenting thorough field observation how males locate females within a tree canopy. We generally have followed the setup of Koch et al. (2008) in the design of cages.

Results and Discussion:

1. Blend Optimization

These evaluations are complete and what is clear is that the "main" component, (*Z,Z*)-11,13-hexadecandienal, presented alone does not attract males to the source. Addition of the C23 compound is crucial to achieving a modest level of attraction, and addition of the ZZ-OH component further increases lure attractiveness. A 4^{th} previously unknown component added to these 3 further increases attraction. As well, this 4^{th} component can augment the attractiveness of the (*Z,Z*)-11,13-hexadecandienal-C23 compound two-component mixture, in essence substituting for the ZZ-OH component. Unlike many other multi-component systems in moths in which component ratio is a crucial feature of blend attractivity, the ratios of these 3 components to (*Z,Z*)-11,13-

hexadecandienal has surprisingly little effect on the attractiveness of a lure. All of the other potential pheromone components reported by Leal et al. (2005) did not prove to augment the level of attraction when added to our 4-component mixture. These wind tunnel trials entailed testing of some 5,000 individual males in the wind tunnel. These findings have been written up for publication and are now undergoing internal reviews by all authors.

We now will utilize this method to compare synthetic pheromones from various commercial sources. Issues of compound purity have long served as an explanation for lure ineffectiveness in the field. Even with the 4-component blend, however, we find in our wind tunnel assay that different sources of synthetic (*Z,Z*)-11,13-hexadecandienal have caused differing levels of attractiveness. This work will be collaborative with Walter Leal of UC - Davis. The immediate goal of this subproject is to develop a synthetic blend that will be useful in traps for field monitoring of NOW populations.

2. Courtship and sound

An unexpected discovery in our studies of NOW mating behavior (Girling and Cardé 2006) was the complexity of the mating sequence. In our wind tunnel observations, females are often quite coy, causing males to chase them until either they lose tactile contact or a successful copulation ensues. We hypothesized that sound produced by the male during courtship might signal to females that they are a genuine suitor and that females were not being touched by a potential predator. Wing fanning produces ultrasonic sound bursts coincident with each wing beat with a frequency up to 75 kHz. The sound is enhanced by the tegulae, small flaps near the base of the forewing. We have now verified the effects of sound on the female's acceptance of a male by muting the male's production of the sound signal by removal of the tegulae. When their tegulae are intact, males are twice as likely to mate and those that do mate do so twice as quickly. Close-range orientation that is mediated in part by sound also may explain how males find females when mating disruptants are used. This study is also complete and is also is undergoing internal review before submission for publication.

3. Field observations of mating and its disruption

A final area of investigation with Brad Higbee has sought to use the large cage method to speed up evaluation of disruptant formulations and the precision with which formulations can be ranked for efficacy of disruption. Although this method has been used with great success in Europe in low canopy vineyards and low trellised orchards (Koch et al. 2008), its use in California almond orchards has provided some unexpected challenges. We have improved our capture rates in a check (untreated) cage with this system, but Brad Higbee has uncovered in the process new factors that clearly affect mate finding and its disruption. We are currently documenting these phenomena.

References:

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.

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.

Koch, U.T., E. Doye, K. Schumann and U. Andrick. 2008. CIRCE – an addition to the toolbox for assessment of mating disruption. IOBC Proceedings, Lund, Sweden, September 2007.

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.

Millar, J., G.G. Grant, J.S. McElfresh, W. Strong, C. Rudolph, J.D. Stein and J.A. Moreira. 2005. (3Z, 6Z, 12Z, 15Z)-pentacosapentanene, a key pheromone component of the fir coneworm moth, *Dioryctria abietivorella*. J. Chem Ecol. 31:1229-1234.

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.