Mating Disruption of the Navel Orangeworm

Project No.: **08-ENTO9-Carde**

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Objectives:

1. Use the large-cage method described by Koch et al. (in press) to compare disruptant formulation types, rates and blend combinations (the complete mixture vs. partial blends).
2. Compare in a wind tunnel the in-flight and close-range male orientation and courtship behaviors evoked by point-source formulations. Determine whether exposure to synthetic pheromone impairs subsequent responsiveness to females and pheromone lures. These wind tunnel studies will help forecast successful strategies for field deployment of formulated pheromone for mating disruption.
3. Measure the rate and ratios of pheromone components released by females.

Interpretive Summary:

Our overall goal has been to improve the efficacy of mating disruption in the Navel Orangeworm moth, *Amyelois transitella*, referred to as “NOW.” To that end we have worked to: 1) improve our understanding of the composition of the pheromone blend used by the female NOW to lure a male; 2) document the courtship behaviors and sensory inputs that occur once the male and female are in proximity; 3) determine if the large field-cage assay method successfully used by Koch et al. (in press) in Europe to compare disruptant formulations in vineyards and trellised dwarf fruit tree orchards can be used in California in almond orchards with their considerably 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 One of our goals has been to verify which of the 9 published compounds (as well as others unpublished) mediate attraction and courtship.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).

Both of these needs justify the effort required to define the optimal blend. To characterize the blend we use a large (3-m-long) wind tunnel and monitor the behavior of individual male NOW moths from take off until landing on a candidate lure, including time on the lure. There are a large number of possible combinations to test. 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 with an additive approach to testing blends, we use 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 the ratio of that component to determine its optimum. Some of the data from a typical test (of more than a dozen performed, testing over 4,000 males) is provided in Fig. 1. In Fig.1B, the four component blend evoked more rapid source finding; other tests (not shown) have demonstrated the C23 compound is crucial to attraction and the compounds ZZ-OH or "A" added to the two-component mix substantially improved percent attraction. Jocelyn Millar and colleagues are preparing this for publication and it will be available shortly. Work on the ratio emitted by females is underway.

An unexpected finding in our studies of NOW mating behavior (Girling and Cardé 2006) was the complexity of the mating sequence—with females often being quite coy, causing males to chase them until either they lose contact or a successful copulation ensues. We wondered what cues might be produced by the male to indicate its identity as a genuine suitor (as opposed, for example, to a predator). We have found that wing fanning produces ultrasonic sound bursts coincident with each wing beat. The sound is enhanced by tegulae, small flaps near the base of the forewing (Fig. 2). We are now verifying the effects of sound on the female's acceptance of a male and its possible role in orientation per se. Close-range orientation that is mediated in part by sound may explain how males find females when mating disruptants are used.

A final area of investigation with Brad Higbee has sought to use the large cage method in the orchard 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. in press), 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. These are being explored.

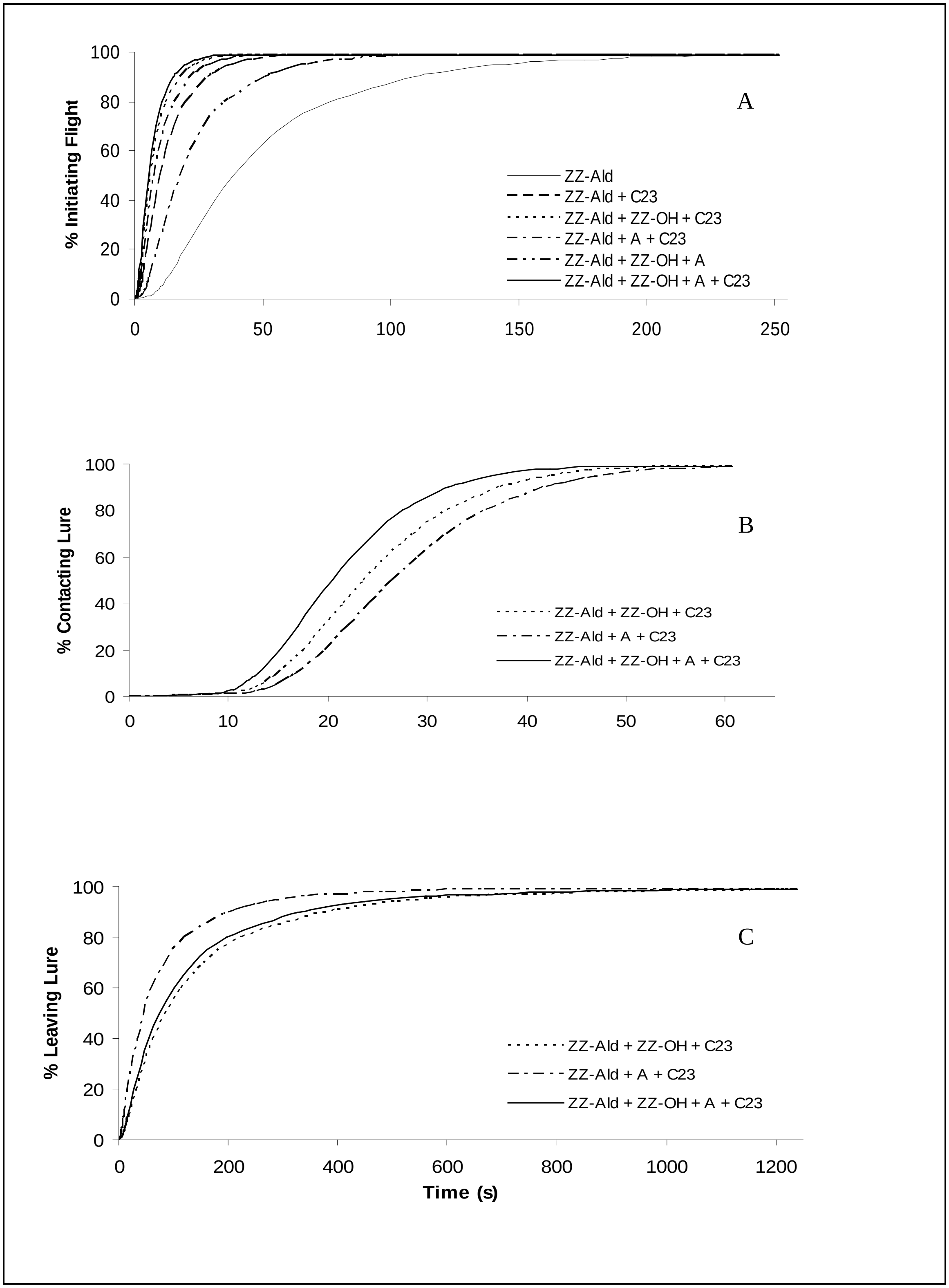

Figure 1. Flight initiation (A), lure contact (B), and contact duration (C) for *Amyelois transitella* with six pheromone blends estimated by Regression for Life Data with a lognormal distribution in Minitab 14.

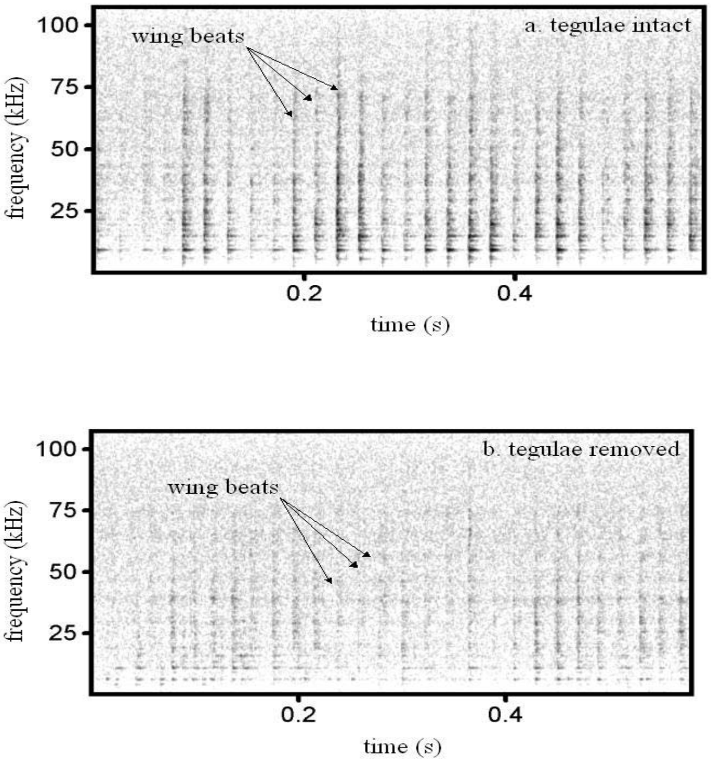

Figure 2. Sequences of sound impulses (including ultrasonic frequencies to 100 kHz) produced by wing fanning of male *Amyelois transitella* in response to a calling female: **a)** untreated (tegulae intact) male and **b)** male with both tegulae removed. Wing beats occur at ~ 0.02 s intervals during wing fanning, and the amplitude of sound is attenuated by removing the tegulae. Males were recorded from a distance of 1 cm with an Avisoft Bioacoustics CM16 / CMPA microphone (frequency range: 10-200 kHz) interfaced with the Ultrasoundgate 116-200 converter and Avisoft SASLab Pro recording software.

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