Standard and Commercial Formulations for Navel Orangeworm Sex Pheromone

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The navel orangeworm (**NOW**, *Amyelois transitella* Walker) is of course the major insect pest of almonds in California's Central Valley, and appeared here in the late 1940's. A dramatic expansion of almond plantings in California has coincided with increased demand for nut products, which has led to an increased demand to reduce NOW damage.

Monitoring insect pests with sex pheromone-baited traps has become a fairly standard practice in US agriculture and an aid to managing insect populations; however, elucidation of the sex pheromone for NOW was elusive. The primary sex pheromone component (Coffelt et al. 1979) is not very attractive by itself to NOW males (Kuenen et al. 2001). A paradigm shift in basic premises of lepidopteran sex pheromone constituents, led to the elucidation of a four component sex pheromone blend that is as attractive as female pheromone glands (Kuenen et al. 2010) and the attractiveness of only these four components was subsequently verified (Kanno et al. 2010).

Preliminary field trials confirmed that the 4-component blend could capture as many or more NOW males than females (Fig. 1); however, it can be seen from this data that lure effectiveness declined rapidly during the 4-day test, compared to trap catch by female-baited traps, even during the cool, short-daylight days of October. Subsequent field tests by Kuenen and Millar (unpublished) could only replicate trap capture equivalent to female baited traps for 1-to-2 days in the summer even though more than 10 formulations were tested.

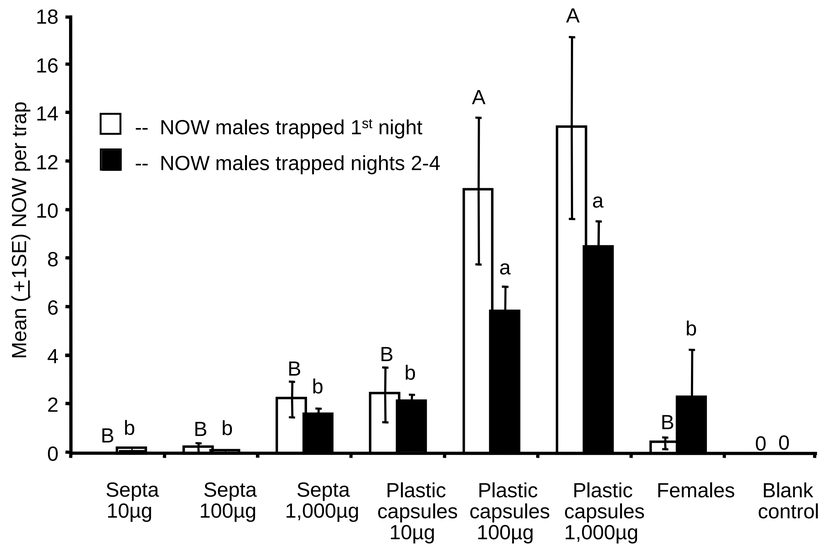


Fig. 2

Synthetic NOW Pheromone With Isomers; GC/MS SIM Mode

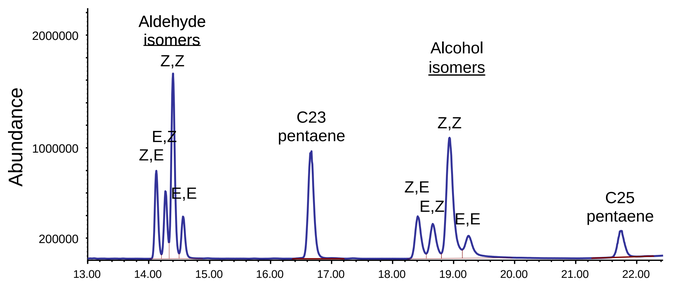
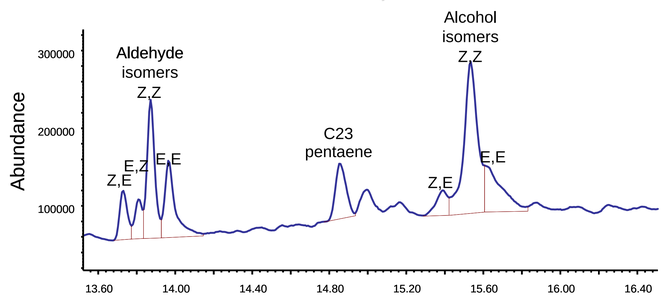


Fig. 3

1 Female NOW Gland Extract; GC/MS SIM Mode



The pheromone components (Fig. 2) emitted by the NOW females are believed to be unstable under field conditions due to their chemical structure (conjugated dienes – prone to isomerization) and the terminal aldehyde moiety of the main component (likely to form trimers). Our first step toward developing a more stable field trap bait was examination of the pheromone components extracted from one gland (Fig. 3) vs. the multiple-glands that were the basis for our pheromone identification. Next, an examination of the ratios of the pheromone components volatilized from the female pheromone glands and prospective trap-bait formulations is required so that release ratios from formulations can be compared to female-emitted ratios to determine how they change over time during normal field use. Pheromone gland extracts were made by extraction of excised glands in hexane for 30 min. Pheromone volatiles are assessed by manual eversion of female sex pheromone glands/ovipositor tips (Fig. 4; after Baker et al. 1981) with volatiles collected on and open capillary tube (after Shani and Lacey, 1984; Fig. 5) that allows us to use small amounts of solvent for collection of adhered pheromone molecules.

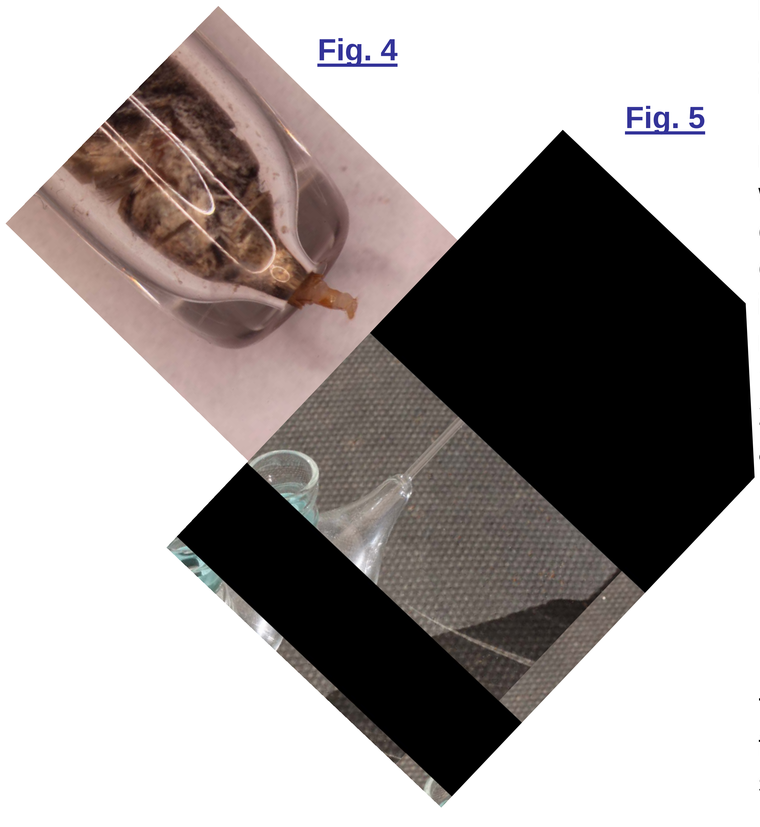


Fig. 4

Fig. 5

Secondly, we cleaned 3 prospective pheromone formulations by solvent extraction (Fig. 6) and retested our pheromone blend in the field during hot summer weather (Fig. 7). The pheromone compounds were chemically stabilized against isomerization and UV degradation, and we noted that traps with cleaned grey rubber septa captured as many males as female-baited traps for one week; moth flight stopped for the next preventing longer assessment.

Fig. 6

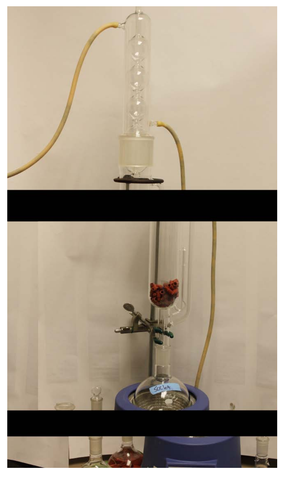
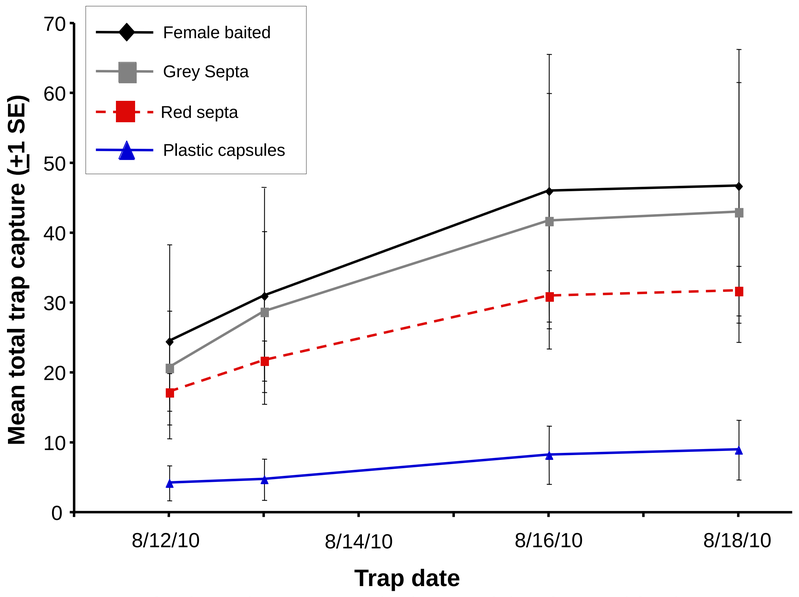


Fig. 7 **NOW trap capture - August 12-18, 2010**
Female baits vs. cleaned pheromone releasers



Trap captures of male navel orangeworm using lures consisting of unmated female NOW, grey rubber septa, red rubber septa or plastic capsules loaded with 1mg of the four-component pheromone blend (*Z*11,*Z*13-16:Ald; *Z*11,*Z*13-16:OH; *Z*11,*E*13-16:OH; 3*Z*,6*Z*,9*Z*,12*Z*,15*Z*-23:H (100;100;5;5) with female-baited positive control traps; tests were conducted August, 2010.

We collected volatiles from filter paper disks that we employed in windtunnel bioassays during the pheromone identification process, by placing the disks in the volatile collection device (Fig. 5). We applied synthetic pheromone components in the same ratios as for the bioassays (Aldehyde:Alcohol:Pentaene -- 100:100:5) and found that the release ratios were (100:8.8:0.8 ; N=4). These ratios are close to those found in female gland extracts (100:5:5) and as noted by Kanno et al. (2010) the ratios of the components is not critical for to elicit NOW upwind flight and source contact, which were able to perform regularly in our bioassays with this blend (plus 5% ZE isomer of the alcohol component). Since the relative release rate of the pentaene was very low, we tested septa with equal loadings of all three pheromone components (above) from which we can calculate the release ratio of the pentaene. A chemically cleaned gray septum was loaded with 1mg of each of the pheromone components in 300ul solvent and then held in a fume hood except for volatile collections. Although we are not rigorously quantify the release rate from the septum the release rate of the aldehyde remained largely unchanged for 29 days (last measurement), whereas the ratio of the alcohol and pentaene fluctuated during this time period, below.

AGE(day)	Ald%	OH%	Pent.%
1	100	57	8.9
3	100	62	10.2
9	100	66	18.0
14	100	33	16.6
29	100	48	20.0

The alcohol release ratio was much higher then from lab assay filter paper whereas corrected pentaene measurements will be similar.

References:

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