Standard and Commercial Formulations for Navel Orangeworm (NOW) Sex Pheromone

Project No.: **10-ENTO12-Kuenen/Walse**

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Objectives:

1. Test "standard" laboratory-based matrices for the navel orangeworm (NOW) sex pheromone after exhaustive cleanup and stabilization procedures.
2. Compare lab-based matrices to appropriate proprietary commercial matrices as carriers for the 4-component NOW sex pheromone as time permits.
3. Develop a stabilized formulation that will provide effective and reproducible NOW lures with reasonable field longevity.

Interpretive Summary:

We exhaustively extracted (Soxhlet) rubber septa (those commonly used in pheromone lures for field traps) and plastic vials for formulating pheromone sources for lab and field tests. The first test to determine if our approach was valid was conducted in a field trapping experiment August, 2010. Cleaned red and grey septa plus plastic vials were loaded with purified pheromone components left over from our pheromone identification studies (Kuenen et al. 2010). As before, these lures were treated with chemical stabilizers to reduce degradation by heat and UV light. These lures plus female-baited traps and blank control traps were tested in the heat of summer (**Figure 4**) and found to be effective at trapping navel orangeworm (NOW) for at least one week compared to female-baited traps.

In addition, we collected sex pheromone from female NOW pheromone glands during the moths' optimal pheromone release time (Coffelt et al. 1979), and found that ratios of the four key compounds were variable among individuals. We also developed methods to collect and analyze pheromone components evaporating from female sex pheromone glands. In both sets of pheromone analyses, ratios of the four compounds (and their isomers) were affected by steps in the analytical process as well as the variability among individuals. These analytical issues have slowed our progress toward our end goal of a field lure, but are now largely resolved; the analyses of these volatiles are critical to development of longer lasting lures.

Materials and Methods:

Our current approach to developing a field lure for monitoring NOW is to use purified pheromone components placed on various "standard" and commercial formulation materials that have been thoroughly cleaned by chemical means. Then we measure NOW's trap capture by lures over time vs. traps baited with unmated females. We are also measuring the ratios of the pheromone components released from the lure formulations as they age in order to correlate trap capture changes with changes in chemical release profiles so they can be adjusted to obtain a longer lasting field lure.

We chemically cleaned rubber septa (those commonly used in pheromone formulations for the field) and plastic vials in a Soxhlet device for three days with hexane for formulating pheromone sources for lab and field tests. All cleaned substrates were held in clean hexane until they were used. ZZ-aldehyde was purchased from Bedoukian Research Inc. (Danbury, CT) and solutions of components remaining in our freezer from the project that identified the four component pheromone blend (Kuenen et al. 2010). Experience in the laboratory had shown that the main pheromone component obtained from Bedoukian was less active than the same compound synthesized and purified by Dr. Jocelyn Millar (JGM; UC Riverside) thus all bioassays were conducted with material obtained from JGM.

Assessment of chemical release ratios from female pheromone glands, rubber septa and other substrates was initiated with a hybrid all-glass collection device designed after Baker et al. (1982) that allowed insertion of extruded female pheromone glands and lure formulations. This was combined with an on-tube collection process demonstrated by Shani and Lacey (1984) which allowed us to use the least amount of solvent to remove the pheromone compounds for subsequent analyses. HPLC-grade hexane was used throughout these experiments. Volatiles collected in the device were eluted with hexane and analyzed by gas chromatography/mass spectrometry (GC/MS) and/or gas chromatography/flame ionization detector (GC/FID).

Initially, we experienced difficulties 1) resolving all isomers of aldehyde and alcohol components of the pheromone and 2) preventing isomerization of these two compounds due to handling techniques. While working to solve these issues we determined that a field trial using extracted substrates was required to determine if our basic approach was valid with standard laboratory lure formulations. We removed septa and plastic capsules from hexane storage and after three days they were loaded with one milligram of the ZZ-aldehyde and ZZ-alcohol components plus 5% of each of the other two components. Four red (Aldrich cat. # 712435-4) and four gray septa (West pharmaceuticals) and four plastic vials (1ml polyethylene BEEM capsules) were loaded with these compounds in 100 microliters hexane to which BHT and Sumisorb 300 were added to stabilize the pheromone components against degradation by heat and UV light. The solvent was allowed to evaporate (about 3 hrs.) and then lures were placed in our freezer at -20^{o}C. The following day, traps baited with these lures plus female-baited traps and blank control traps were placed in a pistachio orchard in a randomized complete block design. Traps were checked for male capture after the first night and then every 2-to-3 days for three weeks.

Results and Discussion:

After perusing GC column catalogs we found a column that separated all four geometrical isomers of the aldehyde and alcohol components. A mixture of synthetic pheromone components plus the C25 analog of the C23 pentaene were analyzed by GC/MS to verify the separation and identity of each component (and isomers where appropriate; **Figure 1**). Clearly all four isomers of the aldehyde and alcohol components were separated by the column and analysis of mass spectrometry (MS) spectra matched that of expected spectra for these authentic compounds. Pheromone compounds extracted with hexane (20 min) from female pheromone glands showed the presence of all four isomers of the aldehyde component and three isomers of the alcohol component (**Figure 2**) and the C23 pentaene was also clearly present. A pooled collection of pheromone volatiles from six females (**Figure 3**) also showed the presence of all four isomers of the aldehyde component and three isomers of the alcohol component, although the latter three did not fully match those from the gland extract.

Refining our techniques for working with these highly labile compounds and to verify that the female releases the same compounds as those we discovered in our identification of the pheromone components for this insect are crucial for developing a lure; we found that even the slightest mechanical perturbation of the solutions can lead to altered isomeric ratios of the aldehyde and alcohol components. In addition, the amount of pheromone emitted by this insect is so low that we had to pool samples or analyze the material from individual insects at near the limits of detection of our instruments. Both situations have the potential to reduce the accuracy of our measurements which will be crucial when we assess volatiles emitted from pheromone lures. We are now confident that our methods and protocols will not yield inappropriate results from lure volatile collections.

The field test was conducted, as noted above, to validate the premise that chemically cleaned lure substrates will catch male moths in the field for more than a couple of days. Lastly, since clean pheromone on chemically cleaned septa was effective for one week in the field (**Figure 4**) we conclude that we are moving in the right direction to obtain a viable field lure. We have also consulted other pheromone researchers with experience in preparing lures with unstable compounds and their advice will be incorporated into future work to lengthen the field life of NOW lures.

Research Effort Recent Publications:

References Cited:

Baker, T.C., Gaston, L.K., Pope, M.M., Kuenen. L.P.S., and Vetter, R.S. 1981. A high efficiency collection device for quantifying sex pheromone volatilized from female glands and synthetic sources. J. *Chem. Ecol.* 7: 961-968.

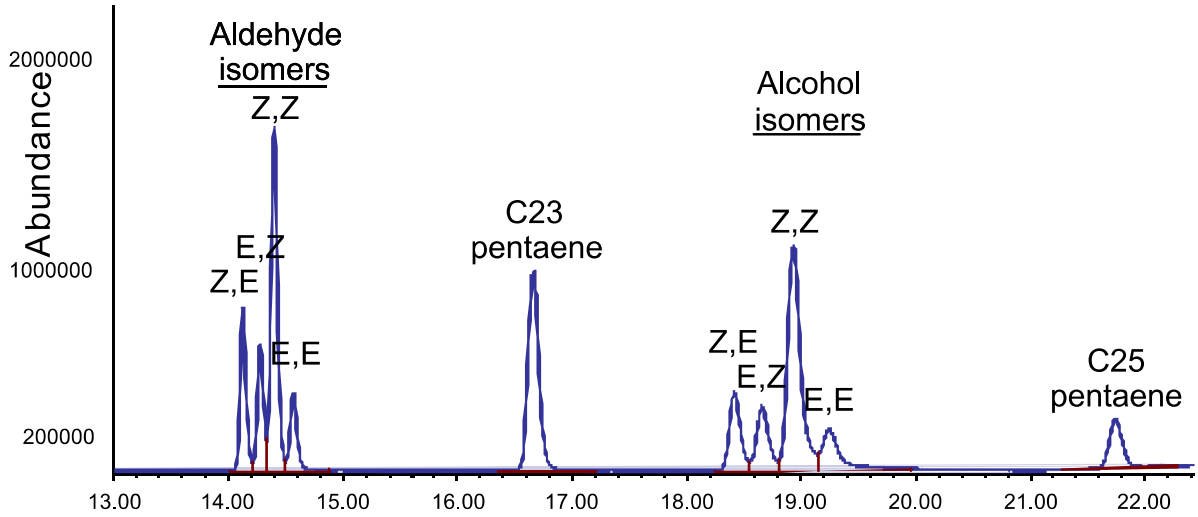
Coffelt, J. A., K. W. Vick, L. L. Sower, and W. T. McClellan. 1979b. Sex pheromone mediated behavior of the navel orangeworm, *Amyelois transitella.* Environ. Entomol. 8: 587-590.

Kuenen, L.P.S., McElfresh J, S. and Millar, J.G. 2010. Identification of critical secondary components of the sex pheromone of the Navel Orangeworm (Lepidoptera: Pyralidae). Journal of Economic Entomology. 103(2):314-330.

Shani, A., and Lacey, M.J. 1984. Convenient method applicable to single insects for collection and measurement of blend ratios of airborne pheromone from artificial sources. J. *Chem. Ecol.* 10:1677-1692.

Fig. 1

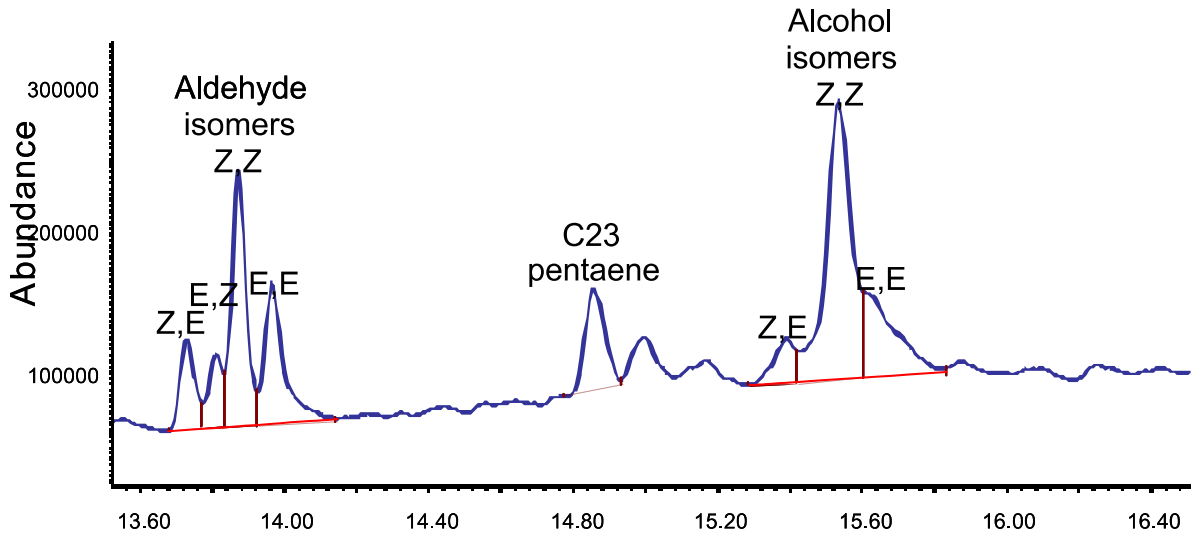
Synthetic NOW Pheromone With Isomers; GC/MS SIM Mode



Chromatogram of mixture of synthetic pheromone components and their isomers

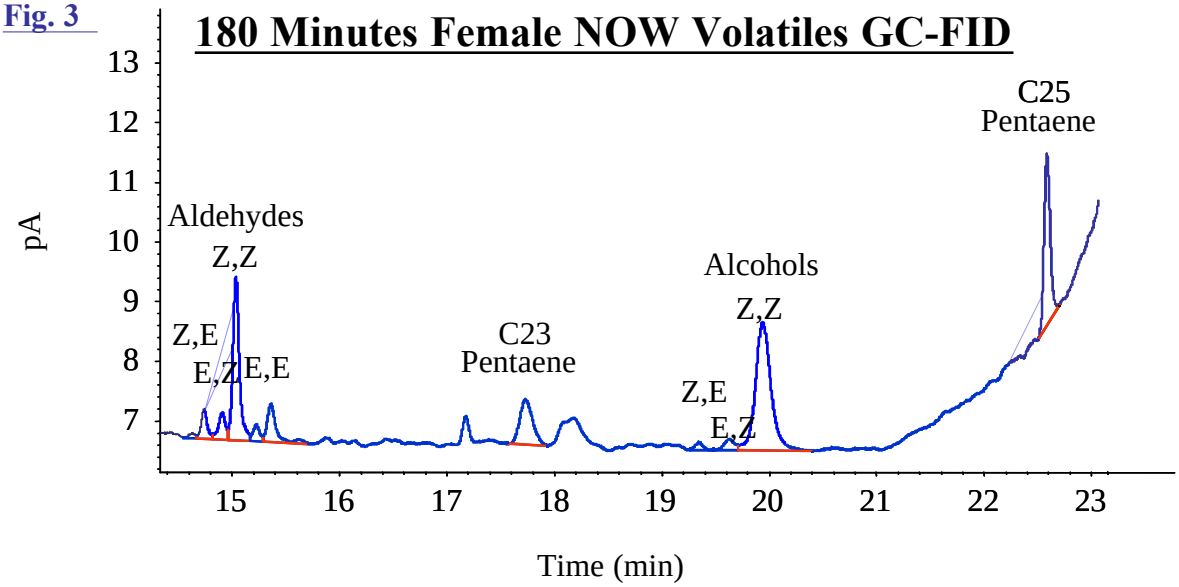
Fig. 2

1 Female NOW Gland Extract; GC/MS SIM Mode



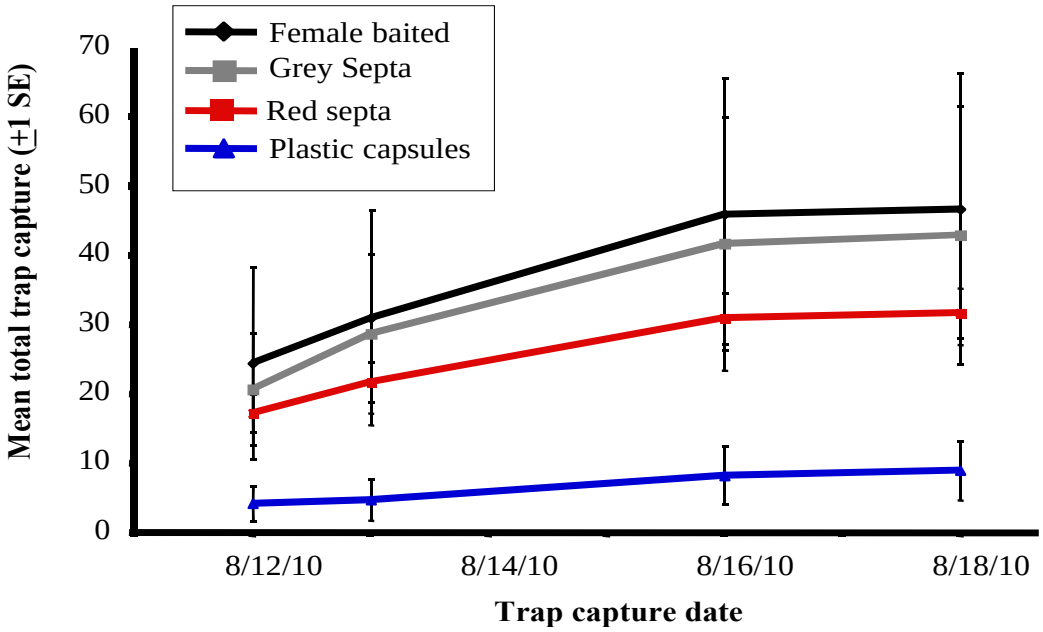
Chromatogram of the extract from a single female pheromone gland, showing pheromone components and their isomers.

Fig. 3



Chromatogram of sex pheromone components volatilized from six female pheromone glands (30 min each) showing pheromone components and their isomers.

Fig. 4. 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.