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

Project No.: **11-ENTO12-Kuenen/Walse**

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Objectives:

1) Develop laboratory-based matrices (with purified pheromone components) for male navel orangeworm (NOW) attraction after chemical extraction and stabilization procedures.
2) Isolate the inhibitory compound(s) in the sole commercial source of the ZZ-aldehyde.

Interpretive Summary:

In mid summer 2010, we field tested rubber septa as navel orangeworm (NOW) lures after exhaustive solvent extraction of the septa; there was one week activity-equal-to-females. We planned further field tests for 2011 which would be co-monitored chemically for changes in pheromone release ratios and/or changes in component isomer ratios; however, during 2011 we found that previously published methods (used and reported by us) led to inaccurate measurements of volatile release ratios due to isomerization of the highly labile pheromone components. Field tests in 2012 did not improve on the one week activity from 2010.

We obtained new rubber septa made of several different rubber compounds from West Company that can be standardized and tracked for future research and use. They were exhaustively extracted (Soxhlet) with polar and non-polar solvents and they are ready for field testing. At the 2012 Almond Board Conference, Suterra, LLC announced they would release a pheromone lure for the 2013 season. Since then they have obtained exclusive license to the patented pheromone components, therefore we did not continue our field work for the 2013 season. Although a commercial pheromone source is available we worked this year to determine the cause of pheromone component isomerization as volatile ratios will likely have to be examined in the future to help extend lure longevity. We found that nearly all handling procedures can lead to isomerization, but all can be avoided by use of new or nearly new syringes and clean glassware. However, during gas chromatographic analyses a retention gap that is part of the analytical process must be new and checked regularly with synthetic standards as solvents can quickly destroy its neutral state and isomerization of the labile pheromone components occurs again.

Materials and Methods:

We obtained septa (from West Company [née West Pharmaceuticals]) made from butyl rubber, chloro-butyl rubber, and isoprene (natural rubber). Batches of each rubber type were exhaustively extracted in a Soxhlet device (two days with dichloromethane and one day with hexane) for formulating pheromone sources for lab and field tests. All cleaned septa are held in clean hexane until needed.

Our approach to developing a field lure for monitoring NOW was use of purified pheromone components placed on various "standard" and commercial formulation materials such as the septa, above, that have been thoroughly cleaned by chemical means. Then we assess NOW's trap capture by lures over time vs. traps baited with unmated females. In addition we planned to assess the release ratios from these lures to correlate to trap capture rates with changing pheromone component ratios. As noted in previous reports, the diene pheromone components (3 of the 4 pheromone components) are very labile. Therefore, we loaded cleaned gray, butyl-rubber septum with 300ul of a mixture of (11*Z*,13*Z*)-hexadecadienal, (11*Z*,13*Z*)-hexadecadien-1-ol, and (3*Z*,6*Z*,9*Z*,12*Z*,15*Z*)-tricosapentaene (C23 pentaene); 1mg each to examine their release ratios. This was determined using an all glass volatile collection device at 6 time-intervals spanning 29 days. The septum was held at room temperature (21-25^{o} C) in a fume hood between measurements.

Additional ZZ-aldehyde and ZZ-alcohol was purchased from Bedoukian Research Inc. (Danbury, CT). Small (nanogram) quantities of the ZZ-aldehyde were separated into three fractions by collecting volatiles eluting from our gas chromatograph. These fractions are, 1) all volatiles eluting before the ZZ-aldehyde, 2) the 3-second ZZ-aldehyde peak, and 3) all volatiles eluting for 15 minutes after the ZZ-aldehyde peak. These fractions have been combined with the other three pheromone components (synthesized by Jocelyn Millar – UCR) remaining in our freezer since identification of the NOW pheromone (Kuenen et al. 2010). Purification of the ZZ-aldehyde and ZZ-alcohol in quantities required for field work is still in progress.

Results and Discussion:

The release ratios from our test septum are shown in **Table 1**. The ratio of pentaene to ZZ-aldehyde increased steadily during the sampling period whereas the ratio of ZZ-alcohol to ZZ-aldehyde remained fairly constant for a week and then dropped during the following 3 weeks. During these analyses we found that even the "new" all-glass-materials and methods we used could lead to inconsistent results in isomer ratios (not shown) of the diene components. We found that the primary causes of isomerization of the pheromone components was due to older glassware, syringes and gas chromatographic columns. Further, purification of the pheromone components was too tedious with our methods and we had not requested enough funds for syntheses by other sources.

We developed new procedures for pheromone component analyses. We now employ new glassware, plastic pipette tips, teflon-tip syringe needles, and new pre-columns on our analytical GC column (this pre-column must be replaced often as indicated by increased isomerization in calibration runs of a purified stock solution). A Stabilwax®-DA analytical

column (30 M, 0.32 mm ID, 0.25 µm film thickness; Restek Corporation) gives full baseline separation of all four isomers of the ZZ-aldehyde and the ZZ-alcohol. These methods, plus careful handling of all samples typically ensure lack of isomerization of the diene compounds and should be helpful to anyone analyzing NOW pheromone components in the future.

Suterra, LLC released an effective pheromone lure for the 2013 season. Since then, they have obtained exclusive license for the patented pheromone components. Therefore, we focused the last of our work on the abot – obtaining a methodology to analyze NOW pheromone components. This will be useful in the future to determine if lure longevity can be increased.

Table 1. NOW pheromone component release ratios from a rubber septum loaded with 1 mg each Z,Z,11,13-16:Ald, Z,Z,11,13-16:OH, and C23 pentaene.

AGE(days)	Ald %	OH %	Pentaene %
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

References Cited:

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.