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

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

Project Leader: Lodewyk P. Kuenen
USDA/ARS SJVASC
Parlier, CA 93648
559.596.2762
Bas.Kuenen@ars.usda.gov

Project Cooperators and Personnel:
Spencer Walse, USDA, ARS, Crop Protection and Quality Unit
James Bettiga, S&J Ranch

Objectives:

The overall goal of this work is to develop a higly attractive lure to be used to monitor navel orangeworm. Specific objectives for 2011 were:

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

Interpretive Summary:

Our current approach to developing a field lure for monitoring NOW continues to use purified pheromone components placed on various “standard” and commercial formulation materials such as septa 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 will assess the release ratios from these lures to correlate to trap capture rates.

In mid summer 2010, we field tested rubber septa as navel orangeworm (NOW) lures after exhaustive solvent extraction of the septa. We planned further field tests which would be co-monitored chemically for changes in pheromone release ratios and/or changes in component isomer ratios; however, during 2011- 2012 we found that previously published methods (and used and reported by us) led to inaccurate measurements of volatile release ratios due to isomerization of the highly labile pheromone components. We believe that newer methods that are approaching completion will resolve these issues.

We obtained new rubber septa made of several different rubber compounds from West Company that can now be standardized and tracked during future research and use. They were exhaustively extracted (Soxhlet) with polar and non-polar solvents and they are ready for field testing. Laboratory issues with bioassays and purification of larger amounts of pheromone components have kept us from completing our goals so far. Further results of this work will be reported at the Annual Conference.

Materials and Methods:

This year's progress on bioassays to isolate inhibitory compound(s) from the sole commercial source of the ZZ-aldehyde has so far been greatly restricted by unknown issues with our insect colonies. Male NOW from the "Kuenen" colony, used since 2000, largely failed to respond to female pheromone gland extract (which, as always, was taken from our research unit's base culture, originated in 1966). We obtained NOW males (for flight assays) and female NOW (for pheromone extracts) from a separate NOW colony maintained by Dr. Burks in our research unit (it was established in 2005).

As we worked to resolve the bioassay issues, above, we continued our work on pheromone substrates and pheromone volatile ratios. Most publications refer to rubber septa by description (size and color) plus a catalog number – finding a reference to some of these septa is no longer possible since the company and/or product are no longer available. This year we consulted experts at West Co. (née West Pharmaceuticals) to obtain more septa and to learn more about the compounds from which they are made and any possible adjuvants that give rubber and plastics many of their properties (last year's rubber septa were obtained as a gift without identifying information). We obtained septa made from butyl rubber, chloro-butyl rubber and isoprene (natural rubber). The new septa can now be tracked for future use and reference. Batches of each rubber type were again 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 current approach to developing a field lure for monitoring NOW continues to use 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 will assess the release ratios from these lures to correlate to trap capture rates. As noted in previous reports, the diene pheromone components (3 of the 4 pheromone components) are very labile. Therefore, we loaded a 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. Release ratios (and approximate release rates) were determined using the all glass volatile collection device, described last year, 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 at the time of this report (08/01/12) and will be field tested later this 2012 season.

Results and Discussion:

After cross checking male responsiveness and pheromone extracts, we determined that female pheromone gland extract from all three Parlier colonies elicited full male responsiveness as long as males from the 2005 strain were employed. Thus, we concluded that an unknown change occurred in our 2000 strain, likely due to severe overheating of the growth chamber during a holiday weekend (more than 50% of individuals in the colony had died and our max/min thermometer indicated a high of 119^{o} F). We subsequently started a colony of NOW derived from eggs from the 2005/Burks strain, which we can now use for our bioassays. We expect to complete laboratory assays of the ZZ-aldehyde fractions prior to the 2012 Almond Board of California (ABC) annual conference.

The release ratios (and approximate release rates) from our test septum are shown in **Table 1**. These date were obtained using our updated lab methods from last year; the ratio of pentaene to ZZ-aldehyde increased steadily during the sampling period whereas the ratio of ZZ-alcohol to ZZ-aldehyde remainded fairly constant for a week and then dropped during the following 3 weeks. However, we found that even the "new" all-glass-materials and methods led to inconsistent results in isomer ratios (not shown) of the diene components. Newer protocols are nearly complete and will be detailed by publication and a future report to the ABC.

We are continuing our efforts to isolate inhibitory compounds in the commercial source of ZZ-aldehyde by lab bioassays and will field test chemically cleaned rubber septa when we have sufficient quantities of purified sex pheromone components. Trap catch in the field experiments will be correlated with volatile ratios from randomly selected field lures. This data may indicate how changes in volatile ratios affect trap capture and then we will attempt to change septa loading to compensate and produce a longer lasting field lure.

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

Research Effort Recent Publications:

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.