PHEROMONE-BASED MONITORING AND MATING DISRUPTION OF
NAVEL ORANGEWORM

Jocelyn Millar and Harry Shorey

ABSTRACT:

The navel orangeworm (NOW), Amyelois transitella, is a key pest of nut crops. Despite intensive study, this insect continues to be a problem for many growers. The major sex pheromone component for NOW was identified as Z11,Z13-hexadecadienal in 1979 (Coffelt et al. 1979), but the pheromone has not been used to any extent either as a trap bait or for mating disruption. The goal of this project is to solve the problems which have hindered use of the pheromone, so that it can be used for both monitoring and mating disruption control of NOW.

During the first five months of this project, technical grade NOW pheromone dispensed at timed intervals from aerosol spray devices ringing 40 acre blocks of almonds, pistachios and walnuts was shown to effectively shut down traps baited with virgin female moths. These results indicate that the pheromone disrupted moth communication, and that mating disruption of NOW may be feasible with the release of pheromone at constant doses and consistent rates for long periods of time. Laboratory studies of the pheromone showed that male moth antennae respond only to one small group of compounds consisting of the major known pheromone component, Z11,Z13-hexadecadienal, and several closely related compounds, which have been identified by comparison with synthesized standards. Identification of the most attractive blend of these compounds may not be important for mating disruption, but could be crucially important in developing the most attractive lures for monitoring purposes.

OBJECTIVES:

- Determine whether there are previously unidentified components in the NOW pheromone blend.

- Synthesize and test potential synergists and antagonists of the NOW pheromone.

- Develop methods of stabilizing NOW pheromone under field conditions.

- Work out practical, field details of pheromone-based monitoring systems for NOW, and mating disruption tactics for NOW.
PROCEDURES:

1. **Insects**: Navel orangeworm cultures are maintained on a bran and honey diet in the laboratory at both UC Riverside and Kearney Ag. Center, as previously described (Coffelt et al. 1979a). Male and female pupae were separated, and the emerging adults were maintained in 30 cm square screen cages until needed. Virgin females were used either as trap baits in field tests, or for preparation of pheromone gland extracts. Male insects were used for conducting coupled gas chromatography-electroantennogram detection (GC-EAD) studies at UCR. All insect cultures were maintained at 20-25°C.

2. **Preparation and analysis of pheromone extracts**: Virgin female insects were put on a reverse light cycle, as they normally call at the end in the couple of hours before dawn. Pheromone glands were dissected out of 1-3 day old virgin females at the end of the dark cycle. Briefly, the abdomen of the female was gently squeezed to extrude the gland on the end end of the ovipositor, and the gland was clipped off with iris scissors. The gland was soaked in pentane (25 microliters) for 10 min, and the pentane was then transferred to a clean vial. Extracts from about 1000 females were consolidated and concentrated by passive evaporation of most of the pentane from the open vial in a fume hood.

   Pheromone gland extracts were analyzed by gas chromatography on several capillary columns of differing polarity (DB-5, DB-WAX, DB-17), and by coupled GC-EAD, using DB-5 and DB-WAX columns, and by GC-mass spectrometry. Compounds were identified by comparison of retention times and mass spectra with those of synthetic standards, prepared as described below.

   Consolidated extracts containing several hundred pheromone gland equivalents were fractionated by high pressure liquid chromatography, using a Supelcosil LC-SI column (3 mm I.D. x 15 cm, particle size 5 microns) eluted with a gradient of 0-50% tetrahydrofuran in hexane. Fractions were concentrated by fractional distillation of the solvent under nitrogen atmosphere.

3. **Syntheses of pheromone compounds and analogs**: Technical grade Z11,Z13-hexadecadienal (Z11,Z13-16:Ald, approx 84% pure), the major component of NOW pheromone, was purchased from Hercon Environmental, from company archives. The technical grade material was cleaned up by reduction to the alcohol (Z11,Z13-16:OH) with sodium borohydride, followed by low temperature (-20°C) reduction of the corresponding alcohol from hexane, giving material of >99% purity. The purified alcohol was then reoxidised to the aldehyde using the Swern oxidation protocol.

   E11,Z13-16:OH and E11,E13-16:OH were prepared as a mixture of isomers using a multistep synthesis (Scheme 1). The alcohols were separated by liquid chromatography on a silver-ion coated ion exchange column eluted with methanol (Houx et al. 1974). The separated alcohols were then oxidised to the aldehydes by the Swern protocol.
Z11,E13-16:OH and E11,E13-16:OH were prepared as a mixture of isomers as shown in Scheme 2. The alcohols were separated by silver-ion chromatography, and oxidised by the Swern protocol to the aldehydes.

4. Field Experiments: Technical grade Z11,Z13-16:Ald was purchased from the chemical archives of Hercon Environmental. The pheromone was diluted with ethanol, BHT was added as an antioxidant, and the pheromone was loaded into spray canisters pressurized with butane and propane. The loaded canisters were fitted with a battery-operated timing mechanism, which sprays a short and adjustable burst of pheromone at an adjustable time interval (1-60 min) onto a cloth target (15 cm diam) fastened on a loop of wire placed 15 cm in front of the spray nozzle. A portion of the pheromone is absorbed onto the cloth target, which acts as a slow release device until the next puff of pheromone. These devices, henceforward referred to as puffers, were hung in trees at about 2/3 the height of the canopy (to ensure good mixing of released vapor throughout the canopy), and positioned at 40 meter intervals around the perimeter of 40 acre test blocks (total of 40 puffers/block). Each puffer was equipped with a large S-shaped metal hook so that it could be easily hung from a tree branch. Puffers were run 24 hours a day, with a 30 min interval between puffs, delivering about 10 mg of pheromone per acre per day.

Mating disruption of NOW using the puffer devices was tested in two pistachio orchards in Madera Co., two almond orchards in Fresno Co., and a walnut orchard in Tulare Co. These five tests were run on separate weeks during August and September, 1995. Tree heights were approx. 2.5 m for pistachios, 6 m for almonds, and 12 m for walnuts.

A second trial was conducted for an additional week in a smaller walnut block (10 acres), with 28 puffers stationed around the perimeter of the block, and using a puff interval of 10 min instead of 30 min.

The effectiveness of the pheromone at disrupting NOW pheromone communication was monitored by placing 2-4 sticky traps baited with four live virgin female NOW in the center of each block, and at the same elevation as the puffers (2/3 tree height). Dead females were replaced as required. Traps were also placed in untreated (with pheromone) control blocks situated at least 1 mile from the pheromone block.

During several of the trials, virgin female NOW were tethered to mating tables (2 moths/table) made from pheromone trap bottoms without stickum, placed in trees (2/3 of canopy height) in the center of blocks. Female moths were dissected the next morning to determine whether mating had occurred (presence or absence of spermatophore).

The degree of mating disruption was assessed by comparing the numbers of moths caught in traps in untreated blocks with the numbers caught in pheromone-treated blocks, and by comparison of the numbers of tethered female moths which mated.
RESULTS AND DISCUSSION

Field trials:
In a number of preliminary trials carried out in 1994, we found that small quantities of navel orangeworm pheromone freshly placed in glass capillary tube dispensers, which were then arrayed in orchards, one per tree, in a 6 x 6 tree grid, prevented male moths from finding virgin female moth baits in traps suspended from trees in the centers of the grids. For 1995, we originally planned to continue and refine these studies, optimizing factors such as release rates, dispenser height, and so forth. In the event, results with the puffer technology for pheromone release in other crop systems looked so promising that we decided to take a big step forward and test this technology for NOW mating disruption. It must be stressed that the puffers have a number of major advantages over more traditional methods of mating disruption, not least of which being that they can be applied at the rate of only one to three per acre, in contrast to the several hundred to many thousand point sources/acre used with other pheromone devices such as twist ties, or sprayable beads or fibers.

Consequently, we made a jump to 40 acre plots, using a small number of widely separated aerosol puffers as pheromone release devices. These devices release comparatively large doses of pheromone at timed intervals. The puffs of pheromone permeate the orchard atmosphere due to mixing by air movements, and the portion of the pheromone puff sprayed onto the cloth target slowly evaporates to provide essentially continuous release. Furthermore, the aerosol puff of pheromone is adsorbed onto the orchard foliage, and slowly rereleased, further helping to provide a continuous blanket of pheromone within the treated area.

In the total of four week-long trials in pistachios and almonds, trap catches in the pheromone-treated blocks were completely shut down, indicating a complete disruption of moth communication. During the same time period, analogous traps placed in blocks without pheromone treatment caught significant numbers of moths (6-60 moths/ trap/night). Furthermore, three of 15 tethered virgin females were mated in control blocks, while 0/14 tethered females were mated in the pheromone-treated blocks. These numbers are too low for rigorous statistical comparison, but they do further suggest that males were unable to find females in the pheromone-treated blocks.

In the first trial in walnuts, with much larger trees and consequently a much larger air volume to saturate with pheromone, and puffers spaced at 40 m intervals around the outside of the 40 acre block, trap shutdown was 98% effective as compared to trap catches in control blocks. When the interval was shortened to 10 min, and the puffer density was increased to 28 puffers ringing a 10 acre block, complete trap shutdown was again achieved for the week-long test period.

These experiments have demonstrated two important points. First, the 100% trap shutdown indicates that NOW mating disruption has a good chance of success. Second, complete trap shutdown was obtained using a limited number of large sources of
pheromone, spaced around the outside of blocks, rather than the large number (160-400 or more/acre) of smaller point source dispensers which are typically used in mating disruption. Use of only one or a few large point sources per acre, as can apparently be done with the puffers, should help to keep application costs down.

There are several other advantages to using the puffer technology. First, the NOW pheromone is known to be unstable, rapidly degrading upon contact with air and sunlight. However, with the puffers, the pheromone is protected inside the aerosol can from light and air until the actual moment of release, so that degradation before release is minimized or eliminated. Once the pheromone has been sprayed onto the target degradation can be slowed by incorporation of stabilizers into the formulation. Second, the release rate of most other pheromone dispensers which are currently available decreases exponentially over time. Consequently, these dispensers release a lot of pheromone initially, but the release rate drops off rapidly, so that pheromone coverage of the crop becomes progressively weaker as the dispensers age. In contrast, the puffers release a constant, measured dose of pheromone with every puff, so that the release rate remains constant, and coverage of the crop with pheromone is as good on the last day as on the first day.

The puffers have several other potential advantages over all other types of pheromone dispensers. For example, the release mechanism can be fitted with a timer, so that pheromone is only dispensed during a daily time period bracketing the activity period of the moths. NOW females are known to call in a few hour period around dawn, which would be the critical time period for saturating an orchard with pheromone. Thus, setting the puffers to only release pheromone during an eight or even twelve hour period bracketing the activity period of the moths would result in a saving of materials of 66 or 50% respectively. Furthermore, considerable fine tuning of the pheromone release rate can be done very easily by simply adjusting the size of the puffs, and/or the number of puffs per hour.

We expect to rapidly extend this work during 1996, to include both seasonlong trials with full damage assessments at harvest, and trials with larger blocks.

Laboratory studies:

1. Syntheses of chemicals for testing. The syntheses of all four isomers of the navel orangeworm pheromone were completed on a small scale (Schemes 1 and 2) for testing in wind tunnel experiments, and as baits in field traps. However, the compounds proved to be less stable than anticipated, and interconverted readily, even when stored at -20°. Thus, three of the four isomers rapidly developed isomeric impurities at levels of 1-7%, and cannot be used for the field tests we had planned. These tests involve adding small amounts of the various isomers to the major component of the pheromone to see whether attraction is enhanced or inhibited. The syntheses will be repeated using modified routes to generate the high purity materials required for testing next spring. However, the materials that we have on hand have been used to examine female pheromone gland
extracts, and to identify and determine the amounts of the minor isomers which are present in the extracts.

2. Analyses of pheromone glands. We have dissected and extracted approximately 1000 NOW pheromone glands, preparing both extracts from individual females to determine individual variability, and pooled extracts for fractionation and wind tunnel testing.

Because of the demonstrated instability of the pheromone compounds, we conducted a preliminary gas chromatography (GC) study to ensure that the compounds were not interconverted during analysis. It was found that GC injector temperatures above 200° C resulted in detectable interconversion. Consequently, all GC analyses of insect extracts were conducted at or below 200°.

Individual and pooled extracts have been analyzed by gas chromatography on two GC columns, and by coupled gas chromatography-electroantennography, which uses a live male moth antenna as the detector. We have not detected antennal responses to any compounds other than Z11,Z13-hexadecadienal and its isomers in the extracts, suggesting that these may be the only compounds constituting the pheromone. However, we cannot yet rule out the possibility that the extracts may contain unstable compounds which do not survive gas chromatography, even at reduced temperatures. There are several precedents for such unstable pheromone components in other moth species.

To determine whether there are thermally unstable components to the pheromone which are destroyed by GC, we have fractionated pooled extracts of several hundred pheromone glands by liquid chromatography. By bioassaying judiciously selected combinations of these fractions, we hope to determine whether or not there are any "hidden" components to the pheromone, which act to synergise Z11,Z13-16:Ald and its isomers. The fractionations have been carried out, and we anticipate carrying out wind tunnel bioassays of the fractions early in 1996.

Acknowledgments:

We thank the California Pistachio Commission, the Almond Board of California, and the Walnut Marketing Board for joint support of this project.

Literature Cited:


Scheme 1

Cl-CH₂-CH₂-CH₂-CH₂-CH₂-CH₂-CH₂-CH₂-CH₂-OH

Cl-CH₂-CH₂-CH₂-CH₂-CH₂-CH₂-CH₂-CH₂-CH₂-OH

-CH₂-CH₂-CH₂-CH₂-CH₂-CH₂-CH₂-CH₂-CH₂-OH

HO-CH₂-CH₂-CH₂-CH₂-CH₂-CH₂-CH₂-CH₂-CH₂-OH

HO-CH₂-CH₂-CH₂-CH₂-CH₂-CH₂-CH₂-CH₂-CH₂-OH

H₂C=CH-CH₂-CH₂-CH₂-CH₂-CH₂-CH₂-CH₂-CH₂-OH

H₂C=CH-CH₂-CH₂-CH₂-CH₂-CH₂-CH₂-CH₂-CH₂-OH

-CH₂-CH₂-CH₂-CH₂-CH₂-CH₂-CH₂-CH₂-CH₂-OH

E₁₁, Z₁₃-16:OH  E₁₁, E₁₃-16:OH

E₁₁, Z₁₃-16:Ald  E₁₁, E₁₃-16:Ald