1988 ANNUAL PROGRESS REPORT
ALMOND BOARD OF CALIFORNIA

Project no. 88-A9 - Navel Orangeworm Attractants and Carob Moth Pheromone

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Objectives:

3) Develop controlled release formulations of acidulated almond oil plus insecticide to attract and kill egg-laying navel orangeworm female moths;

4) Optimize NOW female moth lures for use in black monitoring traps;

5) carob moth pheromone - 1) determine the blend of synthetic sex pheromone components recently identified from carob moth females that will optimize male attraction.

Note: Progress on objectives 1 and 2 of the project will be described separately by P.L. Phelan.

Interpretive Summary:

3) *NOW attracticide.* Field studies conducted this year on attracticide formulations were inconclusive due to high levels of orchard infestation (>60%), even in insecticide-treated plots.

4) *Black traps.* The relative effectiveness of acidulated almond oil (two concentrations), synthetic fatty acids (two concentrations), and 10% crude almond oil in presscake (CAO/PC) was tested in black adult traps in the field. The CAO/PC proved most attractive, with female catch showing very good correlation with egg deposition on egg traps.

5) *Carob moth.* Three components were identified from the pheromone gland of carob moth females, the major component being a novel compound never before identified from any moth species. When combined, these were effective in eliciting upwind flight and source location in a wind tunnel, although at a somewhat lower level than the natural pheromone extract.

Materials and Methods.

3) *Attracticide.* A formulation of free fatty acids plus insecticide (isomerically pure Fenvalerate) in a slow release carrier was developed by Bio-Control, Ltd., of Australia and shipped to Riverside for testing on Roberts Farms near Bakersfield. The formulation was applied to the trunks of trees in the following treatments: 1) 4 grams of attracticide per tree; 2) 12 grams of attracticide per tree; 3) 4 grams of formulation without insecticide (hereafter called "attractant" per tree; 4) two untreated check plots per block. The material for the 4 gram treatments was applied from a caulking gun in 4, 1-gram dabs on different branches at head-height, each dab smeared to smoothness on the bark using a putty knife. For the 12 gram treatments, again four different dabs were applied, but the amount in each was increased to 3 grams of

formulated material. The treatments were applied to trees in blocks of 10 trees by 10 trees (Fig. 1), and there were 4 replicates of each treatment in a randomized, complete-block design. Oviposition activity was monitored each week using two

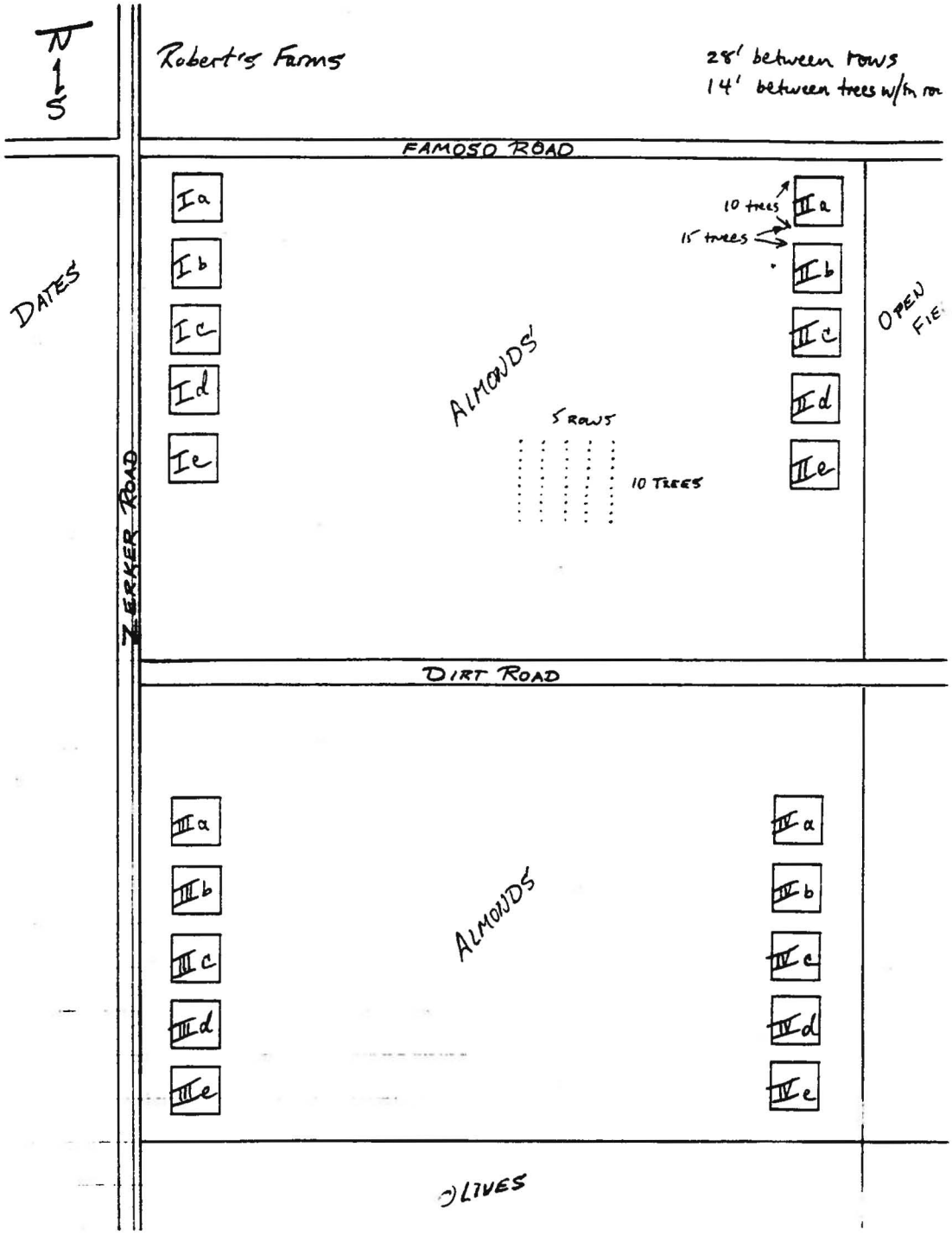


Figure 1. Design of Attracticide test, Roberts Farms. 4 grams attracticide per tree in plots Ib, IIc, IIIb, Iv b; 12 grams attracticide per tree in Ic, II b, IIId, IVe; 4 grams attractant alone in plots Ie, IIe, IIIe, and IVb; the rest were check plots.

black egg traps, one placed on a tree near the center of each 10 by 10-tree block and one placed on an edge tree. Although the formulation was never meant to be used around hull split and harvest to prevent damage, nut samples were taken anyway at harvest in case there were any effects. The final harvest counts of 100 nuts per tree, ten trees per treatment per block were taken beginning on August 20th.

4) *Black traps and attractants in field.* In the first experiment, the efficacy of the black traps in capturing adult females was compared with counts of eggs on black egg traps. The untreated control blocks from the attracticide test on Roberts Farms was used to gather this data beginning 5/17/88 and ending 9/1/88. The lure in the adult female black Pherocon ^{R} traps was 20 mg free fatty acids from almond oil applied to green construction paper, which was replaced every week. The egg traps were loaded with 13.5 g almond press cake plus 1.5 g crude almond oil, which was replaced every 4 weeks. Traps were sampled weekly, the adult females in the black traps removed and checked for eggs, and eggs deposited on the egg traps were counted and removed.

In a second experiment, different mixtures of attractants were tested in the field for attractancy to gravid females, using the black Pherocon ^{R} traps developed in our previous studies. Four replicates of 6 treatments were deployed in Roberts Farm in a randomized, complete-block design beginning 7/5/88 and ending 9/13/88. Traps were sampled weekly and the blocks re-randomized after sampling each week. Females in the black traps were removed and checked for eggs. Attractants were replaced weekly after sampling.

In a third experiment, lures were tested for their attractiveness after aging under field conditions on Roberts Farms. Free fatty acids from almond oil (20 mg) were placed on either green construction paper (standard lure from wind tunnel) or rubber septum dispensers and exposed to field conditions in traps for 2, 4, 6, 8, or 10 weeks. The lures were then removed and placed in clean black Pherocon ^{R} traps during the interval from 9/13/88 to 9/20/88 and the captures during this period compared to blank control traps and traps containing a fresh lure of presscake plus crude almond oil (13.5 g + 1.5 g). Traps were deployed in a randomized, complete-block design with 25 m separating traps within a block.

Carob moth sex pheromone. Materials and methods will be described along with results in Results section.

Results.

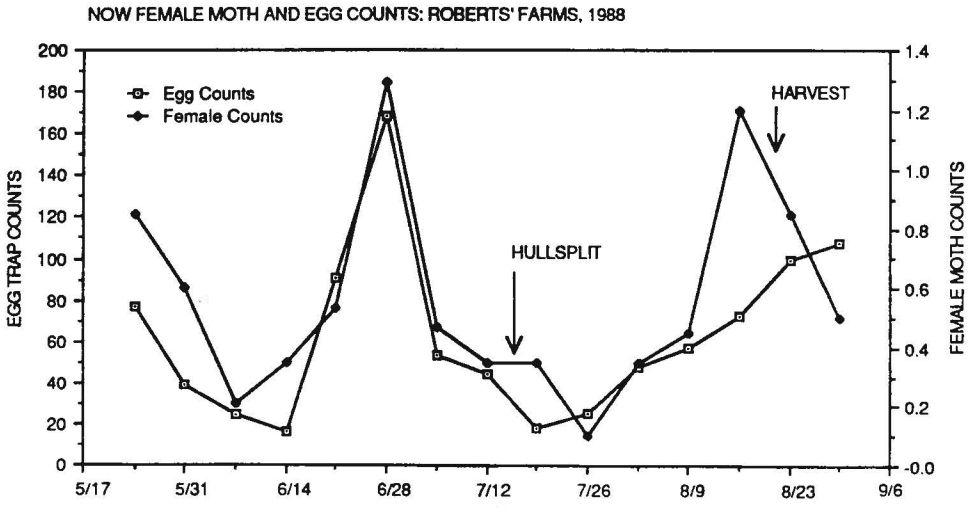
Attracticide. There was a slight trend for the two attracticide formulations to reduce egg deposition on egg traps in the first month following the application of the material in the field. In the first two weeks following application, egg deposition only increased in the 12 gram attracticide plots by 1.6 times, and in the second two-week interval by 2.4 times. This occurred despite the fact that these plots had had the heaviest pre-treatment moth pressure in the two weeks before application, as indicated by egg trap count (Table 1). Also, in the 4 grams per tree attracticide plots the increases

Table 1. Number of eggs (+ S.D) deposited on egg traps in attracticide plots before and after application of attracticide or attractant formulation (N=8 for each mean, 16 for check means).

	Before Appl., 2 week avg.	After 0-2 weeks	After 2-4 weeks
4 g attracticide	22.5 + 20.4	54.6 + 17.3	69.5 + 30.8
12 g attracticide	30.1 + 18.3	49.1 + 22.5	72.4 + 25.5
4 g attractant	9.1 + 9.3	42.1 + 21.1	104.9 + 25.1
untreated check	23.3 + 26.7	58.2 + 32.8	97.5 + 42.7

were only 2.4 and 3.1 times at the first and second post-application two week intervals. In contrast, the increases in the untreated control plots were 2.5 and 4.2 times the pre-treatment levels. The 4 grams of attractant alone without insecticide failed to reduce the increase in egg deposition; the post-treatment increase was 4.6 and 11.5 times during the first and second two-week post-treatment intervals (Table 1). Although the attracticide formulation was only designed for early-season use, and not to protect the nuts close to harvest, we took damage readings anyway. 1000 nuts per block (4000 nuts per treatment) were cracked and examined for damage. Due to the extremely high infestations, not much can be said about the effects of the attracticide on egg deposition on nuts and subsequent damage. Damage in the check plots averaged 62.3%, whereas that in the 4g and 12g per tree plots averaged 58.2% and 54.5%, respectively. Damage to nuts in the 4g of attractant alone plots without insecticide averaged 61.8%.

Black traps and attractants in field. The first experiment demonstrated that the captures of female NOW in black traps were highly correlated with the intensity of egg deposition on the standard black egg traps (Fig. 2). The coefficient of



correlation (r) throughout the experiment from June until September was 0.765; for the 13 sampling dates prior to harvest r was 0.809, and for the 8 dates prior to hullsplit r was 0.907. The numbers of females captured per trap per week were not high, but they were consistent with the egg counts throughout the experiment, as the correlation coefficient demonstrates. In this test the lure for the black adult female traps had been determined early in the season based on wind tunnel tests to be 20 mg free fatty acids on green construction paper, and we had to continue with this lure to the completion of the test in order to follow the experimental design. Possibly the use of the presscake plus almond oil lure (CAO/PC), later found to be a better lure (second experiment below, started at later date), might have increased the actual numbers of females captured per trap.It would be difficult to improve the correlation between egg traps and adult female traps much more, however. Thus, the captures of adult females appear to be highly correlated with egg traps counts throughout the season. Perhaps increased detection sensitivity might be gained by using the CAO/PC rather than the 20 mg free fatty acid lure.

The second test, that of various attractants for adult females, demonstrated that females were optimally attracted to a mixture of press cake (13.5 g) plus almond oil (1.5 g), compared to other treatments (Table 2). A mixture of three synthetic fatty acids identified from almond oil and similar to the mixture that was active in wind

Field test of various attractants for female NOW moths

Treatment	Amount	Female catches	
		Total	Mean/Trap/Week
Control	---	12	0.1
PC/CAO	13.5/1.5g	57	1.6
FFA_L	20 mg	19	0.5
FFA_H	100 mg	16	0.4
Oleic/Lin./Palm.(Syn_L)	20/5/5mg	16	0.4
Oleic/Lin./Palm.(Syn_H)	100/25/25mg	22	0.6
Orthogonal Contrasts		*F*-statistic	
Treatments		8.7 ***	
Control *vs.* all treatments		7.0 *	
PC/CAO *vs.* FFA & Syn treatments		35.5 ***	
FFA *vs.* Syn treatments		0.3	
FFA_L *vs.* FFA_H		0.1	
Syn_L *vs.* Syn_H		0.9	

tunnel experiments also captured significant numbers of females. Although a few females were captured in blank traps, not one of them was gravid, whereas all of the females captured in the traps baited with an attractant contained eggs.

In the experiment testing the aging of various lures, it was clear that the 20 mg of free fatty acid, regardless of whether it was on construction paper or on rubber, lost effectiveness within two weeks in the field (Table 3). Thus, improvement in longevity would be needed if this lure were to be used in the field. The freshly prepared presscake plus almond oil lure again out-performed the low amount of free fatty acids, indicating that more work must be done in the coming year to develop this lure for use in the adult female trap.

Table 3. Field aging of lures: Roberts' Farms, 9/13/88 - 9/20/88. Treatments having no letters in common are significantly differetn according to an analysis of variance followed by Tukey's test ($P < 0.05$).

Treatment	X No. Females/Trap
Blank Control	0 c
Fresh PC/CAO (15 g)	5.5 a
Fresh free fatty acids (FFA) on paper	2.5 b
FFA on paper, 2 weeks old	0.3 c
" " " , 4 weeks old	0.5 c
" " " , 6 weeks old	0.3 c
" " " , 8 weeks old	0 c
" " " , 10 weeks old	0.3 c
Fresh FFA on grey rubber septum	0.3 c
" " " , 2 weeks old	0.5 c
" " " , 4 weeks old	0 c
" " " , 6 weeks old	0 c
" " " , 8 weeks old	0 c
" " " , 10 weeks old	0.5 c

Carob moth sex pheromone isolation and identification.

The sex pheromone glands of 2-to-5-day-old virgin female carob moths, *Ectomyelois ceratoniae*, were excised into *ca.* 50 µl of CS_2 in a micro-test-tube during the normal period of female pheromone emission in the fourth through sixth hours of darkness. The glands from up to 100 females were extracted for 15-45 min. and then the solvent was recovered with a syringe and the extract was pooled with other similarly collected samples and stored at -20°.

Analysis of the extract by combined GC/EAG[1)] consistently revealed three peaks with strong EAG activity on two different types of 30 meter x 0.25 mm i.d. fused silica columns, the stationary phase being either immobilized methyl silicone (DB-1) or polyethylene glycol (DB-wax)[2)]. The order of elution was the same on both columns; amounts of EAG-active compounds I and II were similar and reached about 10% of that of compound III. Retention times of compounds II and III relative to compound I were 1.043 and 1.048 on DB-1 and 1.142 and 1.274 on DB wax respectively.

Combined GC/MS analysis of the EAG-active peaks was performed using a Hewlett-Packard 5970 instrument coupled with a Hewlett-Packard 5890 gas chromatograph. We used a 30 m DB-wax column under temperature program identical to that employed for the GC/EAG analysis. The extract from *ca.* 40 female glands was used after being concentrated under a gentle stream of nitrogen. The mass spectrum of compound I in the natural extract and retention times on both columns were identical to those of synthetic (Z)-9-tetradecenal. The EAG-active peak II had a base peak at *m/z* 67 and an abundant *m/z* 208. The later fragment could be interpreted as the molecular ion of a doubly unsaturated 14-carbon aldehyde with conjugated double bonds. Conjugated double bonds will stabilize the molecular ion in long-chain aliphatic compounds, whereas M^+ is virtually missing in monounsaturated analogues or analogues with isolated double bonds[3)]. The EAG-active peak III had a mass spectrum characterized by a base peak at *m/z* 79 and an abundant *m/z* 206. This corresponded to a triply unsaturated 14-carbon aldehyde, again with at least one pair of conjugated double bonds. The preliminary assignments of compound II and III were supported by their retention times relative to (Z)-9-tetradecenal and various homologous alcohol and acetate references available in the laboratory collection. Microozonolysis of compound III collected from gas chromatographic separations on either a DB-wax or DB-1 column, produced a compound identical to that of ozonized (Z)-9-tetradecenal. This implied that compound III should have no double bonds below the nine position, and that any additional two double bonds would have to be in the 11 and 13 positions. At this stage the only possible alternative structure would include a conjugated double bond and a triple bond in the 9 and 11 positions.

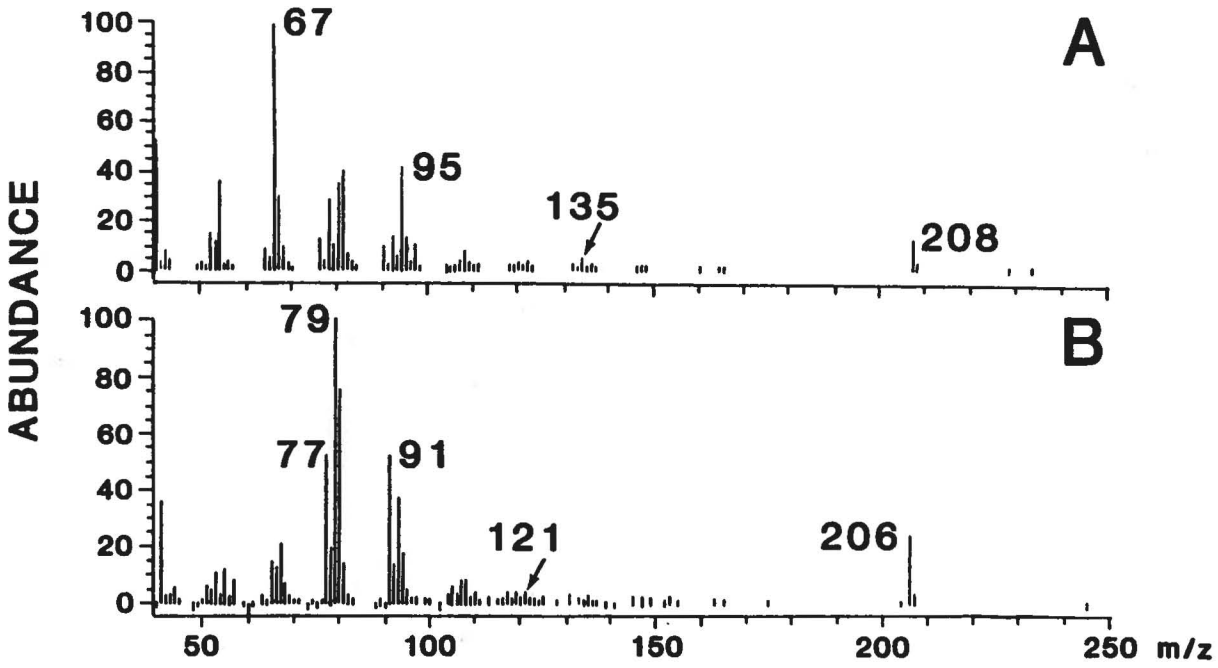


Fig. 1. Mass spectra of compounds II (A) and III (B) of *E. ceratoniae* (Hewlett Packard 5970).

EAG tests with an extensive series of monounsaturated straight-chain acetates and alcohols 12 to 16 carbon atoms in length (10 µg on filter paper cartridges) revealed that the greatest EAG amplitudes were evoked by the (*Z*)-9- and (*E*)-11 isomers in the 14-carbon chain-length series. The total evidence was thus strongly suggestive that compound II was (*Z,E*)-9,11-tetradecadienal and that compound III was (*Z,E*)-9,11,13-tetradecatrienal.

(*Z,E*)-9-11-Tetradecadienal can be prepared from commercially available (*Z,E*)-9,11-tetradecadienol (SIGMA) by oxidation with PDC/molecular sieve[4]. The synthesis of (*Z,E*)-9,11,13-tetradecatrienal was carried out as a C9 + C5-sequence: Oleylalcohol or, preferably, 9-decen-1-o1 (Aldrich) was silylated with tert. butyldimethylsilychloride (TBDMSCl, Aldrich)[5], which after ozonolysis yielded the TBDMS-derivative of 9-hydroxynonanal (1) (bp. 120°C/0, 5mm)[6]. Ethyl (*E*)-2,4-pentadienoate (Aldrich) was converted to 1-bromo-(*E*)-2,4-pentadiene through reduction with $LiAlH_4$ and subsequent treatment of the resulting alcohol with triphenylphosphonium dibromide[7]. Refluxing 1-bromo-(*E*)-2,4-pentadiene and triphenylphosphane in benzene furnished the respective Wittig salt (2) (mp 207-208°C)[8]. Wittig reaction of 1 (alternatively, 9-acetoxynonanal[9] could also be used as a C9-unit) with 2 followed by cleavage of the silylether group with tetrabutyl

ammonium fluoride[10] led to a mixture of geometrical isomers of 9,(*E*)-11,13-tetradecatrienol (bp 108-110°C/0, 1 mm) which could be separated by HPLC[11]. The first eluting component (M^+ = 208) showed the NMR-data given in Table 1 ($J_{H9,10}$ = 11,2 Hz: cis- and $J_{H11,12}$ = 14,8 Hz: trans-) and thus proved to be the (*Z,E*)-isomer. Using PDC/molecular sieve[4] the compound could be oxidized to (*Z,E*)-9,11,13-tetradecatrienal. NMR data of the double bond system of the aldehyde were the same as for the alcohol.

C-Hx	1	2-7	8	9	10	11	12	13	14,14'
∂	3.36	1.15-1.45	2.15	5.46	6.09	6.58	6.18	6.37	5.00 5.13
Hx/Hy			8/9 8'/9	9/10	10/11	11/12	12/13	13/14 13/14'	14/14'
∂ [Hz]			8,0 8,0	11,2	11,2	14,8	10,4	10,4 16,8	1,0

Table 1. ^{1}HNMR (C_6D_6) of *(Z,E)*-9,11,13-tetradecatrienol; chemical shifts and coupling constants.

The mass spectra of the synthetic aldehydes and retention times on both columns were identical to those of the natural compounds II and III respectively. Combined GC/EAG on a 30 m Supelcowax fused silica column demonstrated that (*Z,E*)-9,11tetradecadienal had a retention time idential to compound II and that it was EAG active while the later eluting *E,E*-isomer was not. Likewise, (*Z,E*)-9,11,13-tetradecatrienal proved to be EAG-acative and its retention time matched that of compound III, while the non-active (*E,E*)-isomer eluted later.

Due to the sensitivity of the aldehydes, which easily rearranged even under very mild conditions, no pure compounds were available for behavioral tests. Samples of both the dienal and trienal used for bioassays contained considerable proportions (*ca.* 30% or more) of the respective non-EAG-active (*E,E*)-isomers and traces of the (*Z,Z*)- and (*E,Z*)-isomers as well.

Behavioral tests were conducted in a 3.5-m-long wind tunnel at 4-6 hours into scotophase, the optimal activity period for this species. In an initial test, compounds I and II (0.5 ng each) were inactive by themselves in evoking upwind flight (0/18 and 0/19 males flew upwind, respectively) but 0.5 ng of the triene alone did cause significant upwind flight and source location by males (9/23 flew upwind, 4/23 touched the source). In a second experiment, a blend of the three compounds was nearly as good as 5 FE of gland extract in eliciting upwind flight in males from 3 m downwind (Table 2). Thus, addition of the other two compounds tended to slightly increase the percentage of males touching the source, although not significantly so under these conditions and numbers of males tested (Table 2). The slightly lower activity of the synthetics compared to the natural extract may be due to the synthetics' contamination with other isomers such as (*E,E*), or to suboptimal ratios of I, II and III.

Table 2. Upwind flight and source location from 3 m away by male *E. ceratoniae* in response to synthetic compounds I, II, and III, and to extract from female sex pheromone glands.

	5 FE Extract	0.5 ng III +0.05 ng II + I	0.5 ng synth. III +0.05 ng II	0.5 ng synth. III
% Male Flying Upwind	53% (80)	42% (100)	36% (100)	39% (100)
% Males Touching Source	48% (80)	28% (100)	13% (100)	21% (100)

Among sex pheromones of moths, only a few compounds show more than two double bonds and only recently conjugated trienes were reported: (*E,E,E*)-10,12,14-hexadecatrienyl acetate is a sex pheromone of the mulberry pyralid, *Glyphodes pyloalis*[12] while (*E,E,Z*)-10,12,14-hexadecatrienal was identified as a component of the sex pheromone of females of *Manduca sexta*[13].

1) H. Arn, E. Stadler, S. Rauscher, Z. Naturforsch. 30c 722 (1975)

2) GC conditions: Injector temperature 250°; oven 80°, 2 min hold, 10°/min to 230° final temp; FID temp 250°; H2 carrier gas flow 1.5 ml/min.

3) C. Lofstedt, G. Odham, Biomed. Mass Spec., Vol. 11, 3 (1984)

4) J. Herscovici, M.J. Egron, K. Antonakis, J. Chem. Soc., Perkin I 1967 (1982).

5) E.J. Corey, A. Venkatiswarlu, J. Am. Chem. Soc. 94 6190 (1972).

6) All NMR-spectra were obtained with a Bruker WM 400 at 400 MHz. 1H NMR (CDCl3): alpha + 0.05 (s, 6H); 0.86 (s 9H); 1.26-1.4 (m, 8H); 1.5 (m, 2H); 1.6 (m, 2H); 2.4 (m, 2H); 3.57 (t, 2H); 9.72 (1H).

7) G.A. Wiley, R. Hershkowitz, B.M. Rein, B.G. Chung, J. Am. Chem. Soc. 86 964 (1964).

8) 1 H NMR (CDCl3): alpha = 4.90 (m, 2H); 5.13 (m, 2H); 5.5 (m, 1H); 6.18 (m, 1H); 6.45 (m, 1H); 7.68 (m, 6H); 7.75-7.95 (m, 9H).

9) H.J. Bestmann, R. Range, R. Kunstmann, Chem. Ber. 104 65 (1971)

10) E.J. Corey, B.B. Snider, J. Am. Chem. Soc. 94 3549 (1972).

11) HPLC conditions: RP-18; low pressure gradient; methanol/water

12) K.Y. Seol., H. Honda, K Usui, T. Ando, Y. Matsumoto, Agric. Biol. Chem. 51 2285 (1987)

13) J. Tumlinson, presented at the 16th Int. Symp. Chem. Nat. Prod. (IUPAC), Kyoto, May 29-June3, 1988.

ALMOND BOARD PROJECT PROPOSAL - 1989

Project Title: Investigations of Almond Volatiles for Navel Orangeworm Host Finding

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Objectives: 1) improve field-longevity of formulations for disruption of NOW oviposition in almonds through use of fatty acid blends and various chemical additives, and
2) continue large-scale field testing of improved NOW disruption formulations

Progress to date: In a field study this summer involving approximately 50 acres of almonds, we investigated the potential for two formulations of oleic acid to disrupt NOW oviposition. Five-acre plots were treated during hullsplit with either of the two experimental disruption formulations using a speed sprayer at the rate of 40 gal/ac (1.6 gal oleic acid/ac). These treatments were replicated three times, with each replicate also including a five-acre check plot. Egg-laying on black egg traps was reduced by 94% and 98% respectively by the two treatments during the 4 weeks after application relative to egg-laying during the 8 weeks prior to application. Egg-laying in check plots only declined 15% during this period. During the fifth week after application, egg-laying was not significantly lower in the treated plots relative to untreated ones. These results suggest that: 1) a broadcast application of oleic acid can be effective in significantly reducing NOW oviposition, and 2) as presently formulated, this material will retain its efficacy for approximately one month. These preliminary conclusions are further supported by investigations of nut damage. When measured at 5 weeks after treatment application, NOW nut infestation was 0.13% in Formulation 1 plots, 0.53% in Formulation 2 plots, and 2.0% in check plots; however, 8 weeks after application, there were no differences in nut infestation between treated and untreated plots. We are certainly encouraged by these initial field trials, which suggest that formulations of ovipositional attractants hold promise for a non-toxic NOW control strategy. Future work will seek to improve the chemical stability and field longevity of these formulations and will provide more data on field efficacy.

In previous work, I identified 14- to 18-carbon fatty acids

(oleic 77%; linoleic 17%; palmitic 5%; and myristic 1%) as the primary volatile constituents of crude almond oil (CAO) responsible for upwind attraction and oviposition by NOW females. The levels of long-distance attraction of females to CAO or other sources of almond odor could be duplicated by synthetic mixtures of these fatty acids or even oleic acid alone; however, we could not confidently conclude that these were the only constituents involved because NOW females frequently became arrested in flight 5-10cm downwind from synthetic fatty acids. When natural odor sources were used, females were much more likely to land and even oviposit. This difference in response has now been clarified by work carried out this year by R. Youngman and T. Baker. In wind-tunnel studies, response to acidulated almond oil (composed almost solely of free fatty acids from almond oil) could be duplicated using a synthetic blend of oleic, linoleic, and palmitic acids in a 4:1:1 ratio. The key to this improved response to synthetics appeared to be the use of a lower concentration in later experiments. This lower concentration was not as effective in luring NOW females from a long distance, but once females were attracted, it allowed them to continue their flight up to the odor source. Thus, 5mg of acidulated almond oil evoked source approaches (i.e., flights to within 0.5m of source) in 30% of females tested and source landing in 18% of females. Whereas a blend of 2mg oleic, 0.5mg linoleic, and 0.5mg palmitic evoked approach in 34% and landing in 24% of females released. Although oleic acid alone was usually as effective as either the synthetic blend or the oil in eliciting odor approaches, all three fatty acids were necessary for optimal landing response.

One result inconsistent with our contention that NOW attraction and oviposition is modulated solely by a blend of these three fatty acids is that when placed in adult traps in the field, both acidulated almond oil (at either 20mg or 100mg) and the synthetic fatty acids (at comparable concentrations) were significantly less attractively than the standard presscake (PC)/CAO (13.5g/1.5g) egg-trap lure. We believe this difference to be due to greater longevity in volatile release in the PC/CAO mixtures, although this is an aspect of NOW host finding that we hope to investigate further in the ensuing year.

Proposed Research: The research proposed for the coming year is an extension of the previous year's work. Although the results of the field trials on ovipositional disruption look promising, these can only be considered preliminary, and more work is required to definitively demonstrate the efficacy of this approach to NOW control. In addition, there is still room for improving the formulations for increased longevity, increased efficacy of disruption, and lower price of production.

1. Laboratory Studies. From talking to a number of interested growers at this year's Almond Board Research Conference, it was indicated that for most growers, a field-longevity of 5-6 weeks would be of sufficient duration to protect their crop from hullsplit to harvest. To increase field-longevity, most of our

attention will be focused on the use of chemical additives. Due to the chemical nature of the behaviorally active long-chain fatty acids, they are susceptible to oxidative decomposition, particularly when subjected to the high temperatures frequently experienced in almond orchards. Chemical breakdown also occurs due to exposure to UV light from the sun. Therefore, the chemical additives of interest are anti-oxidants and UV inhibitors. Fortunately, many of these additives are effective at a rate of less than 1% of the formulation. Therefore, they should not significantly alter either the water solubility of the formulation, nor its price of production. Prior to next summer's field trials, we shall study the volatile release rate characteristics of formulations containing various additives. Test samples will be sprayed on glass plates and held in an environmental chamber at 38°C under UV lights. The intent is not to precisely simulate field conditions, but rather to provide a relative longevity ranking of the formulations under these harsh conditions, as way to predict how they should perform in the field. Release rates from the samples will be measured 2-3 times per week by placing the samples in a volatile trapping system. Collected volatiles then will be analyzed on a gas chromatograph for quantitative determination of the active long-chain fatty acids and their breakdown products. Release-rate curves will then be plotted over time to determine relative life of formulations, effective rates of active constituent release, and percent constituent loss due to chemical breakdown.

As stated in the preceding section, although synthetic fatty acids performed well in wind-tunnel studies, when tested in the field, neither acidulated oil nor the synthetic fatty acid blend was as effective as the standard NOW lure of CAO/PC in attracting females to adult traps. We suspect this difference is due to differential release charcteristics, with the PC/CAO providing more stable release for long periods. Thus, we propose to measure rates of volatile release from various batches of commercially available PC/CAO lures. There are three reasons for conducting this study. First, by standardizing rates of release for the PC/CAO lure and synthetic blend of fatty acids, we can provide a more appropriate comparison of the relative behavioral activity of these two odor sources. If behavioral activity is similar when the same release rates are used, then we have further definitive evidence that all the chemical constituents responsible for NOW host finding have been characterized. Secondly, by looking at differences between batches of this lure, we shall be able to determine the levels of variation in these lures. We already know from preliminary studies that the percentage of free fatty acids in CAO can vary from 1-6%. This variation could significantly affect the number of eggs laid on egg traps and thus reduce the reliability of the traps for monitoring NOW population levels. Thirdly, by knowing optimal rates of fatty acid release, we shall be better able to develop a synthetic NOW lure. With such a lure, we could better standardize rates of release and reduce season-to-season and lure-to-lure variation.

2. Field trials. As in the previous year, we shall investigate in large-scale field plots the potential of almond odors for NOW disruption. Tests will be conducted using those formulations that provide the greatest protection against oxidative and UV-induced decomposition of active constituents. As in the past year, the best two or three formulations will be applied to 5-acre replicated plots and compared with untreated plots for disruption of egg laying in NOW egg traps and for damage to nuts at time of harvest. In addition to the use of chemical additives, this year's formulations will differ from those of the past year by being based on acidulated peanut oil instead of oleic acid alone. Acidulated peanut oil contains a blend of the same fatty acids in almond oil in similar ratios and is cheaper and more readily available than almond oil. Although the wind-tunnel studies of the past two years indicated that NOW attraction was primarily due to oleic acid, both linoleic and palmitic acids evoked low levels of activity. This response to minor constituents may not have been disrupted by the use of oleic acid alone in the past year's field trials, and thus the use of a blend may provide more effective disruption.

Depending on how rapidly our laboratory studies on longevity furnish us with a significantly improved formulation, we would also like to investigate the effect of timing in application. Thus, if improved formulations are available, these will be applied to 5-acre replicated plots: 1) in May, 2) at hullsplit, or 3) at both times. These treatments will be compared with untreated plots for effectiveness in reducing nut damage at harvest.

Proposed Budget:

Graduate Research Associate	$11,960
Formulations, Supplies, & Travel	1,900
	$13,860

Warren C. Mueller 1-17-89
liaison officer