1989 ANNUAL PROGRESS REPORT
ALMOND BOARD OF CALIFORNIA

Project No. 89-A10a- 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.

Interpretative Summary
This year's efforts have concentrated on: 1) providing further field testing of the potential for fatty acid oils to disrupt NOW egg-laying in almonds, and 2) trying to improve the longevity of the previous year's formulations by the addition of chemical additives and by the identification of minor almond volatiles important in eliciting full upwind attraction of ovipositing NOW females. Various chemical additives, including three anti-oxidants and four UV light absorbers, were tested for their ability to protect the fatty acid formulations from chemical degradation in the laboratory. Accelerated oxidation tests, operated under UV light and high temperature, indicated that the addition of 1% tert-butylhydroquinone (TBHQ), an anti-oxidant used in food products, resulted in a >90% reduction in the rate of decomposition of the fatty acid mixture, as evidenced by the monitoring of degradation products.

Three long-chain fatty acid formulations were each applied to 15 acre almond plots just prior to hullsplit this summer and were compared with 15 acre untreated plots for NOW egg-laying disruption. These trials compared two different controlled-release systems, and tested the efficacy of TBHQ as a control against oxidative degradation. NOW egg-laying was monitored using standard NOW egg traps starting two weeks prior to application and continuing for six weeks. All three formulations were effective in reducing egg laying by 70% or more for about 14 days. Although differences in the level of infestation of plots before application make comparisons between treatments difficult, the addition of TBHQ appeared to have extended the duration of disruption when latex was used as the controlled-release system. Sampling of nuts at harvest six weeks after hullsplit showed 30-32% lower damage by NOW in treated plots. Although this level of control is not considered satisfactory, it is significant given that good ovipositional disruption was effected by the treatments for only 2 weeks. We are confident that work presently being conducted on alternative slow-release carriers will significantly increase the field longevity of the formulations and will provide a higher level of nut protection. Alternatively, multiple applications of the formulation should provide this same higher level of protection.

Experimental Procedures

1. Testing of chemical additives: The potential of four UV light absorbers and three antioxidants to reduce the rate of oxidative degradative of the disruptant formulations was examined in the laboratory. The four UV absorbers, produced by American Cyanamid, were Cyasorb UV5411, UV1084, UV531, and UV3346, and the antioxidants were BHA, BHT, and TBHQ, which have FDA approval as food additives. These were tested in an accelerated oxidation study, in which 1ml samples of fatty acid oil were added to open vials, which were held for ten days in an oven at 60°C under a mercury vapor lamp that shone UV light on the samples. At the end of the 10-day period, samples were placed in a volatile-collection device for 4 min and the volatile products of fatty acid oxidation were identified and quantified by gas chromatography/mass spectrometry. Each of these materials was first tested individually at three concentrations, 0.2%, 1%, and 2%, with each replicated three times.

2. Field trials of NOW ovipositional disruption: Three emulsifiable formulations were tested this year in the field for behavioral disruption of the NOW. In these, two different film-forming slow-release agents were compared, and the impact of the antioxidant TBHQ on formulation longevity was measured. The two release agents were: the anti-transpirant VaporGard (Miller Chemical & Fertilizer) and an experimental latex designated GF-30 (Diversitech Corp.) Each treatment, along with an untreated control, was assigned to three 5 acre plots in a split-plot design, as shown in Figure 1. The treated plots were located downwind from the untreated control according to predominant wind direction, and were separated from the control plots by three 5 acre plots. These precautions were taken to reduce the possibility of odors from the treated plots causing disruption in the control plots. Each plot was 17 trees by twenty rows. Two black NOW egg traps baited with 13.5g almond presscake and 1.5g crude almond oil were placed in each plot on June 8 to monitor egg laying throughout the season. On June 23, plots were treated with diazanon to control an outbreak of peach-twig borer. This did not have a noticeable impact on the NOW populations based on egg-laying data. On June 26, when the almond crop was at about 1% hullsplit, formulations were applied using an orchard sprayer at the rate of 100 gal/acre, containing 1.75gal/acre fatty acid oil. Egg baits were changed on July 20, and egg-laying was monitored through August 1. Nut damage was assessed twice, first on July 26, when nut samples were

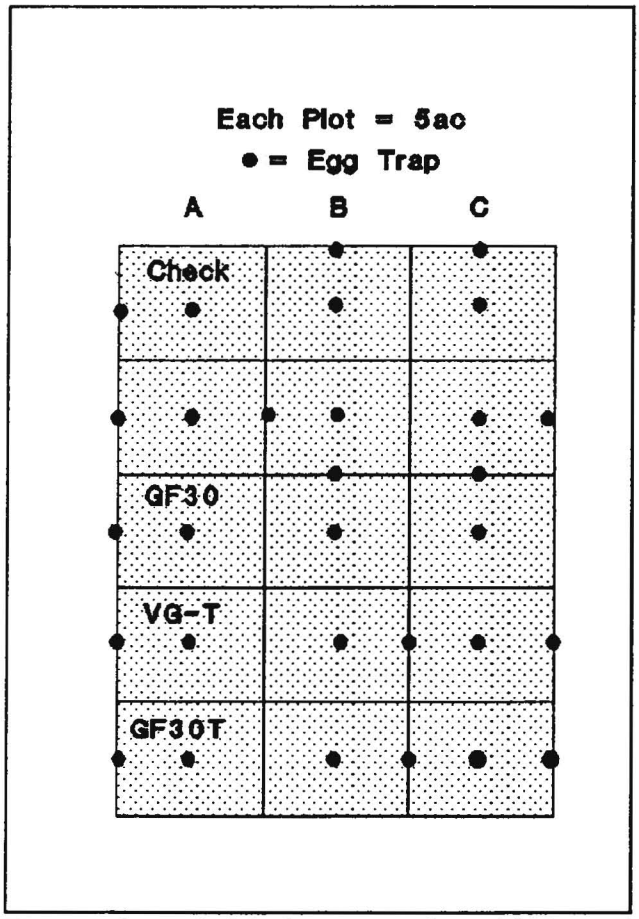

Figure 1. Field lay-out for 1989 NOW ovipositional disruption trials.

collected by knocking them from trees, and then 1000 nut samples were collected from each plot after harvest.

3. Wind-tunnel bioassays of fractionated acid oil: A 1.1cm x 38cm semi-preparative scale column was prepared by packing 25g Silica gel (100-200 mesh) into a 50ml titration buret that had been filled with hexane. About 10ml of hexane were pushed through the column before adding the sample. Then 0.5ml of acidulated almond oil (AAO) was mixed with 5 ml of hexane and this solution was placed on top of the column. The AAO was then eluted with 5ml hexane, 10ml 25% ether:75% hexane, 10ml 50% ether:50% hexane, 10ml ether, 10ml methylene chloride, and ca. 90ml methanol. The first 25 ml eluting through the column was collected and discarded as dead volume, then 12 - 5 ml fractions were collected. Fractions 5 and 7 were slightly yellow and fraction 6 more so. Fractions were bioassayed in a wind-tunnel, for which descriptions and procedures have already been provided in previous Annual Reports.

Results

1. Testing of chemical additives: Of the three antioxidants, TBHQ was found to be the most effective in reducing the rate of fatty acid degradation. Figure 2 compares the quantity of volatile degradation products collected from the headspace over a TBHQ-treated fatty acid oil with that from untreated oil, these

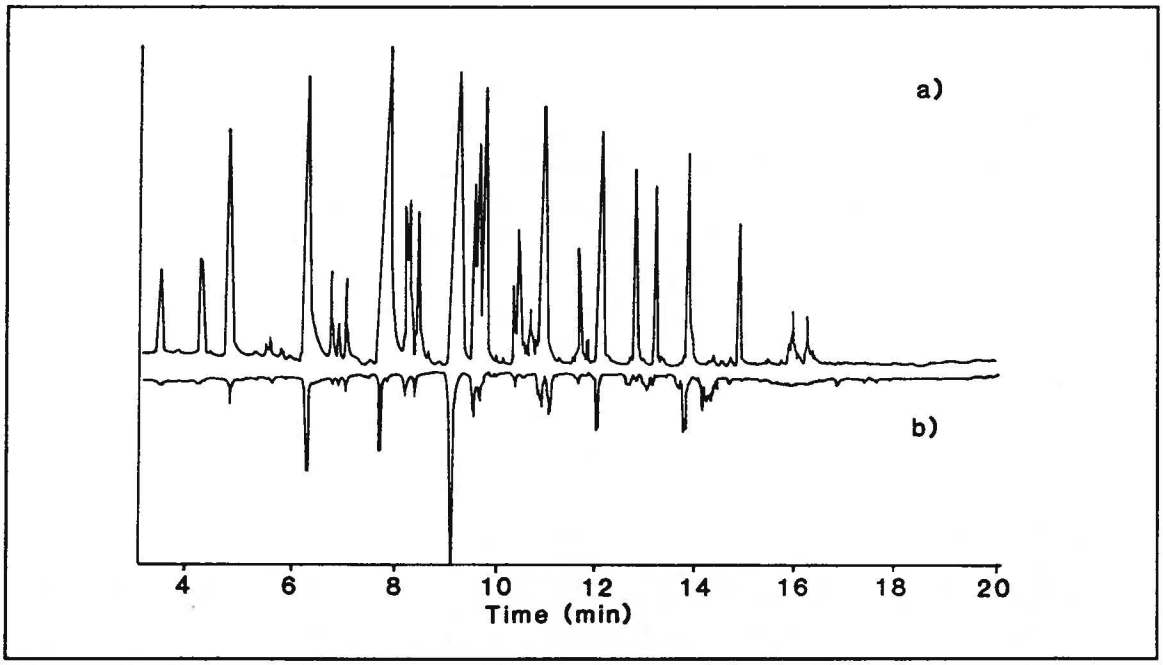

Figure 2. Decomposition volatiles collected from fatty acid oils after accelerated oxidation under UV light for 10 days. Top trace (a) = untreated oil, bottom trace (b) = 1% TBHQ added.

products being mostly short-chain aldehydes. The addition of 1% TBHQ was found to reduce the production of these aldehydes by greater than 90%. The combination of TBHQ with each of the UV absorbers was not found to significantly improve the protection provided by TBHQ alone, even when the accelerated oxidation test was extended to twenty days. Therefore, 1% TBHQ was the only treatment used in field trials of formulations.

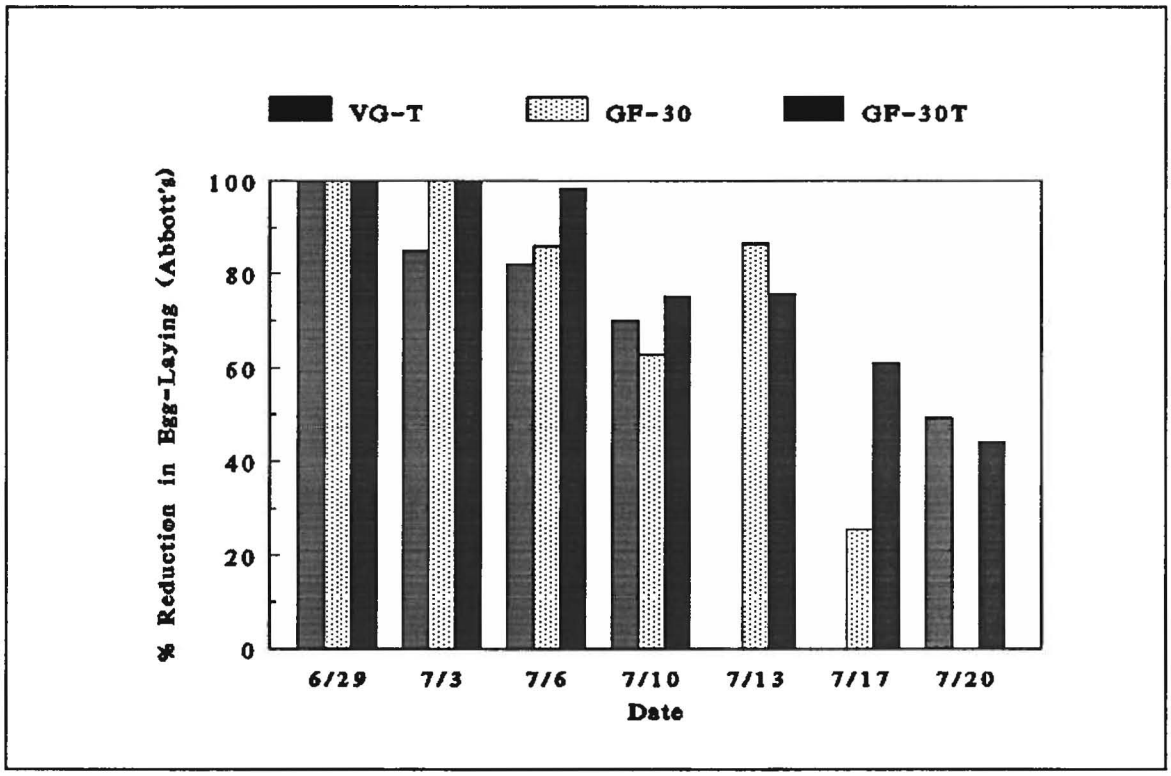

Figure 3. Percent reduction in egg laying by NOW relative to untreated plots during 1989 after application of disruptants on June 26. VG = VaporGard as controlled-release agent, GF-30 = latex as controlled-release agent, T = 1% TBHQ added.

2. Field trials of NOW ovipositional disruption: As in the 1988 field trials, treatment of plots with a slow-released oleic acid oil resulted in a complete elimination of NOW egg-laying on monitoring traps (Figure 3). However, although this suppression was initially absolute, the longevity of disruption was significantly shorter than in the 1988 test for all formulations (Figure 4). In 1989, we see that after ten days (7/6), only GF-30 with TBHQ was near 100% disruption. After this date, the activity of this formulation dropped gradually to 45% on July 20. With VaporGard containing TBHQ, there was a steady decline from 100% at Day 3 (6/29) to 70% at Day 14 (7/10), after which activity was completely lost. For GF-30 without TBHQ, disruption was greater than 80% for four of the first five sampling dates (17 days). Then, as with VaporGard, there was a significant drop in activity. By comparison, in 1988, both the VaporGard and latex formulations reduced egg-laying by 100% during the first three weeks. At four weeks, the VaporGard formulation was still >70% effective, while the latex was negligible in its activity. Trying to assess differences between the formulations in either their level of disruption or their longevity is made

difficult by the differences in rates of egg laying measured prior to treatment applications. This presumably reflects differences in NOW population levels resident in the plots, and these differences are taken into account in values given in Figures 3 and 4; however, this provides enough uncertainty that only large differences in disruption can be considered important. With regard to slow-release matrix, the latex formulations seem to outlast the VaporGard somewhat in 1989; however, just the reverse trend in seen in 1988. Thus, it appears that both slow-release materials were comparable in performance. Similarly, there is no clear trend to indicate that the addition of TBHQ significantly improved the longevity of disruption.

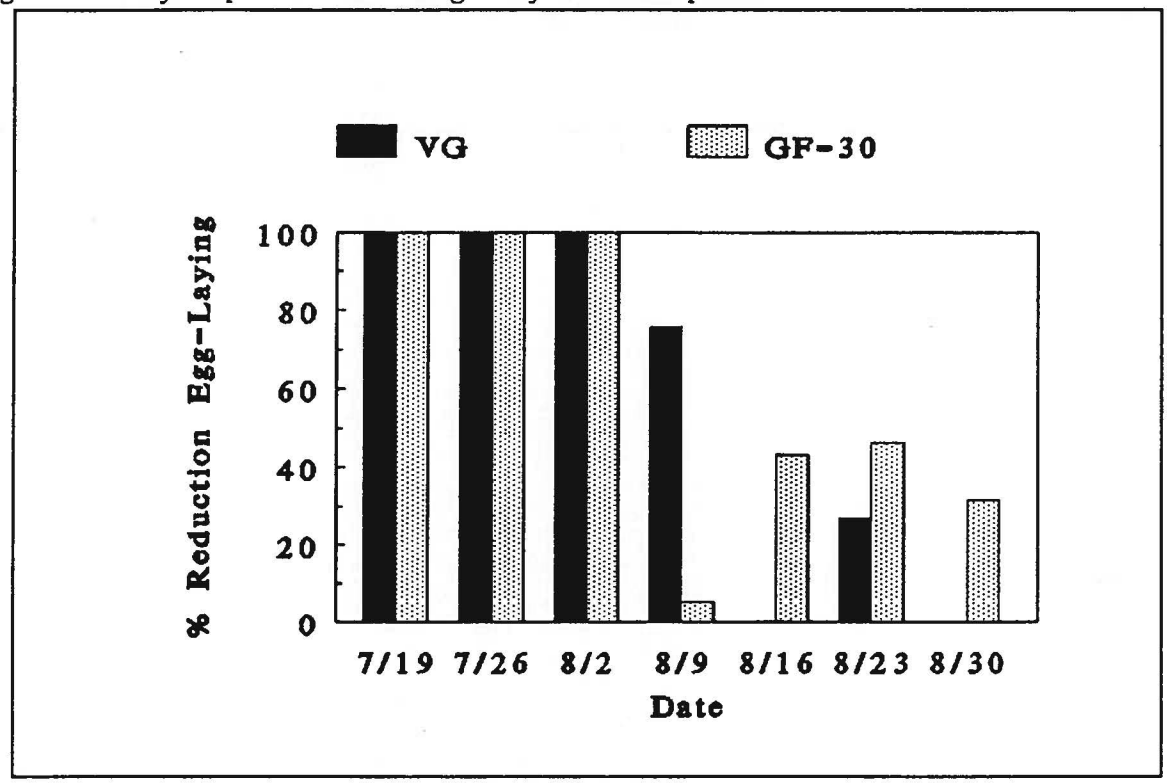

Figure 4. Percent reduction in egg laying by NOW relative to untreated plots after application of disruptants on July 12. VG = VaporGard as controlled-release agent, GF-30 = latex as controlled-release agent.

When nuts were sampled at four weeks for NOW damage this season, no difference was seen between treated and untreated plots; however, when nut damage was assessed at harvest, a 30% reduction was measured in treated plots. This is somewhat puzzling as in 1988, we measured 74% and 94% reductions in damage in treated plots at four weeks, but no difference in damage at harvest. The NOW larvae found in the 1988 harvest were mostly first-instar, suggesting that they had come from eggs recently laid, after the period of effective disruption had past. In this season's study, it is difficult to understand how there could have been no differences in nut damage earlier in the season, but significantly lower damage in all treated plots at harvest. Although we do not consider the 30% reduction in nut damage an acceptable level of protection, it is still encouraging given the relatively short period of effective disruption this season and the high NOW population levels that were in the test plots. Increasing the

period of disruption, either by multiple applications of the disruptant or by increasing the longevity of the formulation should provide singificantly higher levels of protection.

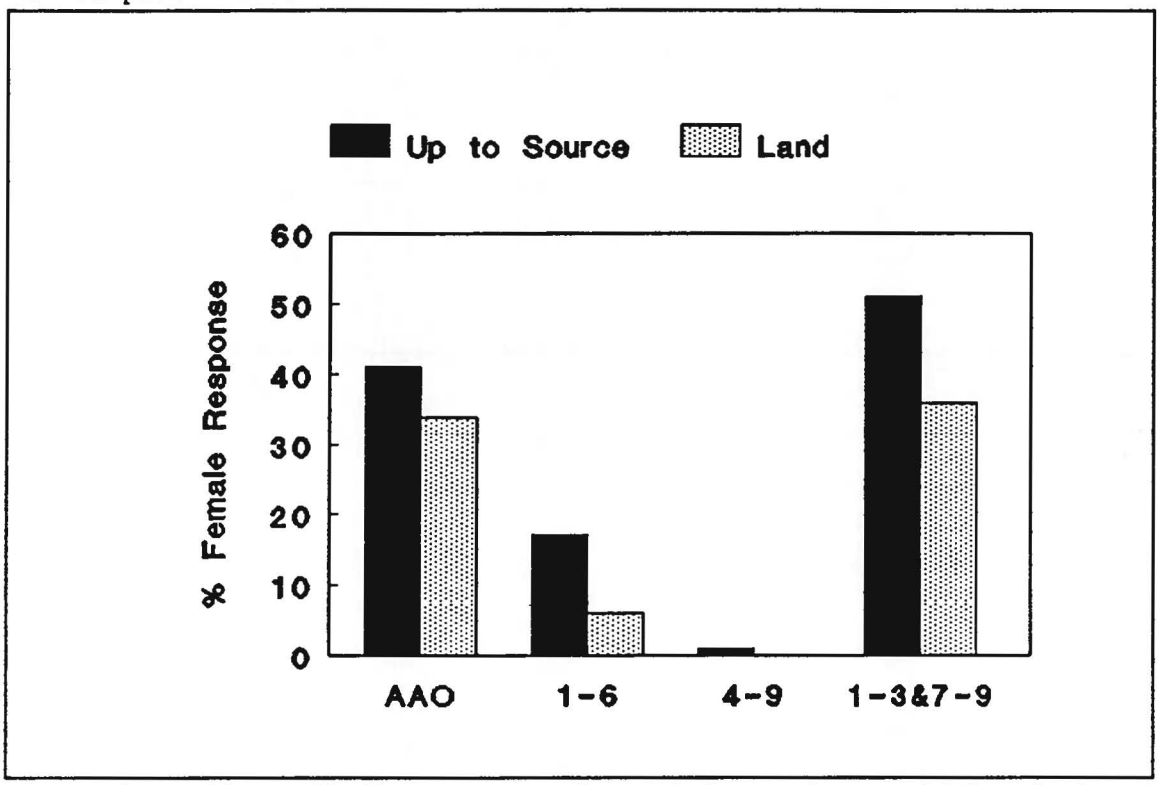

Figure 5. NOW host-finding response in a wind tunnel to acidulated almond oil (AAO) or various combinations of silica-gel fractions of AAO.

3. Wind-tunnel bioassays of fractionated acid oil: The first nine Silica-gel fractions of AAO were submitted to a subtractive wind-tunnel bioassay in which fractions were combined as follows: 1-6, 4-9, and 1-3 & 7-9. Each of these three combinations were presented in the wind tunnel at a rate of 50mg-equivalents of the unfractionated oil and were compared with AAO for activity. The results as shown in Figure 5 indicate two widely separated fractions, neither of which alone evoke full activity, but when combined elicit both upwind orientation and landing at levels comparable to AAO. The long-chain fatty acids previously reported as primary constituents responsible for NOW host-finding are contained in fractions 1-3. Consistent with previous findings, these elicit significant, but not full activity. The later fractions evoke almost no activity on their own, but appear to synergize the activity of the long-chain fatty acids. We have identified a number of constituents in these fractions, most of which are known products of fatty acid degradation. This is the first time that we have been able to duplicate the full activity of AAO using either synthetic compounds or fractionated AAO.

The results of these bioassays suggest a possible explanation for why the disruption formulations were effective for such a shorter period in 1989 than in 1988. The oleic acid oil used in 1989 was of a higher level of refinement than that used in 1988, and thus would have had lower levels of breakdown products, such as aldehydes and other volatile constituents. Work is planned

for this winter and spring to characterize those minor components with significant behavioral activity. Ensuring sufficient levels of these components in the field formulations should provide one avenue for increasing both their level of disruption and their longevity.