NOW Mating Disruption, Dispersal, and Damage Prediction

Project No.: 06-ENTO1-Burks

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Interpretive Summary:

In 2006 we: 1) performed experiments to examine the effect of dispenser density and pheromone formulation on efficacy of mating disruption for NOW in almonds; 2) used a series of mark-capture and mark-release-recapture experiments to examine rates of NOW movement within pistachios and between pistachios and almonds, and 3) examined the relationship between the number of NOW in egg and pheromone traps and subsequent NOW damage in Nonpareil and Monterey almonds.

<u>Effect of formulation and dispenser on efficacy of mating disruption</u> Previous Almond Board research has determined that a timed aerosol dispenser, the Puffer (Suterra, LLC) preserves activity of the principal pheromone component over a longer portion of the field season than hand-applied dispensers such as are commonly used against the Oriental fruit moth. Other Almond Board research has identified minor components of the NOW pheromone. In the 2005 field season we found that Puffers dispensing a multi-component pheromone formulation in areas of high NOW abundance (pistachios) had greater effect on disrupting sexual communication compared to Puffers dispensing only the primary component. However, evidence for reduced NOW damage in almonds using the multi-component formulation was ambiguous.

In 2006 we used 40-acre plots in two almond ranches with historically high NOW pressure to examine whether: a) the multi-component formulation was more efficacious than the single-component formulation when used at 2 Puffers per acre (the rate hitherto used), and b) whether the multi-component formulation would provide adequate efficacy at a lower rate (1 Puffer per acre), possibly thereby being more cost-efficient. The null hypothesis was that a mating disruption treatment or untreated control in a 40-acre treatment plot would result in less NOW damage than in a surrounding 120-acre buffer area treated with residual insecticides (methoxyfenozide, chlorpyrofos, and phosmet).

In one of the two ranches, inclement conditions prevented intended postharvest sanitation following the 2005 harvest. NOW damage in treatment plots in this ranch ranged from 10 to 21% in Nonpareil and 9 to 30% in Butte. The insecticide-treated buffer region had less damage than the untreated control in both Nonpareil and Butte, as expected, and some mating disruption treatment blocks had damage no greater than the surrounding insecticide-treated buffer area. Nonetheless, damage was too high and variable for an adequate comparison of treatments in this location.

At the other location damage ranged from 1 to 4% in Nonpareil and 3-17% in Monterey. In this location we found that:
- In both varieties, NOW damage was greater in the untreated control and the plot treated with one Puffer per acre with the single-component pheromone compared to the surrounding insecticide-treated area.
- In Nonpareil, NOW damage was less than or equal to the insecticide comparison in the plots treated with the multi-component formulation at 1 Puffer per acre and both formulations at two Puffers per acre.
- In Monterey, damage was equal to or less than the insecticide comparison for both formulations at 2 Puffers per acre, but neither formulation performed as well as the insecticide comparison at 1 Puffer per acre.

We conclude:
- The damage data for the multi-component formulation presented here are consistent with the previous data on its effect on NOW sexual communication and, in the long term, such formulations hold promise for improving cost-effectiveness.
- In the short term, using the present Puffer density of 2 per acre provides greater protection to pollinator varieties and is a more reliable approach to overall reduction of NOW damage in almonds.

Comparison of dispersal of NOW males and females in almonds and pistachios We used two techniques to examine movement of NOW. One, referred to as mark-capture involved marking moths in the orchard by applying a protein to trees with an air blast sprayer and using a laboratory assay (enzyme linking immunosorbent assay, ELISA) to detect this protein. The other, referred to as mark-release-recapture, involved feeding lab-reared NOW with a fat-soluble dye and releasing them in the field. Experiments were performed in 640 acres of pistachios in May and June, and in adjacent 160 acre blocks of almonds and pistachios in August.

General findings from these studies were:
- Individual NOW were cable of moving 1100 yards in 24 hours
- However, the probability that an individual would move a given distance depended on resource availability (unmated female NOW for males, suitable oviposition sites for females) and probably on age
- Young male and female NOW exhibit an L-shaped or long tailed curve of number v. distance, indicating that most do not travel far and a few travel a great distance.

- Under summer temperatures, NOW females, on average, live 10 days and lay most of their eggs within three days of mating.
- These observations suggest that, while NOW can travel a great distance, drop-off in damage due to oviposition by mated females should occur over a more intermediate range.

Relationship between NOW trap counts and subsequent damage We placed pheromone traps (i.e., a wing trap baited with three unmated NOW females) and egg traps in 41 40-acre plots (i.e., buffered by at least this distance from other traps, and by a greater distance from NOW mating disruption treatments), of which a central 2 acres were not treated with insecticides. Cumulative numbers of NOW males and eggs for flights 1, 2, and 3 were compared to damage in Nonpareil and Monterey almonds. We found that:
- While percent damage by NOW was, on average, similar between the two varieties, there was a low correlation ($r^2 = 0.18$) of NOW damage in Nonpareil with subsequent NOW damage in Monterey in the same plot.
- NOW damage in Nonpareil was correlated with the cumulative number of males captured in the plot in flights1 and 2, but not with egg counts in either of these flights.
- NOW damage in Nonpareil was positively correlated with harvest date, but the correlation of harvest date and NOW damage in Monterey was at least nominally negative.
- NOW damage in Monterey was not correlated with cumulative counts in males in flights 1 and 2, as for Nonpareil, but was correlated with cumulative males in flight 3. NOW damage in Monterey was also positively correlated with cumulative egg counts in flight 3, and negatively correlated with egg counts in flight 1.

We tentatively conclude that:
- Both egg traps and pheromone traps (when available) have potential for predicted NOW damage as well as timing treatments.
- These data suggest that damage to the Nonpareil examined here was determined by abundance of flight 2 NOW, and damage to Monterey was determined by abundance of flight 3 NOW.
- The low correlation of NOW damage between Nonpareil and Monterey suggests much variability in the relationship between abundance of NOW in flights 2 and 3.

A study of this type should examine at least three years of data; collection of a second year of data is in progress.

Objectives:

1) Compare efficacy for mating disruption of a single- and a multi-component pheromone formulation at two different Puffer densities.

2) Compare dispersal of NOW males and females in almonds and pistachios.

3) Examine the relationship between counts of NOW males in pheromone traps and eggs on egg traps with subsequent damage to almonds.

Materials and Methods:

Effects of formulation and Puffer density on efficacy of mating disruption Two formulations and two pheromone doses were compared to untreated controls using 160 acre plots in two ranches (3700 and 3710). Treatments were: 1) an untreated control (no mating disruption and minimal residual insecticide for NOW control); 2) a multi-component formulation at 42.5 mg/acre AI; 3) a multi-component formulation at 21.2 mg/acre AI; 4) the previously-used single-component formulation at 42.5 mg/acre AI; and 5) the previously-used single-component formulation at 21.2 mg/acre AI. The single-component blend contained only (Z,Z)-11,13-hexadecadienal, the principal component of the NOW sex pheromone, and the multi-component formulations contained components and proportions similar to those described by Leal et al. (2005). The lower doses were achieved by placing fewer Puffers releasing the same amount of material in a more widely-spaced grid; e.g., 80 Puffers (2/acre) at ~50 yard intervals v. 40 Puffers (1/acre) at ~70 yard intervals. Mating disruption emitters were in place and activated by April 11.

The plot arrangement is depicted in Fig. 1. Treatments were assigned to one of five 160-acre treatment plots (880 x 880 yards). The untreated control was placed upwind of the others (in the most northwesterly position), and the other treatments were assigned randomly to the remaining positions. The 160 acre plots were divided into two unequal subplots; a central 40 acre (440 x 440 yards) plot which received either a mating disruption treatment or the untreated control treatment, and the remaining 120 acre surrounding subplot which did not receive the mating disruption treatment, but which did receive a more extensive residual insecticide treatment than the treatment plots. The untreated control and mating disruption plots received an application of 18 oz per acre of methoxyfenozide (Intrepid) on April 18 and 64 oz per acre chlorpyrofos (Lorsban) on May 30. Areas outside the treatment plot received additional treatments of 5.3 lbs per acre of phosmet (Imidan) on May 10-17, 18 oz per acre of methoxyfenozide on June 13-18, and a mixture of 64 oz per acre chlorpyrofos (Lorsban) and 18 oz per acre methoxyfenozide on Jul 28. Because of greater NOW pressure, treatment plots in 3700 also received the June 13-18 methoxyfenozide treatment.

Planned postharvest sanitation (poling) was substantially completed for Ranch 3710, but not for 3700. Data from Paramount Farming's routine sanitation assessment procedures, performed in January to March after the completion of sanitation procedures, were used to compare mummy load between the plots. Each 160 acre quarter-section was divided four 40 acre subdivisions, and then four more 10 acre sub-sub-divisions. Eight tree samples were taken from two of the four 10 acre subdivisions within each 40 acre division. Trees were selected such that each group contained both Nonpareil and a pollinator variety. All mummies were counted in the canopy of these

eight trees and on the ground between them. Mummies from some blocks were collected and examined in the laboratory for evidence of NOW infestation.

Data collected included weekly counts of males in wing traps baited with virgin females and the proportion of nuts damaged at harvest. Female-baited flight traps were placed in the center of each treatment block, and female lures were replaced and data collected weekly throughout the period of mating disruption. The primary purpose of these flight traps was to verify that complete shutdown was achieved, as past experience has shown that complete shutdown is necessary but not sufficient for reduction of NOW damage in almonds. For each variety, samples of nuts were collected from windrows at each of 9 locations in each 40 acre subplot and from each of 8 locations in the surrounding 120 acre subplots (Fig. 1). Harvest dates were August 21 to August 28 for Nonpareil, September 8 for Butte, and October 7 for Monterey. The total number of nuts evaluated from each subplot ranged from 2168 to 2756 for Butte and from 4265 to 6318 for Nonpareil and Monterey. Nuts were held in cold storage pending examination and scoring by Paramount Farming research personnel for NOW damage.

The number of mummies on the ground was compared between ranches with a fixed effects ANOVA using a nested model, with Ranches and 160 acre blocks within ranches as independent variables. The unit of observation was the 40 acre blocks, and the count of mummies on the ground was transformed as $\sqrt{x}$ to stabilize variance. The number of mummies in the tree was compared between ranches using χ^2 statistics for a 2 x 2 contingency table. This method of analysis was also used to compare sums of uninfested and NOW-infested almonds between subplots within each treatment plot, with a comparison-wise α of 0.05.

<u>Comparison of dispersal of NOW males and females in almonds and pistachios</u>
General methods. Female-baited wing traps for males and delta traps (Suterra LLC, Bend OR) baited with an organdy bag containing almond meal were used to capture NOW males and females, respectively. The female-baited flight traps were as previously described (Burks and Brandl 2004) except that they were baited with one virgin female rather than three. These virgin female lures were left in the field for 7 days. Trials were conducted in a square 640 acre block of pistachios starting May 11 and June 13, and in adjoining 160 acre blocks of almonds and pistachios starting August 28.

The protein mark-capture technique used was developed and described by Jones et al. (2006). Briefly, 10% egg white protein was applied to a portion of the orchard. NOW removed from traps inside and outside of this area were examined for the protein marker by externally rinsing them for 3 min in 1 ml of rinse buffer. An aliquot of this rinse buffer was then analyzed using an indirect enzyme-linked immunosorbent assay (ELISA) with a commercially available polyclonal antiserum for chicken egg albumin, as described by Jones et al. (2006). This is an automated colorimetric assay which results in an optical density (OD) reading. Moths with an OD over 4 standard deviations (n = 8) above that of extraction buffer from control NOW were considered 'positive' (i.e., exposed to egg white protein). In some cases insects were stored on trap liners at -20°C prior to extraction.

Moths for mark-release-recapture release studies were marked internally using a fat-soluble dye, Calco oil red. NOW from a pistachio strain colony established in 2005 were reared in the laboratory on standard bran diet containing 0.05% Calco oil red dye (w/w). Eggs were collected over successive 24 hour periods and reared on a minimal quantity of standard bran diet. Dyed diet was added after 7 days and larvae were counted and transferred when 21 days old. Two hundred mixed sex last instar larvae each were placed in release containers (0.5 gallon plastic food container) with a small amount of diet, and the daily cohorts were brought into synchronous emergence by cooling them as pupae for a predetermined number of days at 21.1°C

Mark-capture experiment, first flight. The first experiment was conducted in pistachios because we previously found higher NOW abundance in spring and summer in pistachios compared to almonds. This higher abundance was advantageous for initial examination of frequencies of females captured in delta traps baited with almond meal. This experiment was conducted in a square 640 acre pistachio ranch (Paramount Farming location code 4010) (Fig. 2). Protein was applied in a 22 row by 26 tree area covering 4 acres in the center. A total of 137 female and 17 male traps were used. A grid of 8 north-south rows of 16 female traps was centered around the protein-treated area with traps 107 yards apart north-south and 222 yards apart east-west, and an additional 9 traps were placed in the center acre of the protein-marked area arranged as a 3 x 3 grid, separated by an average of 32 yards east-west and 38 yards north-south. Male traps were placed in a 4 x 4 grid at ca. 430 yard intervals centered on the protein-treated area, with one additional trap in the center of this area. Protein was applied to the treatment area on May 11th and the male traps were subsequently baited with live females. Traps baited with females and almond meal were examined and liners changed daily from May 12 to May 18.

Chi-square tests of homogeneity were used to compare total and marked NOW between quarters of the experimental site (i.e., the 4 male and 32 female traps in each quarter outside of the protein-treated area). Total and protein-positive males were compared between different distances within each day using separate 1-way ANOVAs with males per trap as the dependent variable and distance from the center of the site (8, 217, ~620, or 832 m) as the independent class variable, and the relationship between females per trap (total and protein-positive) and distance of the traps from the center of the study site was examined using Pearson correlation.

Combined mark-capture and mark-release-recapture experiment, second flight In June, 10% chicken egg white was again applied to the same 4 acres in pistachio Ranch 4010, but this time 10,000 mixed-sex dye-marked NOW pupae were released following the protein application and 49 rather than 17 male traps were used (Fig. 2B). The female traps were arrayed as described for the previous experiment, and the male traps were placed in a 7 x 7 grid, with the center trap in the center of the protein-treated area, with inter-trap distances of 222 yards east-west and 213 yards north-south. Protein was applied to the treatment area on June 13th, the dyed moths were placed in the field as 50 containers of 200 pupae each spread evenly throughout the protein-treated area, and the flight traps were baited with virgin females or almond meal, as previously

described. Male and female traps were examined and liners with captured moths were changed daily from June 14 to June 20 (7 days). Bait was replaced in the male and female traps on June 20 and liners were collected on June 27 and trapped moths were evaluated for dye but not for protein.

Data analysis was similar to that for the previous experiment. Chi-square tests of homogeneity were used to compare total and marked NOW between quarters of the experimental site (i.e., the 9 male and 32 female traps in each quarter that were outside the protein marking area and off of the north-south and east-west centerlines of the site). Pearson correlation was used to examine the relationship between trap count and distance from the center of the site in males. Chi-squared statistics were used to compare numbers of females between distance ranges for each day, and fit was examined for linear regression of dyed males on day 4 and dyed females on day 5 on regressed on the reciprocal of the distance from the center of the site.

Dispersal from a crop border, third flight In August protein mark-capture and mark-release-recapture with dye-fed moths was used to examine dispersal from the border between a 160 acre quarter-section of almonds (Ranch 3470) on the north and pistachios (Ranch 4450) on the south (Fig. 3). The mark-release area was a long narrow rectangle (880 x 33 yards, 2 acres, 5 rows) in the portion of the pistachios adjacent to the almonds, instead of a small square in the center of a larger square release area as was done with the previous two experiments, and 7% chicken egg white was applied instead of 10%. The experiment was monitored with 105 female traps and 21 male traps as shown in Fig. 3. Protein was applied in the marking area in August 28 and females were subsequently placed in male traps. On August 30 50 containers, each with 200 dyed pupae, were place in approximately every third tree of the center row in the treatment area. Traps were examined daily from August 29 to September 4, and liners were changed if they contained captured moths.   Chi-squared statistics were used to compare the number of dyed and ELISA-positive males and females found outside the marking/release area.

Relationship between NOW trap counts and subsequent damage A single wing trap (Suterra LLC, Bend OR) baited with three unmated females (Burks and Brand 2004) and two NOW egg traps containing almond flakes (Liberty Vegetable Oil Company, Santa Fe Springs CA) were placed near the center of 41 blocks of 40 acres each of almonds. The wing trap was placed in the center of a 220 yard (30 tree) long block of Nonpareil trees between two orchard roads, and the egg traps were placed five trees north and south of the wing trap within the same row. Within this block the alley containing the traps, and two alleys on either side, were flagged to prevent application of insecticide treatments. Harvest samples taken within this 150 tree (2 acre) area examined damage in the absence of residual insecticide treatments, with Nonpareil samples coming from the middle row far from possible insecticide drift.

Sites were selected to include the varieties Nonpareil and Monterey and to avoid mating disruption treatments, and were located throughout the Kern County portion of the San Joaquin Valley, in an area roughly bounded by Highway 33 on the west, Highway 99 on the east, Seventh Standard Road on the South, and County Line Road on the North.

Traps were monitored weekly beginning February 15 for oviposition traps and March 1 for flight traps and ending on September 29. Each week the unmated females in the trap were replaced, trap liners were changed, and counts of eggs on the oviposition traps and males in the flight traps were recorded.

Harvest of Nonpareil occurred between August 15 and September 3, and harvest of Monterey occurred between September 16 and October 20. Nonpareil harvest samples of ~280 Nonpareil and ~265 Monterey nuts were taken from a 5 locations in each plot. All nuts were examined by Paramount Farming research personnel to determine NOW-specific damage, and the proportion of NOW damage in Nonpareil and Monterey at each plot was calculated directly from the five pooled samples.

Plots of male and egg activity were used to pool weeks according to flights, and damage was compared between the Nonpareil and Monterey using boxplots to examine frequency distribution. Scatter plots and linear regression were used to the examine correlation between percent NOW damage of the two varieties in the same plot, and the relationship between harvest date and percent NOW damage in the two varieties. Prior to regression an arcsine transform of proportional harvest damage was used to normalize the distribution, and harvest dates were transformed as ln(Julian date). Sums of male and egg counts were not transformed because initial examination indication that this gave the best fit. One of the 41 plots examined, one did not contain Nonpareil almonds, eight did not contain Monterey, and 10 did not have both varieties. The frequency distributions of damage are therefore based on 40 plots and 33 plots for Nonpareil and Monterey, respectively, and regression of Monterey on Nonpareil damage is based on 31 plots. Linear regression models were compared by forward selection and by selection for the best adjusted r^2. Diagnostic procedures (condition indices and variance proportions) indicated that multiple collinearity did not occur among the predictor variables.

Results and Discussion:

Effects of formulation and Puffer density on efficacy of mating disruption In the interim report (Burks et al. 2006) we reported that there were 2 males captured in virgin-baited flight traps in the four mating disruption plots in each of the two ranches over the entire season (0.25 males per trap per season), compared to 402 to 942 females per trap over the same time period in traps in the center of the untreated control plots or on the margins between 160 acre plots. Clearly the Puffers reduced the ability of NOW males to find the calling females we placed in traps. In that interim report we also presented data showing that there was little association between eggs on egg traps and subsequent damage, both in this study and that of the association of trap counts with subsequent damage outside of a mating disruption context (discussed below).

There were significantly more mummies in the plots in 3700 compared to 3710, both on the ground ($F = 174.39$, df = 1, 30; $P < 0.0001$) and in the trees ($\chi^2 = 366.22$, df = 1, $P < 0.0001$) (Table 1). Overall the percentage of ground mummies infested was $15 \pm 5.6\%$ (mean $\pm$ SD, n = 36), and the percentage of mummies infested on the ground and in the

tree for the 11 40-acre blocks for which both these data were obtained was 17 ± 4.8 and 24 ± 6.5, respectively. Not surprisingly, there was also more harvest damage in 3700 than in 3710 (Fig. 2).

In all cases the rigorous insecticide regime used in the outer 120 acre subplot resulted in less damage than the inner 40 acre treatment plot for the untreated control (Fig. 4A-D, Unt). At Ranch 3700 the overall damage rate was 10 to 21% in Nonpareil harvested on August 24 and 9 to 30% in Butte harvested. For Nonpareil in Ranch 3700 the damage in the mating disruption treatment subplots was less than or equal to the damage in the insecticide comparison plot in three of the four mating disruption treatments, but the exception in which the insecticide comparison had less damage was the multiple component formulation at 2 Puffers per acre; i.e., putatively the most stringent treatment (Fig. 4A). In Butte in Ranch 3700 damage was no greater in the mating disruption treatment plot than in the surrounding insecticide comparison in only one of the four mating disruption treatments (Fig. 4B).

At Ranch 3710, NOW damage was in the range of 1 to 4% in Nonpareil (Fig. 4C) harvested between August 21 and 28 and of 3 to 17% in Monterey (Fig. 4D) harvested 40 to 47 days later. Under these circumstances the order of efficacy of mating disruption treatments was more similar to that of effectiveness at disruption of NOW sexual communication in pistachio plots with high NOW abundance (Burks et al. 2005). In Nonpareil both the single- and multi-component formulation at two Puffers per acre resulted in less damage in the 40 acre treatment subplots compared to surrounding 120 acre insecticide comparisons (Fig 4C). At one Puffer per acre in Nonpareil the multi-component formulation had no more damage than the insecticide comparison, whereas the single component formulation had greater damage than the surrounding insecticide comparison (Fig 4C). In Monterey in Ranch 3710 both formulations at two Puffers per acre resulted in no greater damage than in the insecticide comparison, whereas this was not true for either formulation at 1 Puffer per acre (Fig 4D).

One possible objection to the design of this experiment is that mating disruption formulations in the inner 40 acre subplot could affect NOW damage in the surrounding 120 acres comprising the insecticide comparison. We believe that this is unlikely in view of distinct differences in NOW damage seen between treatments in 16 20-acre treatment plots in a single 640 acre Latin square arrangement (Burks et al. 2004); i.e., within similar proximity. Moreover, even if the treatment does affect the surrounding 120 acre insecticide comparison, this simply makes equaling or beating the efficacy of the insecticide a more stringent test of efficacy of the mating disruption treatment.

Another possible objection is that the statistical tests employed here do not use the plots as random samples from an entire region to make inferences about response of all almonds in the region to mating disruption treatments. We acknowledge that this is that case; instead, we attempt the more modest objective of determining (with an α of 0.05) whether there is a greater positive difference in NOW damage in [treatment] – [insecticide comparison] than can be attributed to sampling error. It has been noted that "…difficulties in measuring the efficacy of mating-disruption formulations in small plots has led to an overwhelming reliance on conducting such trials in large commercial

settings. The major drawbacks of on-farm experiments have been that the untreated controls are not available and that a high level of replication cannot be accomplished" (Gut et al. 2004). The current design addresses this first problem, but does not entirely address the second. Under these circumstances it is necessary to accept more restricted inference from the damage data, and examine more carefully whether these damage data are consistent with the data on sexual communication; i.e., those on effects of these formulations on males captured in virgin-baited traps and mating in sentinel females (Burks et al. 2006).

The data from Ranch 3710 are consistent with those data on sexual communication (Burks et al. 2006) in that control similar to that of the insecticide comparison was achieved with 1 Puffer per acre with the multi- but not the single-component formulation. It was also similar to earlier experiments (Burks et al. 2004) in that NOW damage proved more difficult to reduce in pollinator varieties harvested later compared to Nonpareil. The data from Ranch 3700 serve primarily as an unintended demonstration of the long-held principal that inadequate sanitation severely compromises efficacy of all other measures to manage NOW damage, including residual insecticides.

Comparison of dispersal of NOW males and females in almonds and pistachios
Mark-capture experiment, first flight In the first two dispersal experiments we compared direction, distance, and rate of movement of male and female NOW. Directional effects were examined by comparing the total number of male and female NOW between traps in the four quarters of the site with the number of ELISA-positive males and females (Table 2). The number of males captured in the southwest quarter comprised 43% of the total of the four quarters for ELISA-positive males but only 28% of this total for all males, a significant differences ($x^2 = 84.25$, df = 1, $P < 0.0001$). The southwest corner also had more ELISA-positive males than the next largest quarter (the northwest, with 31%), and this difference was also significant ($x^2 = 16.11$, df = 1, $P < 0.0001$). For females, the southeast corner had significantly fewer ELISA-positive and significantly fewer total moths compared to the next larges sum ($x^2 = 17.47$, df = 1, $P < 0.0001$; and $x^2 = 10.73$, df = 1, $P = 0.0011$, respectively). However, most ELISA-positive females were captured in the southwest corner whereas most females overall were captured in the northwest corner. This difference in proportion of ELISA-positive and total females captured in the southwest quarter was significant ($x^2 = 13.57$, df = 1, $P < 0.0001$).

Distance and rate were examined by comparing the proportion of males and females marked by day (Table 3) and the number of marked males and females captured by distance for various days (Fig. 5). ELISA-positive males and females differed between days, both in terms of absolute numbers captured and as a proportion of NOW captured (Table 3). In males the proportion of ELISA-positive individuals increased over the week, from 10% on day 1 to 40% on day 7, and in females there was a decrease over the week, with 54% of the ELISA-positive females captured on the first day of the experiment. ANOVA performed separately for each day found no significant differences between distances among all males (i.e, both ELISA-positive and –negative) for day 1 ($F = 0.96$, df = 3, 13; $P = 0.5196$) or for any of days 2-7 ($P > 0.05$). There were significant differences in ELISA+ males captured at different distances for day 1 (i.e., 24 hours after application of the protein label) ($F = 28.23$, df = 3, 13; $P < 0.0001$), but not

for subsequent days ($P > 0.05$) (Fig. 5A-D). In contrast, for females 24 hours after label application there was no correlation between the number of ELISA-positive moths per trap and distance ($r = -0.077$, $P = 0.3661$). The only day in which there was a significant correlation between trap count and distance was day 3, in which only five ELISA-positive females were found and all were within the first 500 m from the trap.

Combined mark-capture and mark-release-recapture experiment, second flight One dyed male and no dyed females were captured on days 1 and 2 (24 and 48 hours after application of the protein label, but dyed moths were a majority of all NOW captured for days 4 to 7 in males and 3 to 6 in females (Table 4). Overall dyed moths comprise 52% of males and 66% of females captured in the first week of the experiment, and for each sex dyed moths were 24% of the total captured in the second week.

Differences in the number of NOW captured between the four quarters of the experiment site were more apparent in males than in females. There were significant differences between the four quarters of the site in the number of feral NOW captured in week 1 ($\chi^2 = 31.38$, df = 3, $P < 0.0001$) and week 2 ($\chi^2 = 35.63$, df = 3, $P < 0.0001$), and there were significant differences in proportions of feral and dyed NOW between the quarters for week 1 ($\chi^2 = 49.60$, df = 3, $P < 0.0001$) and week 2 ($\chi^2 = 62.37$, df = 3, $P < 0.0001$). The traps in the southwest quarters captured 35 and 34% of feral males from the four quadrants for weeks 1 and 2, respectively, and 58 and 68% of the dyed males captured for those weeks (Table 5). Among females there were not significant differences ($P > 0.05$) between the four quarters of the site in either feral or dyed NOW captured in week 1. There were, however, significant differences between these quarters in week 2, both in the number of feral females ($\chi^2 = 22.82$, df = 3, $P < 0.0001$) and dyed females ($\chi^2 = 8.29$, df = 3, $P = 0.00405$). For females the clearest difference in captures among the four quarters was the southeast, which contributed 8% of the feral females and 7% of dyed females captured in the four quadrants in week 2 (Table 6).

Correlation analysis shows a positive association between males captured and distance from the center of the site for days 1-3 and a negative association on subsequent days (Table 7); i.e., for the first three days more male were captured farther from the center and subsequently more males were captured near the center (Fig. 6). This was true not only for feral males, but also for ELISA-positive males which presumably acquired the protein label in the center of the study site, and for dyed males which must have eclosed in the center. While the correlation coefficient for the dyed males on day 3 is not significant (Table 7), the median distance from the center of the study site for the 114 dyed males caught that day was 558 m. On day 4 the correlation coefficient for dyed males was significantly negative (Table 7) and the median distance from the center of the study site for the 226 dyed males captured that day was 197 m (Fig. 6).

The four feral females found on day 3 were captured at distances up to 545 m from the center of the study site (Fig. 7), with the one ELISA-positive female coming from this farthest distance. However, the maximum distance from the center for traps captured dyed females was 41, 110, 702, and 959 m for days 3, 4, 5, and 6, respectively (Fig. 7). When distances were pooled as three categories, there was a significant association

between time after release/eclosion of dyed females and the proportion of females found at near, intermediate, and far distances (Table 8). Regression of the number of dyed males captured on day 4 on the reciprocal of trap distance was highly significant ($F = 78.43$, df = 1, 47; $P < 0.0001$, $r^2 = 0.63$). Regression of females captured on day 5 on the reciprocal of distance for distances up to 263 m (excluding many 0 counts) was also significant ($F = 8.67$, df = 1, 19; $P = 0.0083$, $r^2 = 0.32$).

Dispersal from a crop border, third flight Very few dyed and ELISA-positive males or females were found outside the marking/release area (Table 9). While the ELISA-positive and dyed females captured in pistachios comprise ~60 and ~70%, respectively, of the marked females found outside the mark/release area, this ratio was not significantly different from 50:50 with the few moths captured. It would be necessary to capture ten times more females this ratio to be a statistically significant departure from homogeneity.

Summary and conclusions, dispersal experiments The first two experiments showed consistent results with respect to direction of movement and different results with respect to rate. There were consistently more labeled males captured in the southwest quarter than in the others. Other data (Burks et al., unpublished) indicate that the prevailing wind at night in this region is generally from the southwest, thus these data simply indicate a bias of direction in NOW males, presumably due to a tendency to move upwind toward pheromone sources. Fewer unmarked and marked females were captured in the southeast quarter in both the first and second experiment. This may have been due to differences in sanitation efficiency between parts of the study site; presumably females are less likely to respond to a trap baited with oviposition medium in an area with more mummies.

Results with respect to rate of movement were not consistent between the first two experiments. In the first experiment, the mark-capture technique showed that most labeled males were near the labeled area 24 hours after the label was applied, but males were found at all distances after 48-72 hours. Marked females in the first experiment were found at all distances 24 hours after the label was applied. In the second experiment ELISA-positive and even dye-positive males were found at far distance in the first three days of the experiment, but then more dye-positive males were found near the release site rather than farther away. Together these data suggest that individual NOW of both sexes are able to move 1000 m in 24 hours, but that the probability that a member of a population will move a given distance depends on the distribution of resources (unmated females for males, suitable oviposition sites for females) and perhaps the age structure of the population.

The data from experiment 2 indicate that dispersal of the newly eclosed females across the range of distances examined required at least 2-3 days. This is important because our data on daily fecundity using single NOW pairs held at 26°C constant temperature indicates that 50% of oviposition occurs by day 3 after mating. A reciprocal function is like the negative exponential and Cauchy function, two other functions used to model insect dispersal data, in that it has a very long tail and predicts high values close to the origin; i.e., most individuals don't disperse far but a few disperse very far. These data

suggest that, while NOW females are capable of dispersing over 1,000 m following eclosion, most oviposition will occur within a shorter range.

The data from the third experiment did not demonstrate a greater probability for NOW in border rows of an almond/pistachio edge to go to one crop or the other. If that is the case, then the collective probability of gravid females moving from one crop to the other is a function of the abundance in the two crops. Our data this year indicates that damage to Nonpareil and Monterey almonds are caused predominantly by the second and third flights, respectively (see below), and our previous data indicated that NOW abundance in the Kern County region is generally greater in pistachios than almonds at this time.

Relationship between NOW trap counts and subsequent damage In the interim report we presented an analysis of data on the relationship between eggs in egg traps and males in female-baited traps with damage to Nonpareil almonds, but the last few weeks of trap data and damage data for Monterey almonds were not yet available. Here we present these additional data and compare, between these two varieties, damage patterns and association of damage with eggs on egg traps and males in virgin baited wing traps between the two varieties.

The weekly count data for males in virgin-baited traps and eggs on egg traps are presented in Fig. 8. Here flights are defined empirically, based on egg trap data, with the first week of a sustained increase of two or more weeks considered as the start of the new flight. We acknowledge that these "flights", particularly the later ones, likely include individuals of various chronological ages due to different rates of development on different substrates. Nonetheless, we believe that the flights identified here have an empirical basis and are operationally useful.

Compared to Nonpareil, there was a similar overall distribution of damage between plots in Monterey (Fig. 9A). In contrast, correlation between damage in the two varieties in individual plots is relatively poor (Fig. 9B). Regression of NOW damage in Monterey on that in Nonpareil for all 31 plots containing both varieties resulted in an r^2 of 0.11 and a P value of 0.055. Examination of Fig. 9B reveals two influential observations in which NOW damage was ≤5% in Nonpareil but >18% in Monterey. Recalculation of the regression without these two observations results in an r^2 of 0.18 and a P value of 0.016. Regression of NOW damage in Nonpareil on harvest data revealed a significant positive slope (t = 2.28, df = 38, P = 0.0075) and an r^2 of 0.17. A plot of NOW damage by harvest date for Nonpareil and Monterey indicates a direct association for Nonpareil, and an inverse association for Monterey.

Since we defined flights 1 and 2 differently between the interim and final report, regression statistics for Nonpareil are again presented. In the interim report flight 2 was defined as beginning as soon as flight 1 trap counts returned to a baseline level (i.e., near 0). This is not a traditional definition of a flight, but would allow a manager to conclude data collection and make a management decision sooner. When defined in that manner, males captured in flight two were more strongly correlated with NOW damage to Nonpareil then those in flight 1 (r^2 values of 0.23 and 0.14, respectively). In

the present report, with flights defined more traditionally as starting with the first week of a sustained increase in egg trap counts, flight 1 males were more strongly correlated with NOW damage to Nonpareil than those from flight 2 (Table 10). We also examined the sum of males per trap in the period between peak egg counts for flights 1 and 2, and found that this correlated with NOW damage to Nonpareil almonds more strongly than males in either flight in its entirety, and even more strongly than harvest date. As we reported in the interim report, eggs counts have no predictive value for damage in Nonpareil, and there is little gain in overall model fit with addition of more than two terms. A model based on the sum of males captured in pheromone traps over the first flight and harvest date had better predictive value than one based on males captured in second flight and pheromone traps. However, a model based on harvest date and the sum of males captured in pheromone traps in the period between peak egg traps counts for flights 1 and 2 provided even better prediction of NOW damage to Nonpareil (Table 10).

The relationship between damage in Monterey and the predictor variables examined was very different than that for Nonpareil. Eggs in flights 1 and 3 were significantly correlated with NOW damage in Monterey, whereas there was no significant association of egg counts in any flight with damage in Nonpareil (Table 11). The correlation between eggs in flight 1 and NOW damage in Monterey was negative (Fig. 11A), although there was a positive association between eggs count in flight 3 and NOW damage in Monterey (Fig. 11B). Males in the first and second flight--which were significantly associated with damage in Nonpareil--were not associated with damage in Monterey whereas males in flight 3, not significantly associated with damage in Nonpareil, were significantly associated with damage in Monterey (Table 11). While neither harvest date nor previous NOW damage to Nonpareil were significant predictors of NOW damage by themselves, damage to Nonpareil was more useful than harvest date in multiple-variable models. These data indicate that reasonable prediction of NOW damage in Monterey could be obtained just from egg counts in first and third flights, and that these two egg counts in addition with data on earlier damage in Nonpareil provide even better prediction (Table 11). We hypothesize that the inverse correlation between egg counts in flight 1 and Monterey damage is due to an inverse correlation between mummy density and flight 1 egg counts; i.e., poorly sanitized orchards have more mummies competing with egg traps and therefore tend to have lower egg trap counts in flight 1.

Recent Publications:
Burks, C. S., and D. G. Brandl. 2004. Seasonal abundance of navel orangeworm (Lepidoptera: Pyralidae) in figs and effect of peripheral aerosol dispensers on sexual communication. 8 pp. Journal of Insect Science 4: 40.

Burks, C. S., B. S. Higbee, K. Daane, and W. Bentley. 2004. Mating disruption for suppression of navel orangeworm damage in almonds. pp 1-10 in Proceedings of the 32nd Almond Industry Conference, December 1-2, 2004, Modesto, CA.

Burks, C. S., B. S. Higbee, and D. G. Brandl. 2005. Mating Disruption for Suppression of Navel Orangeworm Damage in Almonds. pp. 1-7 *in* Proceedings of the 33rd Almond Industry Conference, December 7-8, 2005, Modesto, CA.

Burks, C. S., B. S. Higbee, and D. G. Brandl. 2006. Mating Disruption for Suppression of Navel Orangeworm Damage in Almonds. pp. 1-19 *in* Proceedings of the 34th Almond Industry Conference, December 6-7, 2006, Modesto, CA.

Gut, L. J., L. L. Stelinski, D. R. Thomson, and J. R. Miller. 2004. Behaviour-modifying chemicals: prospect and constraints in IPM, pp. 73-121. *In* O. Koul, G. S. Dhaliwal and G. W. Cuperus [eds.], Integrated Pest Management: Potential, Constraints and Challenges. CAB International, Wallingford, UK.

Jones, V. P., J. R. Hagler, J. F. Brunner, C. C. Baker, and T. D. Wilburn. 2006. An inexpensive immunomarking technique for studying movement patterns of naturally occurring insect populations. Environmental Entomology 35: 827-836.

Leal, W. S., A.-L. Parra-Pedrazzoli, K.-E. Kaissling, T. I. Morgan, F. G. Zalom, D. J. Pesak, E. A. Dundulis, C. S. Burks, and B. S. Higbee. 2005. Unusual pheromone chemistry in the navel orangeworm: novel sex attractants and a behavioral antagonist. Naturwissenschaften 92: 139-146.
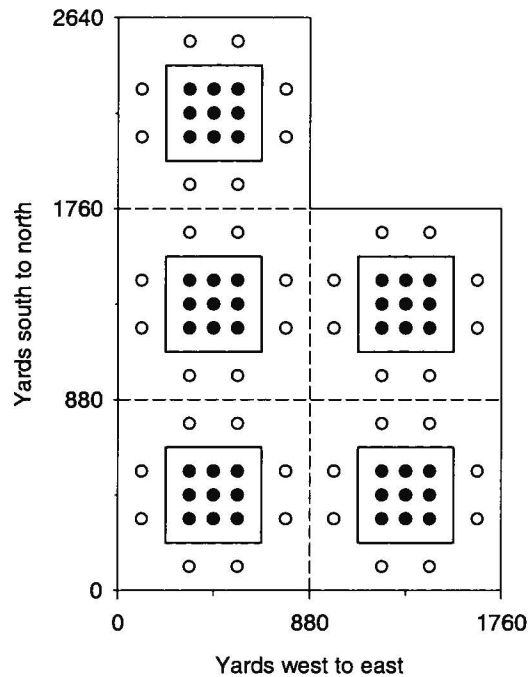
Fig. 1. Plot arrangement for mating disruption experiment examining the effects of pheromone formulation and Puffer density on NOW damage to Nonpareil and pollinator almond varieties. Plots of 160 acres were subdivided into an inner area of 40 acre and an outer area of 120 acres. Four mating disruption treatments (Puffers containing the primary component or a blend, at a density of 1 or 2 Puffers per acre) were randomly assigned to inner 40 acre subplots of the four lower positions, and an untreated control treatment applied to the upwind (northwest) 40 acre subplot. The surrounding 120 acre subplots received an insecticide treatment withheld from the treatment plot. Damage in the treatment subplot was compared to that in the surrounding insecticide-treated subplot. This experiment was replicated in two sites.
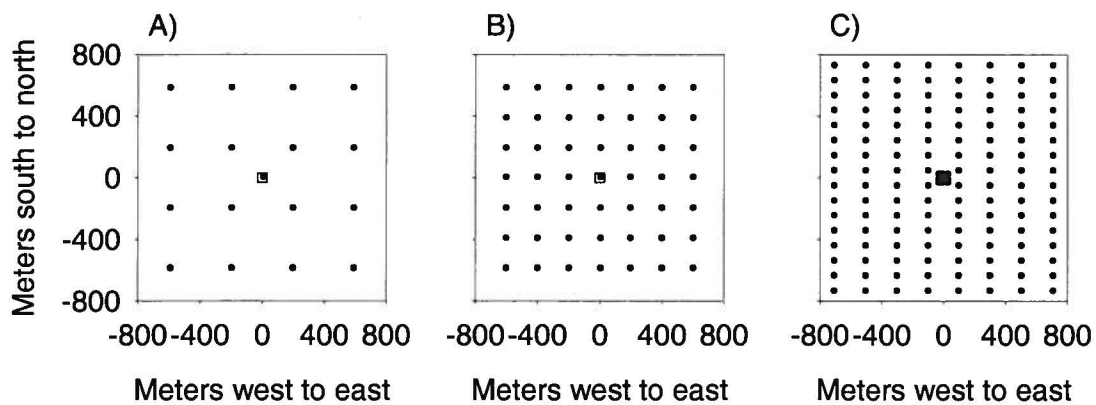

Fig. 2. Plot arrangement for mark-capture and mark-release-recapture experiments examining movement of NOW in 640 acres of pistachios on May 11 and June 13, 2006. The central square represents a 4-acre area in which chicken egg white was applied to trees with an air blast sprayer (May 11 and June 13), and 10,000 mixed sex NOW pupae were placed for emergence (June 13 only). Male traps (wing traps baited with virgin females) were placed in a 4 x 4 grid with an additional trap in center on May 11 (A), and in a 7 x 7 grid on June 13 (B). Delta traps, baited with almond meal for capturing NOW females, were placed in eight north-south columns of 16 traps each for both May 11 and June 13 (C). An additional 9 delta traps were placed in a 3 x 3 grid in the mark/release area.
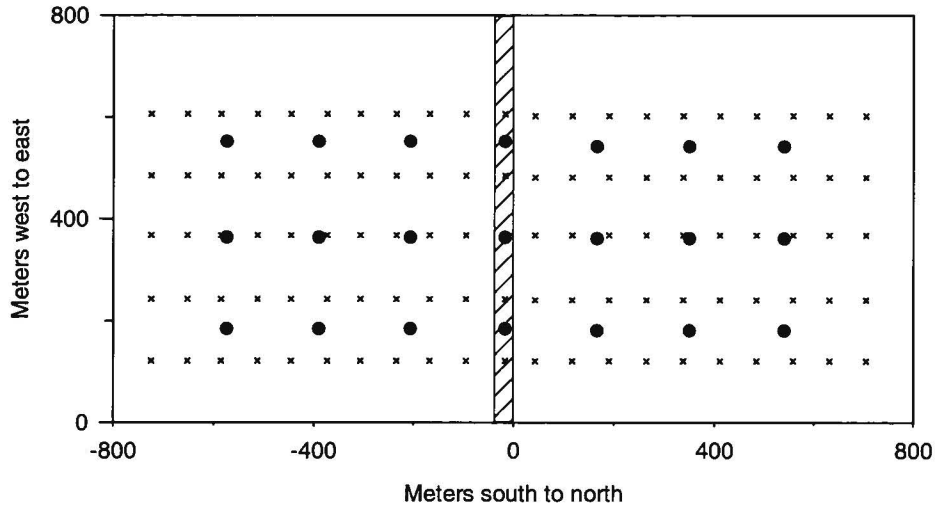
Fig. 3. Plot arrangement for mark-capture and mark-release-recapture experiment examining movement of NOW in adjacent 160 acre blocks of almonds and pistachios on August 28, 2006. The dots and x's represent traps for males and females, respectively (see previous figure). The pistachios were to the south (negative numbers on X-axis), and almonds were to the north. Protein application and release of dyed moths were performed in a 2-acre area comprising the first five pistachios rows, represented here by the cross-hatched square.

Table 1. Mummies per tree (mean ± SE) in the canopy and on the ground in 160 acre treatment plots for comparison of mating disruption treatments

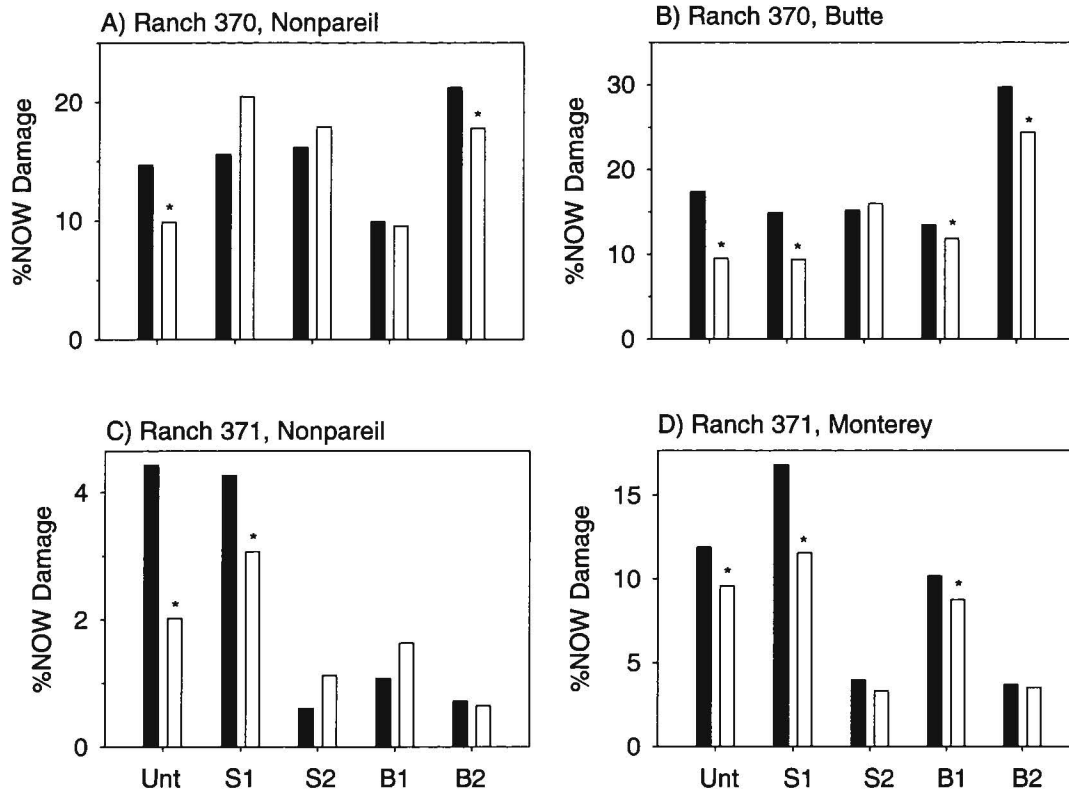| Ranch	Pheromone formulation	Puffers per acre	Mummies on the ground	Mummies in the canopy
3700	Untreated control	-	6.4 ± 1.1	21 ± 4
	Single component	1	8.6 ± 1.2	41 ± 4
		2	29.2 ± 2.6	33 ± 5
	Multiple component	1	7.7 ± 0.9	0 ± 0
		2	35.9 ± 2.3	42 ± 6
3710	Untreated control	-	9.8 ± 1.4	0 ± 0
	Single component	1	5.5 ± 0.8	0 ± 0
		2	2.5 ± 0.5	0 ± 0
	Multiple component	1	4.0 ± 0.6	0 ± 0
		2	4.5 ± 1.0	7 ± 3

Fig. 4. Comparison of damage between plots receiving minimal insecticide (black bars) and either no other treatment (Unt) or a mating disruption treatment (S1, S2, B1, B2) in comparison to the surrounding 120 acre area (white) treated more extensively with residual insecticides but not with mating disruption. The mating disruption treatments were Puffers containing either the primary component (S1, S2) or a more complete blend (B1, B2) placed at either a density of 1 Puffer or 2 Puffers per acre (S1, B1, and S2, B2, respectively). Asterisks indicate that the proportion of infestation in the surrounding insecticide comparison plot is significant less than that of the central treatment plot (χ^2, $P < 0.05$).

Table 2. Total NOW males and females captured and number with detectable egg white protein in the mark-capture experiment commencing on May 11, 2006, by part of the 640 acre pistachio site

	Males			Females		
Part	No. traps	No. NOW	No. ELISA+	No. traps	No. NOW	No. ELISA+
Northeast	4	2067	109	32	608	66
Southeast	4	1831	100	32	353	14
Northwest	4	1951	249	32	669	53
Southwest	4	2234	347	32	489	70
Center	1	369	204	9	80	15

Table 3. Total NOW captured and number with detectable egg white protein by day for mark-capture experiment in 640 acres of pistachios commencing on May 11, 2006

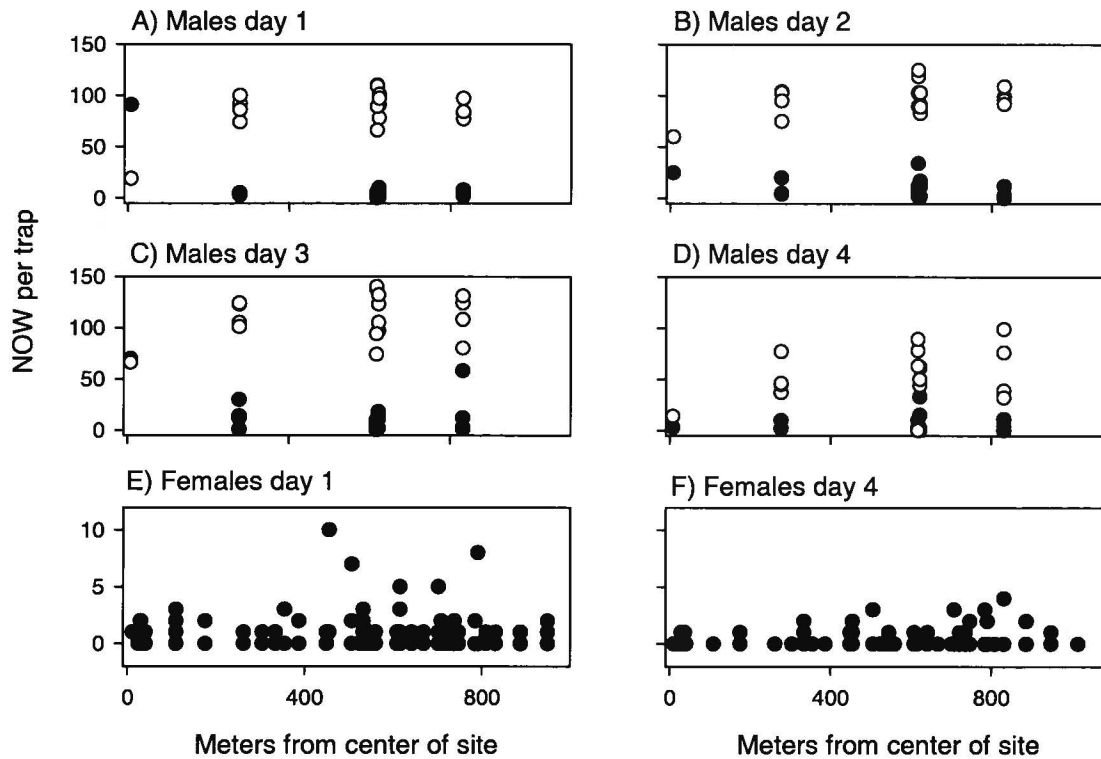|	Males			Females	
Day	No. NOW	%ELISA+		No. NOW	%ELISA+
1	1584	159		743	118
2	1719	174		575	47
3	2021	270		253	5
4	944	99		301	41
5	722	112		141	3
6	505	109		108	3
7	209	86		63	1

Fig. 5. Number of NOW males (A-D) and females (E, F) v. distance from the center of the 256 ha study site the May 11 mark-capture experiment. Black dots indicate number of ELISA-positive moths per trap, and white dots (A-D only) indicate the number of ELISA-negative moths per trap.

Table 4. Total NOW captured and percent with detectable egg white protein and with dye, by day, for combined mark-capture/mark-release-recapture experiment in 640 acres of pistachios commencing on June 13, 2006

Day	Males			Females		
	No. NOW	%ELISA+	%Dyed	No. NOW	%ELISA+	%Dyed
1	111	45	1	0	-	-
2	143	1	0	0	-	-
3	243	7	47	5	20	40
4	328	34	69	16	25	63
5	368	20	64	44	27	91
6	204	16	67	55	38	60
7	123	15	60	29	31	41
14	1329	-	24	163	-	24

Table 5. Feral, dyed, and ELISA-positive NOW males captured in the mark-capture/mark-release-recapture experiment commencing on June 13, 2006, by part of the 640 acre pistachio site

Week	Part	No. traps	No. feral (not dyed)	No. Dyed	No. ELISA+
1	Northeast	9	111	34	16
	Southeast	9	96	39	19
	Northwest	9	120	74	52
	Southwest	9	197	201	55
	Center	1	24	149	59
2	Northeast	9	138	12	-
	Southeast	9	177	23	-
	Northwest	9	180	13	-
	Southwest	9	251	101	-
	Center	1	10	30	-

Table 6. Feral, dyed, and ELISA-positive NOW females captured in the mark-capture/mark-release-recapture experiment commencing on June 13, 2006, by part of the 640 acre pistachio site

Week	Part	No. traps	No. feral (not dyed)	No. Dyed	No. ELISA+
1	NE	32	8	8	4
	SE	32	9	7	5
	NW	32	11	10	5
	SW	32	5	16	6
	CN	9	19	56	27
2	NE	32	45	12	-
	SE	32	11	2	-
	NW	32	54	9	-
	SW	32	37	5	-
	CN	9	16	11	-

Table 7. Pearson correlation coefficient of distance from the mark/release area with all NOW males, males with dye, and males with detectable protein for the June 13, 2006 mark-capture/mark-release-recapture experiment

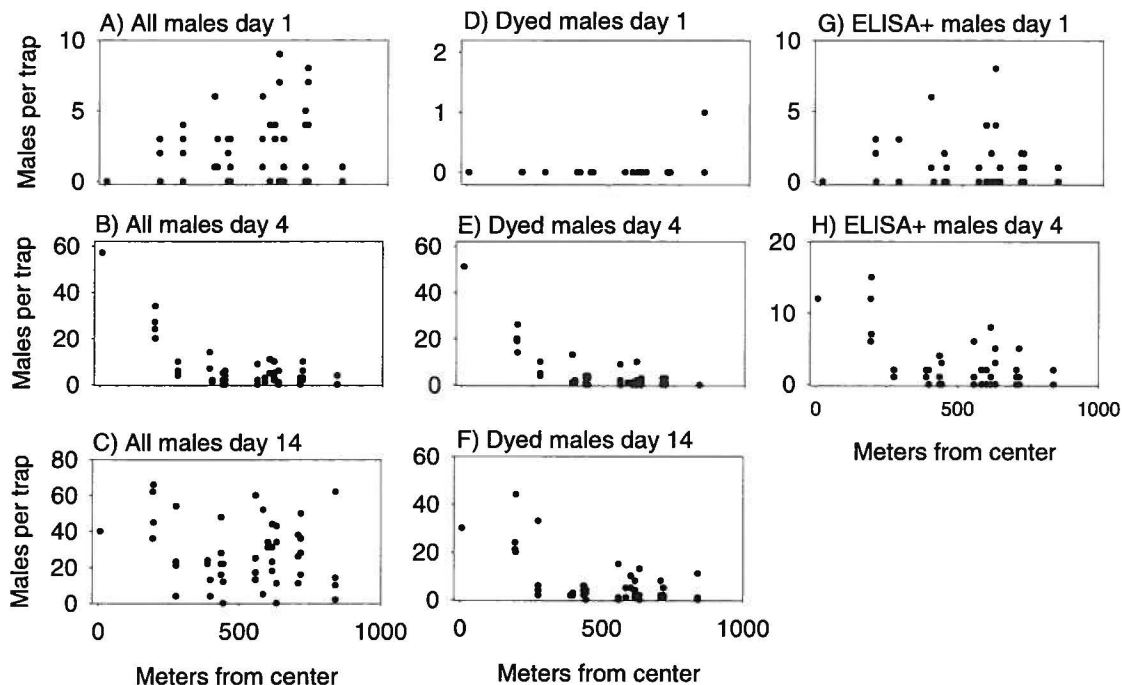| Day	All males	Dyed males	ELISA% males
1	0.134	0.234	-0.061
2	0.327*	-	-0.183
3	0.069	0.114	-0.241
4	-0.657***	-0.681***	-0.536***
5	-0.663***	-0.695***	-0.477***
6	-0.484***	-0.526***	-0.310*
7	-0.256	-0.306*	0.031
14	-0.212	-0.586***	-

*$P < 0.05$, ***$P < 0.001$

Fig. 6. Total number of NOW males (A-C), dyed males (D-F), and males with detectable egg white protein (G-H) v. distance from the protein-treated area on days 1 (A, D, G), 4 (B, E, H) and 14 (C, F) for the June 13 mark-capture/mark-release-recapture experiment.
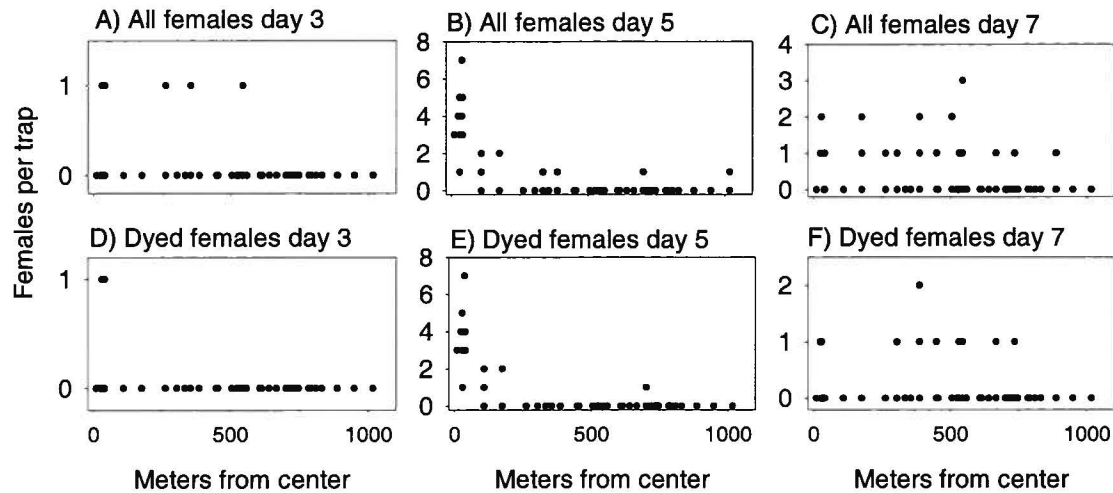
Fig. 7. Total number of NOW females (A-C) and dyed females (D-F) v. distance from the protein-treated area on days 3 (A, D), 5 (B, E) and 7 (C, F) for the June 13 mark-capture/mark-release-recapture experiment.

Table 8. Number of dyed NOW females recaptured at various distances and times during the June 13, 2006 mark-capture/mark-release-recapture experiment

Day	Distance from center of study site		
	0-41 m	41-450 m	450-1017 m
3	2	0	0
4	9	1	0
5*	34	5	1
6	9	16	8
7	2	6	4
14	11	12	16

*Proportion of counts in the three categories on day 5 is significantly non-homogenous (χ^2, df = 1, $P < 0.0001$), and, between days 5 and 14, there is a significant association between rows and columns (χ^2, df = 1, $P < 0.0001$)

Table 9. Total NOW and NOW with dye and detectable egg white protein for the mark-capture/mark-release-recapture experiment in adjacent almonds and pistachios commencing August 28, 2006

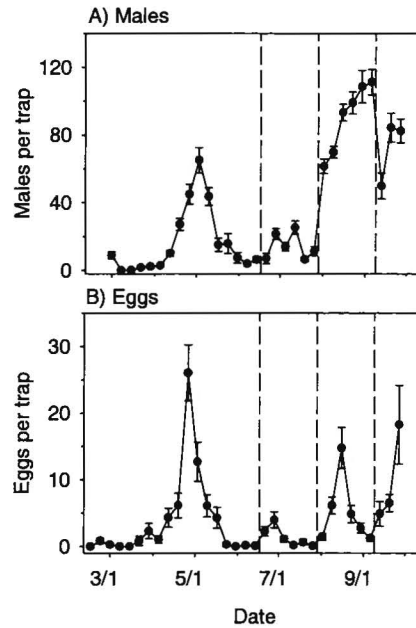| Trap Bait	Part of Site	No. Traps	No. Feral	No. Dyed	No. ELISA+
Almond meal	Pistachio	50	12	5	7
	Mark/release	5	11	10	8
	Almond	50	15	2	4
Unmated females	Pistachio	9	85	5	20
	Mark/release	3	94	35	37
	Almond	9	194	4	22

Fig. 8. Comparison of weekly trap counts in 41 40-acre plots distant from mating disruption sites. (A) Males (mean ± SE) captured in wing traps baited with virgin females. (B) Eggs (mean ± SE) on egg traps. Vertical dashed lines represent the beginning of cohorts (flights), defined as the beginning of a sustained increase in egg trap counts.
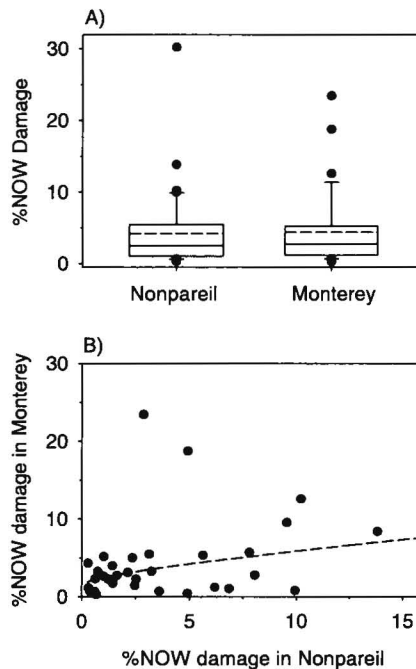

Fig. 9. Comparison of distribution of NOW damage between Nonpareil and Monterey. (A) Boxplots depicted the frequency distribution of NOW damage for Nonpareil (n = 40) and Monterey (n = 33). The mean is represented by a dashed line, the solid line in the center represents the median, the bottom and top of the box represent the 25th and 75th percentile observations, the bottom and top whiskers represent the 10th and 90th percentile, and individual observations outside the 10th and 90th percentiles are represented by dots. (B) Now damage in Monterey v. preceding damage in Nonpareil for plots in which both varieties occurred (n = 31). The dashed line is a back-transformation of the values predicted by linear regression.
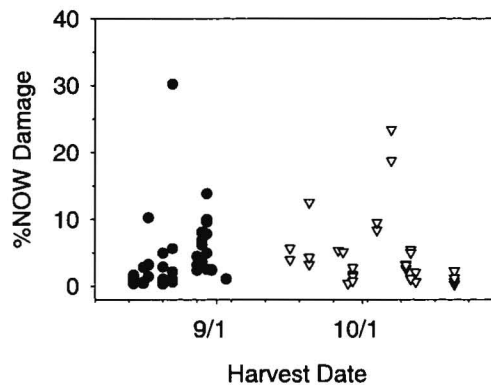
Fig. 10. Percent NOW damage in Nonpareil (dark circles) and Monterey (open triangles) almonds v. harvest date. Minor ticks on X axis represent Mondays.

Table 10. Comparison of linear regression models of NOW harvest damage to Nonpareil almonds based on day of harvest (Day), males in flights 1 and 2 (M1, M2), and males in the period between peak egg counts for flights 1 and 2 (Inter)

Predictors	F	P	r^2
Inter Day	13.17	<0.0001	0.42
M1 Day	10.75	0.0002	0.37
M2 Day	9.05	0.0006	0.33
Inter	9.87	0.0033	0.21
Day	8.06	0.0072	0.18
M1	7.14	0.0098	0.16
M2	4.80	0.0374	0.11

Table 11. Comparison of linear regression models of NOW harvest damage to Monterey almonds based on NOW damage to Nonpareil (Np), males in flight 3 (M3), eggs in flights 1 and 3 (E1, E3), and day of harvest (Day)

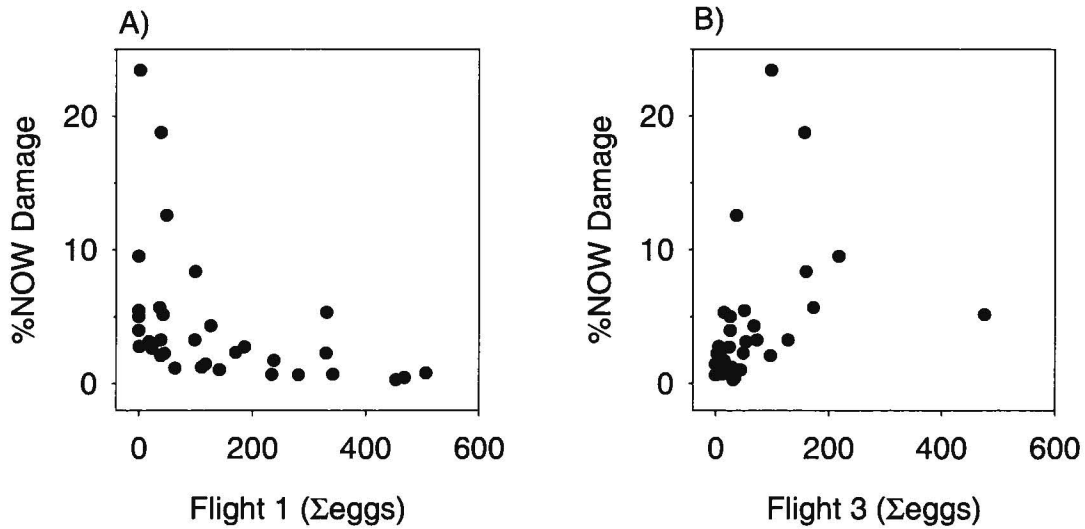| Predictors	F	P	r^2
M3 E1 Np	8.89	0.0002	0.48
E3 E1 Np	8.14	0.0004	0.46
E3 E1 Day	6.38	0.0018	0.40
M3 E1	11.72	0.0002	0.44
E3 E1	9.07	0.0008	0.38
E1	13.68	0.0008	0.31
M3	11.29	0.0021	0.27
E3	6.55	0.0157	0.17
Np	3.99	0.0547	0.11
Day	2.99	0.0883	0.06

Fig. 11. Scatter plots of percent NOW damage to Monterey almonds v. number of eggs found on traps over the entire flight for (A) flight 1 and (B) flight 3. Regression of damage on egg counts showed a significant negative slope damage v. eggs in flight 1, and a significant positive slope for damage v. eggs in flight 3. There was no significant association between eggs in any flight and damage to Nonpareil almonds.