Mating Disruption for Suppression of Navel Orangeworm Damage in Almonds

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Executive summary

We used traps baited with unmated navel orangeworm (NOW) females to monitor abundance from March to November in 8 ranches comprising 1 square mile each, 4 planted in almonds and 4 in pistachios, and to compare the impact of mating disruption treatments with a currently-used insecticide (Guthion) and untreated controls. Sanitation was practiced in all ranches, both almond and pistachio. These data were used to:

1) compare abundance and seasonal variation of abundance of NOW adults between the two crops;
2) compare the effectiveness of 3 mating disruption treatments to Guthion, and the conditions under which insecticide and mating disruption can reduce NOW damage; and
3) examine under which conditions favorable for mating disruption and how a synthetic pheromone lure, when one becomes available, might give useful information to managers.

We found that:

1) In almonds, NOW abundance was generally low until >20 July, i.e., during the first and second flights. In pistachios, in contrast, there was high NOW abundance throughout the year.
2) In the three almond ranches in which the abundance of NOW adults was low from mid-July to mid-August, neither mating disruption nor Guthion reduced damage. In the ranch with high NOW abundance during this period, both the most effective of the mating disruption treatments and the Guthion treatment significantly reduced damage. In pistachios, harvested during the fourth flight, there was far less damage in the Guthion-treated nuts than in those receiving mating disruption treatments or the untreated controls. Monterey almonds, also harvested during the fourth flight, represented an intermediate situation in which there was less damage in nuts treated with the most effective mating disruption treatment, and still less damage in nuts treated with Guthion.
3) In Kern county the period of mid-June to mid-July appears to be a critical period with respect to management of NOW damage to Nonpareil almonds. Four traps over 40 acres gave the best predictive capability. Ranches in which no moths were captured over this period had very little damage, and consistent captures of even a few males per trap in pheromone-baited traps over this period indicated potential for significant NOW damage and suggested that treatment for navel orangeworm in almonds would be economically advantageous.

Introduction

The development of efficient systems of monitoring and mating disruption for NOW management has lagged behind that of other lepidopteran pests of horticultural crops, due in part to technical limitations. Only the principle component of the female sex pheromone has been identified. This component, (Z,Z)-11,13-hexadecadianel, is not sufficient to efficiently bring males to a point source and is particularly vulnerable to degradation in the field. Monitoring of this pest is therefore dependent on oviposition traps, which produce numerically skewed results and are out-competed by the presence of a susceptible host in the orchard (Rice 1976, Van Steenwick and Barnett 1985). An aerosol release system, in which the pheromone is stored in a liquid organic solvent prior to being released at timed intervals, has been one method of avoiding problems with degradation (Shorey and Gerber 1996).

Previous studies of mating disruption for control of NOW in almonds have shown that release of (Z,Z)-11,13-hexadecadienal can reduce male capture in female-mated flight traps and mating of unmated females and reduce crop damage (Curtis et al. 1985, Shorey and Gerber 1996). Studies with this and other lepidopteran pests have suggested that aerosol timed release systems placed around the perimeter of a block to be protected could be equally efficacious and perhaps save labor costs compared to such devices placed evenly through the protected block (Shorey and Gerber 1996, Shorey et al. 1996). Studies over two growing seasons, using monitoring with unmated females, showed that the suggested placement of aerosol release devices on the perimeter of 40-acre blocks of figs substantially disrupted mating throughout the treatment block (Burks and Brandl 2003).

In this report we present results of studies using female-baited flight traps and mating assays over 4 square miles each of almonds and pistachios spread through the southern San Joaquin Valley in Kern County. In this report we compare spatial and temporal variance of NOW adults between these two crops, compare the impact of the insecticide and experimental mating disruption treatments on NOW reproductive behavior and damage from NOW in these crops, and examine the possibility of predicting NOW damage in Nonpareil almonds using pheromone-based flight traps.

Materials and Methods

Flight traps and mating assays Male prevalence and the ability of males to locate calling females were monitored using unmated females. Groups of three females were sealed in a mesh bag, which was then suspended from the top of a wing trap (Pherocon IC, Trécé Inc., Adair OK) as described by Curtis and Clark (1984). Cohorts of larvae maintained on a wheat bran diet and held at 26°C 16:8 L:D for 21 days, then last instar larvae were sorted by sex. Males were identified using the testes, visible as a dark spot through the light dorsal cuticle, and discarded. Groups of 100 females were placed in 3.9 l glass jars with the bottom covered with bran diet to a depth of 2 cm, held at 26°C 16:8 L:D. Jars were examined each morning and any moths that had emerged in the previous night were isolated in transparent plastic vials with screen mesh lids, examined to confirm sex, and held for experiments. Where possible females were placed in mesh bags and placed in the field the first morning after they emerged, and moths were always used within 48 hours of eclosion. When it was necessary to use moths eclosed on two different days, they were grouped so that each bag of three moths contained the same number of 1-day-old and 2-day-old females. Mesh bags containing unmated moths were prepared in the morning at the USDA-ARS location in Parlier and delivered to Paramount Farming's Belridge Laboratory, and were distributed to the field on that afternoon or on the following day.

The relative likelihood of insemination of unmated females was determined using modifications of the mating assay of Curits and Clark (1984). A 473-ml round polypropylene cup was suspended from the top of a wing trap by clips, and used to contain a second such cup with the top half coated with Fluon (ICI, London, UK). On the morning of delivery, freshly-eclosed females were briefly anaesthetized with <30 s exposure to carbon dioxide, the distal third to half of the two wings on one side were clipped, and then these females were individually placed in plastic vials for transport to mating assay locations in the field at the same time as the flight traps were tended. The following week, females were again placed in plastic vials for transport to the laboratory where they were evaluated by egg development or dissection. Initially both the presence and color of eggs were noted for all females, and then they were dissected. When we observed red (i.e., fertile) eggs, this was considered diagnostic of a mated status, otherwise we determined mating status by dissection.

Experimental locations and plots We examined eight square 256 ha (640acre) ranches, four of almonds and four of pistachios, located across the southern San Joaquin Valley (Fig. 1). The almond ranches each contained 50% Nonpareil and 50% pollinator varieties (Carmel, Fritz, or Monterey) and the pistachios were all Kerman. Each section contained four 16 ha (40acre) treatment blocks centered in each quarter. Cultural control methods were performed throughout the section to the best of the ability of the managers of the ranches involved, and the portion of the observational sections outside the treatment blocks all received a hull-split azinphos-methyl treatment.

Spatial and temporal variation in abundance Nine female-baited flight traps forming intersecting east-west and north-south transects across the center of section (Fig. 1A) were used to compare seasonal variation in NOW abundance between crops and between ranches within each crop, and to examine distribution of moths between central and peripheral trap positions at different levels of abundance. Each trap was examined on a weekly basis. Mesh bags containing unmated females were replaced, and empty liners were replaced if dirty. Data were recorded as the data when the unmated moths were placed in the field, and analyzed using standardized weeks beginning on Monday 5 March 2003 and ending ending 12 November 2003, i.e.; 37 weeks.

In order to compare these data with prior phonological models, degree day (DD) accumulations were calculated with DDU (DNAR 1990), using the double triangle and vertical cut-off options, and presuming 607DD°C (1092.6DD °F) for flights (i.e., cohorts) in which oviposition occurred prior to hullsplit, and 410DD °C (738DD °F) for subsequent flights (Sanderson et al. 1989). Climate data were for stations 5, Shafter; 54, Blackwell's Corner; 138, Famoso; 146, Belridge; and 172, Lost Hills were obtained from the California Department of Water Resources, http://wwwcimis.water.ca.gov/. March 1 was used as a nominal biofix, since NOW were already active and flying in pistachios on the first sampling date of 3 Mar 2003.

In order to examine spatial distribution of NOW between the transect trap positions and the effect of overall abundance on this distribution, the sum of moths captured in the 9 transect traps was calculated for each ranch by week combination. The 268 cases in which this sum was greater than 0 were separated in four ~equal groups as follows: those with sums of 1-29 moths (53 almond, 14 pistachio), those with sums of 30-133 moths (35 almond, 33 pistachio), those with sums of 134-345 moths (16 almond, 51 pistachio), and those with sums of 347-949 moths (27 almond and 39 pistachio). These comprised a total of 268 ranch by week combinations and of 2,412 trap positions. Individual trap positions within each ranch by week combination were re-coded as a proportion of the sum of all traps, and ANOVA was used to examine the effect of overall abundance variation between proportions of moths captured at locations within ranch by week combinations.

Mating disruption Four treatment blocks in each ranch were used to compared two or three experimental mating disruption treatments with treatment with the insecticide azinphos-methyl and the untreated control plot. In almonds the four treatment plots contained: 1) controls receiving no insecticide or mating disruption treatment; 2) mating disruption with aerosol dispensers placed around the perimeter (the Suterra Puffer, Suterra, LLC), hereafter referred to as "Puffers"; 3) mating disruption with Puffers gridded throughout the block; and 4) mating disruption with membrane dispensers (CheckMate NOW, Suterra LLC, hereafter referred to as CheckMate membranes) placed throughout the block. In pistachios no membrane emitters were used, and instead one of the treatment blocks was treated with insecticide at hull split. Mating disruption treatments were applied from the beginning of April through mid-October in the case of almonds, or mid-September in the case of pistachios. Puffers were placed peripherally or evenly throughout the experimental block at a density of 5 dispensers per ha (2 dispenser per acre), and emitted ca. 0.7 mg AI every 30 minutes from 6PM to 6AM PDT. CheckMate membranes were placed on each tree at the beginning of April, and a second dispenser was placed on each tree in mid-July. The release rate was 6.5 g active ingredient (AI) per acre for the season. In almonds dispensers were moved between varieties in adjacent rows as first Nonpareil and then pollinator variety nuts were harvested, and in pistachios the dispensers were removed from the orchard shortly before time of harvest.

To examine the impact of the treatments on the ability of males to find calling females, four flight traps were placed at in each 40-acre treatment plot 1.5 m above the ground, 200 m from the nearest other traps, and 100 m from the edge of the block (Fig. 2A). These traps were tended and the females replaced weekly for 24 consecutive weeks in pistachios and 23 weeks in almonds, from 31 March to 8 September 2003. One week was discarded from the almond data set and 2 weeks from the pistachio data set because of missing observations. We additionally used the previously-described mating assays, placed at the center of each treatment plot (Fig. 2A) to examine the impact of the treatments on the relative probability of mating of NOW females.

We also sampled the crops at harvest to determine the impact of the treatments on crop damage. For each crop and (within almonds) each variety, samples of ~500 almonds or ~1,000 pistachios were taken at harvest at 16 points within each of the treatment blocks and an additional 4 points around the perimeter of each treatment block, for a total of 80 samples (Fig 2B). Each nut was opened and examined by Paramount Farming personnel and the presence or absence of damage consistent with NOW was noted.

Relationship between NOW abundance and subsequent crop damage The flight trap and crop damage data sets from untreated control plots were used to examine how pheromone-baited flight traps might best be used as a monitoring tool to predict NOW damage, and to compare the relative importance of moth abundance and harvest timing as factors affecting NOW damage in Nonpareil and Monterey almonds and pistachios. To examine spatial scale, regressions were performed on one of 4 ha (10 acre), 16 ha (40 acre), or 256 ha (640 acre) scales. For the 4 ha scale, each of the 4 flight traps in the untreated block was regressed on damage from the 4 nearest nut samples in that quarter of the treatment plot. For the 16 ha scale, the pooled capture from the 4 traps were regressed against the pooled damage from the 16 nut samples from the entire control treatment plot. For the 640 ha scale, the sum of moths capture in the 9 transect traps spanning the entire ranch was regressed against the pooled damage from the 16 nut samples of the control plot. This regression was done for data from individual weeks, and to examine the effect of time prior to harvest it was repeated for weeks throughout the season. To compare effects of NOW abundance and harvest time on nuts, abundance at harvest was represented by pooling the moths captured in traps on the first through fourth weeks prior to harvest. Effects of time were examined by regressing the Julian date of harvest on damage.

Statistical procedures Statistical inference was made using the SAS System (SAS Institute 2002). Weekly flight trap totals and damage proportions were transformed using log [$\log_{10}(X+1)$] and angular [$\arcsin((y/n)^{0.5})$] respectively (Sokal and Rohlf 1995). To compare seasonal variation in abundance between crops and weeks, individual transect trap captures were transformed as square root of log10(x+1), and analyzed as a nested arrangement on a completely randomized design with repeated measures and subsampling. Factors included crop and ranch within crop, with the crop within ranch mean error used to test the hypothesis of difference in abundance between crops. This design was analyzed using PROC MIXED, with an autoregressive moving average covariance structure and with degrees of freedom calculated as by the method of Kenward and Roger (1997). The effect of overall abundance on the proportion of moths trapped at each position was initially examined using PROC GLM to perform a 2-way factorial ANOVA, with crop and abundance as factors. The variation due to abundance was highly significant ($F = 18.71$, $P_{3,\,2404} < 0.0001$), whereas variation due to crop and the crop by abundance interaction were not significant. A series of 1-way ANOVAs was then used to examine variation and contrast among ranch by week combinations within each level of abundance. The effect of mating disruption and insecticide treatments on the number of NOW captured in flight traps was examined separately for each crop using PROC GLM to perform a 3-way factorial arrangement on a randomized complete block design. The ranches served as blocks and treatment, east-west position, and north-south position of the traps within the block were examined in factorial combination. Pair-wise tests of least-squares means (LSMEANS) were used to examine, within weeks, the hypothesis that the number of moths captured per trap was greater than zero and it differed between treatments. The effect of these treatments on mating status of NOW females in assays was examined by pooling the data for all assays throughout the season, and by using PROC FREQ and Fisher's exact test to pair-wise tests of the hypothesis of differing proportions of the categories "not mated" and "mated" between treatments. In addition, linear regression (PROC REG) was used to linear regression of proportion of females mated from the transect and untreated control plot assays in each crop on Julian date and the average weekly trap catch from transect and untreated control plot flight traps for that ranch. The effect of mating disruption and insecticide treatments on crop damage were examined using PROC GLM to perform a randomized complete block design with subsampling. Factors used were treatments, with ranches as the blocking factor, and the ranch by treatment mean square was used for hypothesis testing. Separate analyses were performed for Nonpareil almonds and pistachios. Among pollinator almonds, Monterey was the only variety with sufficient replication to be analyzed in this manner. PROC REG was used to examine effect of time prior to harvest and spatial scale on subsequent damage to Nonpareil almonds and the effect of abundance in the four weeks prior to harvest on NOW damage at harvest to Nonpareil and Monterey almonds and to pistachios. Means and standard errors of untransformed data are shown in figures and tables.

Results

Temporal and spatial variance in abundance The flight trap data from the transects show very different trends in seasonal abundance of NOW the almond and pistachio ranches in this study (Fig. 3). The abundance of NOW in pistachios was significantly greater than that in almonds in 20 of the 21 weeks examined between 3 March and 28 July (the exception being that of 31 March). Of the subsequent 15 weeks examined, the differences between the mean flight trap capture almonds and pistachios are only significant in 3 weeks (those of 1 September, 20 October, and 10 November). While the difference between the mean numbers of NOW captured in the two crops is large in this latter portion of the study, so is the variance.

The median estimated date for the start of the second, third and fourth flights was 21 June, 12 August, and 16 September, respectively, with data from the 5 climate recording stations yielding estimates over range of 6-7 days for each of these values. According to this model and these assumptions, progeny of fourth flight females could complete development by 1 November at two of the 5 stations. If a development time of 410DD is supposed for the second flight, then the median estimate for the start of the second, third, fourth, and fifth flights are 27 July, 30 August, and 10 October, respectively. There is a significant difference between the mean numbers of NOW captured in transect traps in almonds on the weeks of 21 and 28 July. In the other cases there are not significant differences between trap means within crops for weeks proposed as the beginning of flights and the previous week, regardless of which assumption is used about development time for second flight. While the way in which the transect data are analyzed does not allow hypothesis testing concerning differences among ranches with a crop, the mean trap catch for each crop for each of the 5 flights is presented (Table 1) to demonstrate that the variance between crops is far greater than that between ranches within crops.

Examination of a graphical representation of the mean proportion of moths captured at each transect trap position (Fig. 4) suggests that most moths are captured in the western-most and northern-most trap positions. A comparison of the mean proportion of moths captured on the western-most and northern-most trap positions along with the coefficient of variation for all traps, for each level of abundance, shows that these traps are far greater than the average of all positions (11.1%, by definition) at the lowest level of abundance and approach this overall average with increasing abundance (Table 2). The coefficient of variance likewise decreases with increasing abundance. The contrasts of the northern-most position with the remaining positions is not significant ($P>0.05$, $\alpha = 0.05$) at the highest level of abundance, but highly significant ($P<0.0001$) at all other levels of abundance. The same contrast with the western-most position is highly significant at all levels of abundance.

Impact of mating disruption on flight traps and mating assays Among between-subject factors in the ANOVA for NOW males captured in flight traps in almonds over the 23 weeks examined, the effect of location ranch was a highly significant ($F_{3,45} = 6.63$, $P = 0.0009$), and that of treatment was not quite significant ($F_{3,45} = 2.43$, $P = 0.0778$). The east-west and north-south location of the trap and there interactions with the treatment effect were not significant. Among within-subject effects, that of week was highly significant ($F_{22,968} = 1.87$, $P = 0.0009$) as was the interaction between week and ranch ($F_{22,968} = 4.7$, $P < 0.0001$). The interactions of other effects with that of week were not significant. Examination of trap means for the individual weeks shows that the number of moths captured in the peripheral and gridded Puffer plots was not significantly greater than 0 at any time during the study (Table 3). The trap catch in the CheckMate membrane treatment plot was significantly greater than 0 in two of the last 3 weeks of the study, but not in any of the previous weeks. The Checkmate mean was also significantly greater than the Puffer mean trap catches in the final week of the study. The number of moths captured in flight traps in the untreated control plots was significantly greater than 0 and than the Puffer and CheckMate treatment plots in most weeks examined, although, as with the transect traps, low numbers of moths were captured prior to 28 July.

There was a greater difference between treatment effects in pistachios compared to almonds (Table 4). In the case of the pistachios, both treatment and ranch between-subject effects were significant in the repeated measures ANOVA ($F_{3,45} = 123.18$, $P < 0.0001$, and $F_{3,45} = 3.54$, $P = 0.0195$, respectively), but neither east-west nor north-south position nor their interaction with treatment were significant. Among within subject effects, that of week and its interaction with treatment and ranch were all highly significant ($F_{20,900} = 26.25$, $F_{60,900} = 6.72$, and $F_{60,900} = 3.35$; $P < 0.0001$ in each case), and other effects were not significant. As with the pistachio transect traps, high numbers of moths were captured in the untreated control plots and the azinphos-methyl treatment plot throughout the study period. The mean number of NOW males in flight traps in the gridded Puffer treatment plot in pistachios was significantly greater than 0 only on the second of the 21 weeks examined. The traps in the peripheral Puffer treatment plots also captured significantly >0 moths in one week in April, but in addition there were significantly >0 moths captured in 6 of the last 7 weeks examined, and in three of these weeks the trap capture in the peripheral Puffer plots was also significantly greater than that in the gridded Puffer plots. The number of males capture in the untreated control and azinphos-methyl treatment plots was significantly greater than 0 and than the mean trap catch in the Puffer plots in all weeks examined. The Guthion treatment plot mean was also significantly greater than that of the untreated control plot in 16 of the 21 weeks examined.

Over the entire season, the proportions of females mated in assays in mating disruption treatment plots were significantly less than those in assays not exposed to these treatments (Table 5). In addition, there was a significantly greater proportion of mating in the non-mating disruption assay positions in pistachios than in almonds, and there was a significantly higher proportion of mated females from assays in the peripheral Puffer plots in pistachios than in any of the mating disruption treatment plots in almonds. Regressing mating assay data from the transect and untreated control assay positions (8 assays per crop per week) on flight trap data for the transects and untreated control plots from the same ranch resulting in a significantly positive slope in pistachios ($P = 0.004$, $r^2 = 0.2769$), but not in almonds over the entire season (Fig. 5A). However, when performed starting from 7 April, the regression is also significant in almonds ($P = 0.0011$, $r^2 = 0.3782$). Among the 8 ranches (both crops), the regression of proportion mated on average weekly flight traps captures for the season was positive ($P = 0.0148$, $r^2 = 0.6561$)(Fig. 5B).

Impact of mating disruption on NOW damage For Nonpareil almonds, the ranch effect and ranch by treatment interaction were highly significant ($F_{3,12} = 191.1$, $P < 0.0001$; and $F_{12,299} = 2.67$, $P = 0.002$). The treatment effect wasn't quite significant at $\alpha=0.05$ ($F_{4,12} = 2.96$, $P = 0.0649$). A comparison of the mean NOW damage by ranch shows that most one ranch, 3710, incurred commercially objectionable damage whereas the remaining three incurred minor damage that many growers would consider within an acceptable range (Table 6). Planned comparison of treatment means across the four ranches shows that Nonpareil almonds from the outside areas treated with azinphos-methyl and from the gridded Puffer treatment plots had significantly less NOW damage than the untreated controls, whereas NOW damage in the nuts from the peripheral Puffer and CheckMate treatment plots did not differ significantly either from the controls or from those from the gridded Puffer treatment plots or those which received treatment with azinphos-methyl (Table 7). While the experimental design does not permit testing of differences of means within individual treatment plots (e.g., treatment by ranch), the means and standard errors for treatments within individual ranches are presented in Table 8.

The ANOVA for pistachios showed both the highly significant effects ($P < 0.0001$) for both ranch ($F_{3,12} = 27.58$) and treatment ($F_{3,12} = 20.35$), but not for their interaction ($F_{12,300} = 1.22$). While there was significant difference among the overall mean damage in the four pistachio ranches, all four had damage <2%; i.e., levels which many would consider to be commercially tolerable (Table 9). The two groups of pistachios that received an insecticide treatment (the azinphos-methyl treatment plot and the samples from outside the treatment plots) each had similar levels of NOW damage; and, with around 33% of that of the untreated and mating disruption treatments, was significantly different (Table 10).

Among almond pollinator varieties, only Monterey was represented in each of the treatment plots in more than one of the ranches, and thus was the only variety with sufficient replication for ANOVA using the present experimental design. There effects of ranch ($F_{2,8} = 903.5$) and the Ranch treatment interaction ($F_{8,225} = 12.49$) were both highly significant ($P < 0.0001$), but that of treatment was not ($F_{4,8} = 1.47$). While there were no significant differences among the treatment means of NOW damage for pollinator varieties, mean NOW damage is presented by ranch for all pollinator varieties which were represented in all treatment blocks of one or more ranches (Table 11).

Correlation of flight trap data with damage Comparison of regression of NOW damage to Nonpariel almonds from various weeks on flight trap data over scales of 4, 16, or 256 ha indicate that the best correlation is found on the 10 ha scale (Fig. 6) and between 23 June and 14 July (i.e., second flight) (Table 11). The relationship between mean flight trap capture in second flight and subsequent damage to Nonpareil almonds in illustrated in Fig. 7. The relationship between damage to almonds and pistachios and abundance at harvest is examined in Fig. 8. The regression of Nonpareil almond damage on abundance is significant ($P = 0.0146$, $r^2 = 0.9711$), as is that of pistachio damage on harvest date ($P = 0.0034$, $r^2 = 0.9932$).

Discussion

The principle findings of this study are that NOW population dynamics differed between almonds and pistachios over a wide area, and that mating disruption shows promise for preventing losses in almonds, particularly of the Nonpareil variety. Population density and population dynamics are very important to the success or failure of mating disruption for crop protection (Cardé and Minks 1995). These data are thus less favorable for the prospects of using mating disruption to protect pistachios under the conditions encountered in this study. The data also show that, if a pheromone suitable for monitoring were available, monitoring during the period of mid-June to mid-July could predict damage at time of the Nonpareil harvest.

This study is the largest in which unmated female NOW have been used as a pheromone source to monitor adult abundance in almonds, and we are unaware of any published data of this type concerning pistachios. Rice (1976) compared NOW abundance data obtained from black lights, egg traps, and female-baited flight traps in the same 200 ha (500 acre) almond orchard in Caruthers, and Sanderson et al. (1989) used egg traps to monitor 7 different orchards, for one growing season each, over a period of 4 years. While the authors stated the site of the plots—4.1 to 15 ha (8 to 38 acres)—and that the orchards or plots were 0.4 km (440 yards) from the nearest external source of NOW, the size of the orchards involved was not directly indicated. This is important because the large size of the ranches in the present study, and the replication over a wide area, indicate that the trends we saw are probably representative of these crops in this area. Sanderson et al. (1989) also indicated that only one of the 7 orchards they examined received sanitation, whereas all orchards in the present study—both almond and pistachio—received sanitation. Other data (Higbee, unpublished) indicate that smaller almond plots in the vicinity of pistachios are likely to experience higher NOW abundance in June and July, even with good sanitation.

A model created using NOW development on mummy and new-crop almonds (Sanderson et al. 1989) is here used to examine data from both almonds and pistachios. Laboratory observations suggest that NOW may develop more quickly on pistachios than on almonds (Higbee, unpublished), but this model nonetheless provides a useful perspective for the seasonal abundance data from both crops. The predictions made by this model are qualitatively approximated by the empirical data. This perspective suggests that, at least in 2003, southern San Joaquin Valley Nonpareil almonds were harvested well into the third flight, and that both pistachios and the pollinator almond varieties were harvested during the more abundant fourth flight. Perhaps more importantly, low abundance during the second flight seemed to be a general feature in well-sanitized almonds and even in one ranch with higher abundance in the first flight (i.e., Ranch 394), and conversely trap captures averaging over 1 moth per trap per week in the second flight seemed to predict a potential for high levels of NOW damage in almonds.

The season abundance data from NOW transect traps in pistachios suggest more overlap between the flights in that crop, and in fact suggests that a portion of the NOW population in that crop in the southern San Joaquin Valley overwinters as adults. Inability to detect adult NOW in December, January, and February could come from adult quiescence of adults rather than from their absence. This suggestion would raise the question of survivorship and fertility of overwintered adults. Johnson et al. (1997) found that when adults of the Indianmeal moth *Plodia interpunctella* Hübner, another pyralid of the subfamily Phycitinae, was held at 10°C longevity more than tripled compared to adults held above the developmental threshold of ~15°C. While that study found greatly reduced fertility among females surviving 30 days at such temperatures, they also used individuals from a colony that had been maintained in the laboratory for decades without exposure to cold or diapause-inducing conditions, and moreover, they intentionally examined effects of a constant cold temperature such as is found in a refrigerated storage environment. Other studies have shown that fluctuating temperatures, such as those found in an orchard, can be important in allowing overwintering insects to recover from injury caused by episodic cold (e.g., Nedved 1998), and another recent study provides evidence that photoperiod and diapause status as a larva can influence subsequent oviposition in a Noctuid species (Fontinou et al. 2004). Regardless of the relative importance of adults and larvae in founding the first generation of the year, the data from this study indicate that the pistachios studied, unlike the almonds, did not have a period of low adult abundance that would be desirable for use of mating disruption.

Examination of the susceptibility of almond varieties and pistachios—here defined as the ratio of damage to adult abundance—provides useful insight into the relative importance of low adult NOW abundance and early harvest. As a practical matter, growers do well to heed the current advice to harvest both crops as soon as possible in order to minimize NOW damage. The strong correlation of Nonpareil almond damage with NOW abundance and of pistachio damage with harvest date shows that reduction of damage realized by earlier harvest of Nonpareil almonds is a secondary effect; damage is reduced if the population is increasing locally during the harvest period. In the pistachios in this study, however, all pistachios regardless of harvest date were exposed to very high NOW abundance. The degree to which pistachios resisted infestation was dependent on the degree to which the hull remained intact, and with greater time to harvest more pistachios had non-intact hulls. In this situation, in which high abundance of adult NOW was a given, damage was a function primarily of harvest time. The pollinator almonds were much more variable in their response to both NOW abundance and harvest date.

Differences were also seen between the varieties and crops in their response to the mating disruption and insecticide treatments. Generally, gridded Puffers were the most effective of the three treatments in terms of crop protection. In Nonpareil almonds, both the gridded Puffer and azinphos-methyl treatments significantly reduced damage, whereas the peripheral Puffer and CheckMate treatments did not. In pistachios, despite low levels of damage, azinphos-methyl treatments significantly reduced damage and the two Puffer treatments did not. Variability was high in pollinator varieties of walnuts and no significant differences were seen among treatment differences in the Monterey variety. The numerical trend nonetheless suggested, particularly in the presence of high damage such as in 3710, that CheckMate membranes and peripheral Puffers did not reduce damage, that gridded Puffers did reduce damage, and that azinphos-methyl was more effective than gridded Puffers in protecting these varieties.

The scale of experimental plots appropriate for this study reduced the statistical precision available for discerning differences between treatments on an individual ranch. Generally mating disruption is said to work better when applied to larger rather than smaller blocks, and, in the case of NOW, 16 ha (40 acres) has been suggested as a minimum (Shorey and Gerber 1996). Statistically, an experimental unit (aka. plot) is considered to be the smallest unit over which a treatment can be randomly assigned (Cochran and Cox 1957). Use of multiple samples from within the plot to test the treatment effect is considered pseudoreplication, and is not considered valid. To illustrate the consequence of this statistical consideration, a 1-way ANOVA on the Carmel damage data from Ranch 3710 would tell us that the treatment effect is highly significant ($F_{4,75} = 34.95$, $P < 0.0001$), and Tukey's post-test would tell us that the azinphos-methyl treatments and gridded Puffer treatments are significantly different from each other and the peripheral Puffer, CheckMate, and Untreated controls, which aren't significantly different from each other. However, this would be considered statistically improper. Greater true replication could be obtained within the same labor and budgetary constraints by using more and smaller plots, but there is good reason to believe that smaller plots would decrease the efficacy of the mating disruption treatments.

The flight traps and matings assays also yielded the paradoxical result that the heavier population density in pistachios, which resulted in part in poorer performance of mating disruption for protection of that crop, also provided better separation of treatment means when comparing the effects of mating disruption technologies on moths. For example, there were significant differences between NOW males captured in flight traps in the peripheral Puffer treatment plots in pistachios in 6 of the last 7 weeks analyzed, but no significant differences in any week throughout the study in almonds. Despite the lack of difference in ability to shut down flight traps in almonds, there was very good evidence that the gridded Puffers protected Nonpareil and pollinator almonds better than peripheral Puffers. While the greater population density afforded by the pistachio ranches gave insight into relative efficacy of the mating disruption technologies in preventing mating, it also proved to be the case that complete prevention of mating throughout the block—0 trap catch—was the necessary goal to have the desired impact for crop protection.

A similar phenomenon was seen with respect to the mating assays in which the proportion of moths mated in the absence of mating disruption treatments was proportional to local abundance of NOW. Significantly fewer moths were mated in the untreated controls in almonds than in pistachios, and there was a large difference between the proportion of moths mated in peripheral and gridded Puffers in pistachios but not in almonds. In retrospect, the mating assays gave us no additional information beyond that obtained by using unmated females in flight traps.

Correlation of flight data with subsequent damage were used to examine the spatial and temporal scale over which pheromone-based traps could be used to predict damage. These results indicate that 4 flight traps distributed evenly within a 16 ha block predict Nonpareil damage better than do individual traps in 4 ha sub-blocks, or 9 traps placed over a 256 ha area including the 16 ha block, and that the best correlation of trap capture with subsequent damage is from mid-June to mid-July, i.e., the second flight. The poorer performance of single traps on 4 ha sub-blocks might be due in part to lack of replication and the stochastic nature of count data when low numbers are involved. On the other hand, previous studies have shown that female-baited flight traps affect one another over intervals of 100 m (Burks 2003), and the transect data from the current study suggest this might be true over a scale of 400 m. Thus placing more pheromone-baited flight traps within a 4 ha block may not result in more useful information. It should also be noted that it is likely that the radius of attraction of traps baited with a sex pheromone is different from that of traps baited with oviposition attractants.

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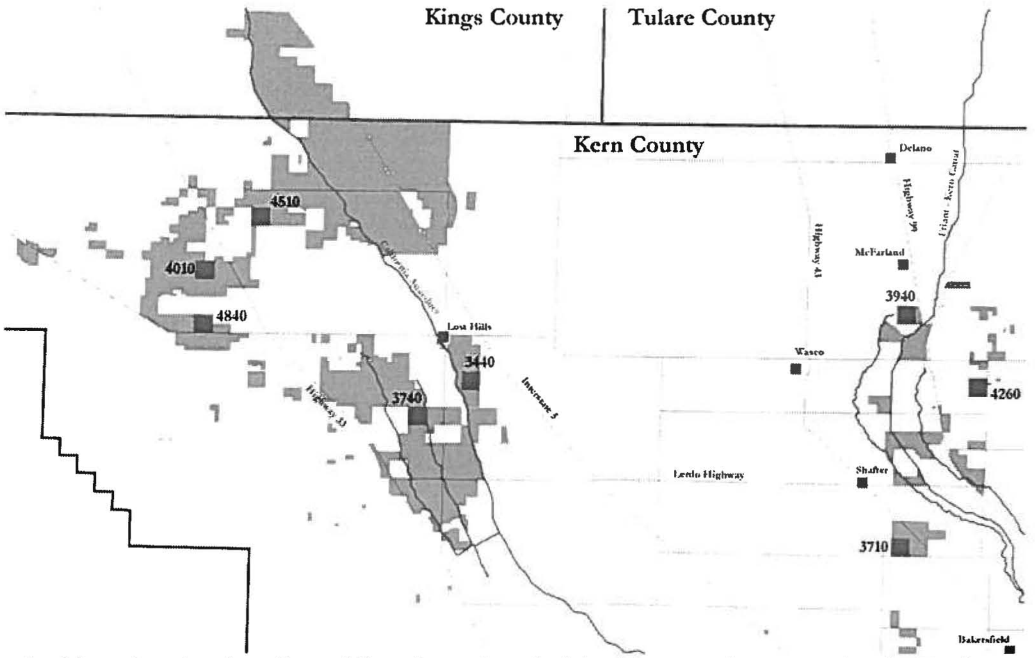

Figure 1. Map showing location of the almond and pistachios ranches examined. Each ranch is 1.6 km (1 mile) squared. Ranches 3440, 3710, 3740, and 3940 are almond, and 4010, 4260, 4510, and 4840 are pistachio.

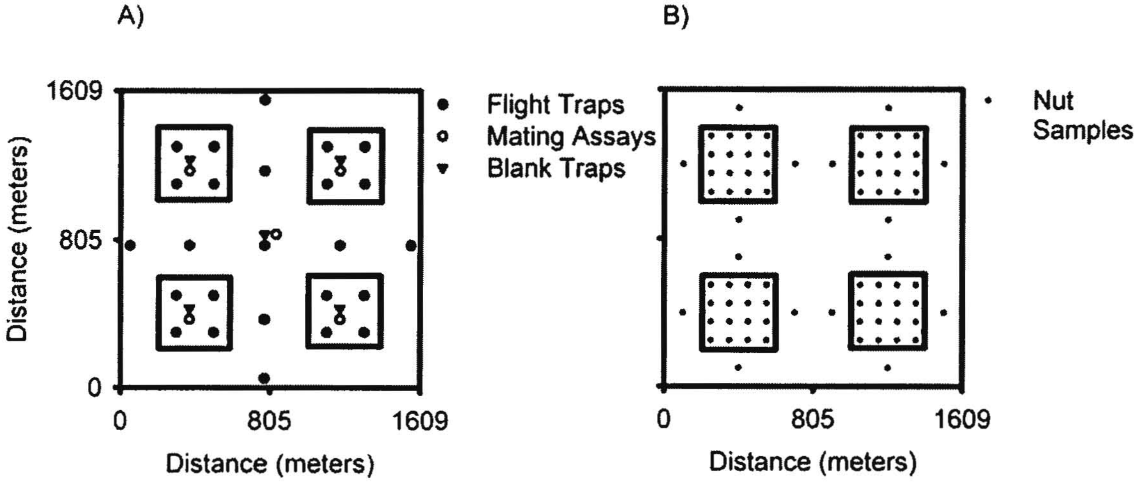

Figure 2. Arrangement of flight traps and nut sampling points at each of the eight research locations. A) There were 9 flight traps baited with unmated females forming north-south and east-west transects across the center of each section. In addition, 4 flight traps were placed in each of the 4 16 ha (40 acre) treatment plots in each section. There was also a mating assay and blank flight traps at the center of each treatment plot and at the center of the section. B) Sixteen samples of ~500 almonds or ~1,000 pistachios each were taken from a 4x4 grid in the treatment plot, with additional samples taken 50 m (165 ft) in each direction outside the treatment plot.

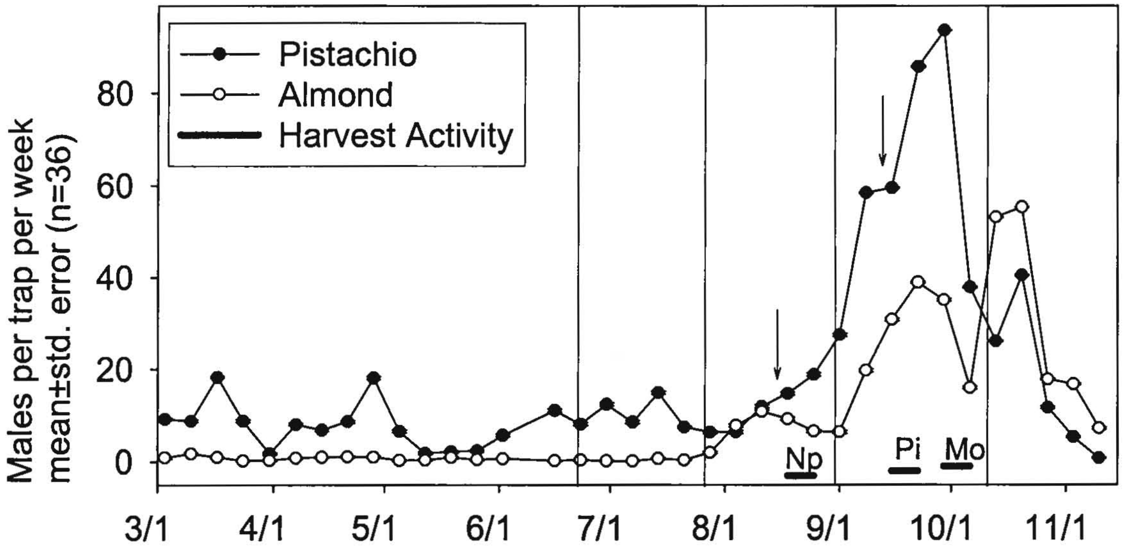

Figure 3. Weekly means of the transect flight trap captures for the 4 almond and 4 pistachio sections Vertical lines show divisions between predicted flights, presuming 607DD°C (1093DD°F) for the first flight, and 410DD °C (738DD °F) for subsequent flights. The arrows show the predicted start of the third and fourth flights if second flight is also presumed to be 607DD. The horizontal bars show periods of harvest for Nonpareil almonds (NP), pistachios (Pi), and Monterey almonds (Mo).

Table 1. Comparison of abundance of navel orangeworm between almond and pistachio ranches for each flight.

Crop	Ranch	Flight 1	Flight 2	Flight 3	Flight 4	Flight 5
Alm.	3440	1.0±0.31	0.3±0.10	12.7±2.5	50.5±5.55	55.6±3.81
	3710	3.5±0.76	3.4±1.51	36.0±4.43	50.2±5.19	22.0±3.42
	3740	0.9±0.18	0.6±0.17	20.2±3.89	54.9±4.86	43.1±4.18
	3940	4.0±1.05	0.1±0.08	8.8±2.56	23.2±3.66	28.2±3.30
Pist.	4010	20.7±2.17	22.5±3.92	20.7±3.72	82.0±4.64	34.3±4.02
	4260	12.2±1.61	18.3±3.49	28.0±4.74	60.9±4.49	12.4±2.37
	4510	16.8±1.92	19.1±2.93	21.7±4.81	72.8±4.70	19.5±2.73
	4840	18.7±2.12	46.3±5.60	44.8±5.27	79.8±4.82	19.1±2.58

Mean±standard error. Sample sizes are ≈144, 45, 45, 54, and 45 for flights 1-5.

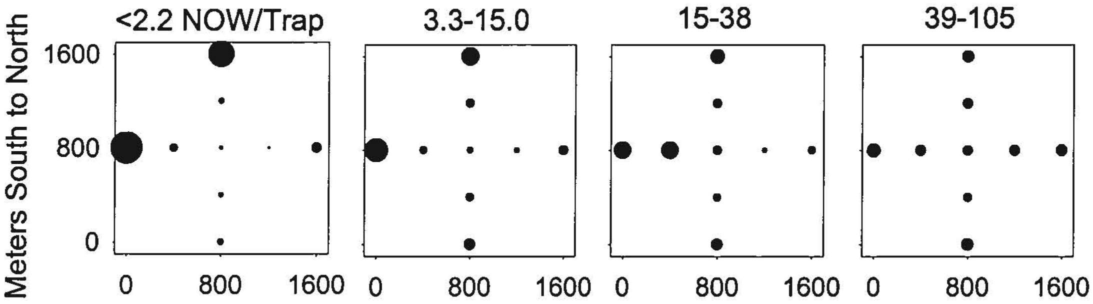

Figure 4. Effect of population density on distribution of navel orangeworm in the absence of mating disruption. The 268 week*ranch combinations are divided into four classes of density, described by the number of males captured per trap per week. The diameter of the dark circles represents the average proportion of moths captured at that trap position.

Table 2. Effect of abundance on proportion of moths captured in western- and northern-most transect traps and the coefficient of variance

		Percent of moths in position:		
Moths per trap	**n**	**Western-most**	**Northern-most**	**Coeff. of var.**
<2.2	67	32.3±4.05	26.1±3.70	180.2
3.3-15	68	23.5±3.07	18.3±2.29	97.4
15-38	67	17.7±1.40	15.3±1.41	65.5
39-105	66	13.9±0.64	11.9±0.74	37.1

Traps mean±standard error of untransformed data; coefficient of variation calculated from arcsine-transformed data.

Table 3. Effect of navel orangeworm treatments in almonds on the number of males captured in flight traps baited with unmated females

	Puffers							
Week of:	Peripheral	n	Gridded	n	CheckMate	n	Untreated	n
31 Mar	0.0±0.0a	1	0.1±0.1a	1	0.0±0.0a	16	0.2±0.1b*	1
7 Apr	0.0±0.0a	1	0.1±0.1a	1	0.0±0.0a	16	2.6±1.3b*	1
14 Apr	0.0±0.0a	1	0.0±0.0a	1	0.0±0.0a	16	0.7±0.3b*	1
21 Apr	0.0±0.0a	1	0.0±0.0a	1	0.1±0.1a	16	1.1±0.5b*	1
28 Apr	0.0±0.0a	1	0.0±0.0a	1	0.0±0.0a	16	2.3±0.8b*	1
5 May	0.1±0.1a	1	0.0±0.0a	1	0.0±0.0a	16	0.1±0.1a	1
12 May	0.0±0.0a	1	0.0±0.0a	1	0.0±0.0a	16	0.1±0.1b*	1
19 May	0.0±0.0a	1	0.0±0.0a	1	0.0±0.0a	16	1.0±0.4b*	1
26 May	0.0±0.0a	1	0.0±0.0a	1	0.0±0.0a	16	0.3±0.1b*	1
2 Jun	0.0±0.0a	1	0.0±0.0a	1	0.0±0.0a	16	1.4±0.6b*	1
9 Jun	0.0±0.0		0.0±0.0		0.0±0.0	8	1.0±0.5	
16 Jun	0.0±0.0a	1	0.0±0.0a	1	0.1±0.1a	16	0.0±0.0a	1
23 Jun	0.0±0.0a	1	0.0±0.0a	1	0.0±0.0a	16	0.9±0.6b*	1
30 Jun	0.0±0.0a	1	0.0±0.0a	1	0.0±0.0a	16	0.5±0.2b*	1
7 Jul	0.0±0.0a	1	0.0±0.0a	1	0.0±0.0a	16	0.2±0.1a*	1
14 Jul	0.1±0.1a	1	0.0±0.0a	1	0.0±0.0a	16	0.5±0.3a*	1
21 Jul	0.1±0.1a	1	0.0±0.0a	1	0.0±0.0a	16	0.0±0.0a	1
28 Jul	0.0±0.0a	1	0.0±0.0a	1	0.0±0.0a	16	7.7±3.9b*	1
4 Aug	0.5±0.2a	1	0.1±0.1a	1	0.1±0.1a	16	22.9±6.6b*	1
11 Aug	1.2±0.6a	1	0.0±0.0a	1	0.8±0.3a	16	27.8±7.4b*	1
18 Aug	0.9±0.7a	1	0.1±0.1a	1	0.9±0.6a	16	27.3±5.9b*	1
25 Aug	0.2±0.1a	1	0.0±0.0a	1	1.4±0.7a*	16	15.8±5.8b*	1
1 Sep	0.0±0.0a	1	0.1±0.1a	1	0.4±0.2a	16	42.6±12.4b*	1
8 Sep	0.4±0.2a	1	0.3±0.2a	1	4.2±1.6b*	16	28.2±8.5c*	1

Mean±standard error. Means followed by asterisks are significantly different from 0, and means within rows followed by different letters are significantly different. Week of 9 June was excluded from analysis.

Table 4. Effect of navel orangeworm treatments in pistachios on the number of males captured in flight traps baited with unmated females

	Puffers							
Week of:	Peripheral	n	Gridded	n	Untreated	n	Guthion	n
31 Mar	0.3±0.1a	1	0.3±0.1a	1	4.9±1.6b*	16	7.3±1.7b*	1
7 Apr	0.6±0.3a	1	1.1±0.4a*	1	17.3±5.6 b*	16	25.2±5.2c*	1
14 Apr	0.4±0.2a	1	0.3±0.1a	1	18.5±6.3 b*	16	28.1±6.3c*	1
21 Apr	0.1±0.1a	1	0.2.±0.1a	1	17.1±6.4 b*	16	30.8±6.4c*	1
28 Apr	1.0±0.3a*	1	0.0±0.0a	1	26.6±7.7 b*	16	48.1±6.5c*	1
5 May	0.1±0.1a	1	0.0±0.0a	1	18.5±5.7 b*	16	32.8±8.1b*	1
12 May	0.0±0.0a	1	0.0±0.0a	1	3.3±1.8 b*	16	25.8±7.7 c*	1
19 May	0.1.±0.1a	1	0.0±0.0a	1	10.7±3.8 b*	16	12.1±3.1 b*	1
26 May	0.1.±0.1a	1	0.0±0.0a	1	4.0±1.5 b*	16	13.9±3.2c*	1
2 Jun	0.3±0.2a	1	0.0±0.0a	1	10.9±2.8 b*	16	20.0±3.6c*	1
9 Jun	0.0±0.0		0.0±0.0		0.5±0.5	2	5.5±5.5	
16 Jun	0.1±0.1a	1	0.0±0.0a	1	2.0±1.4 b*	16	4.3±3.8a*	1
23 Jun	0.8±0.5a	1	0.0±0.0a	1	17.8±5.5 b*	16	41.4±5.8c*	1
30 Jun	0.3±0.3a	1	0.0±0.0a	1	14.2±4.4 b*	16	63.3±5.2c*	1
7 Jul	0.1±0.1a	1	0.1±0.1a	1	25.4±8.6 b*	16	46.9±6.8c*	1
14 Jul	1.7±0.7a*	1	0.1±0.1a	1	31.8±6.5 b*	16	43.8±7.9c*	1
21 Jul	1.5±0.7a*	1	0.1±0.1a	1	7.6±1.9 b*	16	21.3±4.8c*	1
28 Jul	1.5±0.6a*	1	0.0±0.0a	1	15.0±4.3 b*	16	27.1±6.0c*	1
4 Aug	0.0±0.0a	1	0.0±0.0a	1	21.7±8.9 b*	16	41.1±6.1c*	1
11 Aug	0.7±0.5	1	0.0±0.0	1	27.1±6.0	12	61.2±8.2	1
18 Aug	2.9±1.5b*	1	0.3±0.1a	1	34.8±5.4c*	16	48.4±6.6c*	1
25 Aug	3.8±1.3b*	1	0.3±0.2a	1	60.2±5.7c*	16	65.6±7.1c*	1
1 Sep	2.6±0.7b*	1	0.2±0.1a	1	53.3±7.7c*	16	66.3±8.8c*	1

Mean±standard error. Means followed by asterisks are significantly different from 0, and means within rows followed by different letters are significantly different. Weeks of 9 June and 11 August were excluded from analysis.

Table 5. Effect of treatments on female mating status in almonds and pistachios

	Almonds		Pistachios	
Treatment	n	% Mated	n	% Mated
Transect (center)	93	16.1d	80	37.5bc
Guthion	-	-	88	54.5a
Untreated	69	27.5cd	87	51.7ab
Puffers, perimeter	96	2.1f	88	12.5e
Puffers, gridded	99	2.0f	87	5.7ef
CheckMate	97	3.1f	-	-

Percentages within columns followed by different superscripts are significantly different (Fisher's Exact Test, $\alpha = 0.05$).

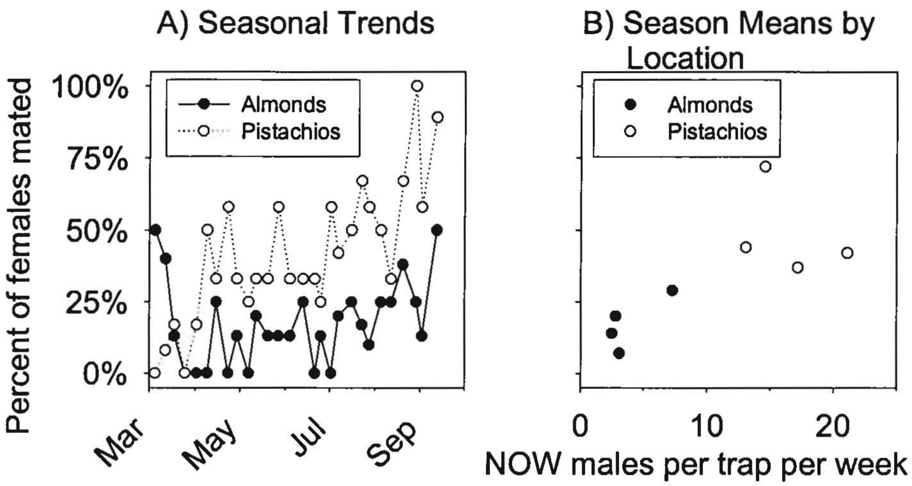

Figure 5. Effects of season and abundance on mating status of navel orangeworm females from center transect and untreated control plot mating assays in almonds and pistachios. A) Percent of females mated weekly in each crop v date, and B) percent mating v. the average weekly trap catch from transect and untreated control plot flight trap over the entire season for each of the 8 ranches.

Table 6. Harvest date and navel orangeworm damage to Nonpareil almonds by ranch

Ranch	Harvest Date	n	% NOW Damage
371	28 August	80	8.51±0.422a
344	28 August	78	1.03±0.100b
374	21 August	80	0.62±0.051b
394	17 August	80	0.24±0.033c

Mean±standard error, means followed by different letters are significantly different at *α* = 0.05.

Table 7. Effect of treatments on navel orangeworm damage to Nonpareil almonds

Treatment	n	Percent NOW Damage
Untreated	64	3.5±0.65a
Peripheral Puffers	64	3.0±0.58ab
CheckMate	62	2.4±0.42ab
Gridded Puffers	64	2.3±0.41b
Guthion	64	1.9±0.34b

Mean±standard error, means followed by different letters are significantly different at *α* = 0.05.

Table 8. Percent navel orangeworm damage to Nonpareil almonds by treatment and ranch

Treatment	Ranch 3710	Ranch 3440	Ranch 3740	Ranch 3940
Untreated	12±0.9 (16)	1.54±0.284 (16)	0.55±0.117 (16)	0.32±0.067 (16)
Periph. Puffers	10±1.1 (16)	0.98±0.234 (16)	0.69±0.077 (16)	0.30±0.098 (16)
Checkmate	7±0.6 (16)	0.99±0.201 (14)	0.80±0.120 (16)	0.15±0.051 (16)
Gridded Puffers	7±0.7 (16)	0.99±0.190 (16)	0.44±0.104 (16)	0.21±0.065 (16)
Guthion	6±0.6 (16)	0.65±0.146 (16)	0.64±0.136 (16)	0.24±0.081 (16)

Mean±standard error, with sample size in parentheses.

Table 9. Harvest date and navel orangeworm damage to pistachios by ranch

Ranch	Harvest Date	n	% NOW Damage
4840	25 September	80	1.32±0.122a
4260	24 September	80	1.28±0.144a
4510	17 September	80	0.63±0.079b
4010	16 September	80	0.36±0.048c

Mean±standard error, means followed by different letters are significantly different at $\alpha = 0.05$.

Table 10. Effect of treatments on navel orangeworm damage to pistachios

Treatment	n	Percent NOW Damage
Untreated	64	1.22±0.165a
Peripheral Puffers	64	1.24±0.140a
Gridded Puffers	64	1.17±0.131a
Guthion treatment plots	64	0.48±0.059b
Outside samples (Guthion)	64	0.37±0.058b

Mean±standard error, means followed by different letters are significantly different at $\alpha = 0.05$.

Table 11. Effect of treatments on navel orangeworm damage in almond pollinator varieties

	% Damage by variety, ranch, and harvest date				
Treatment	Fritz 344 20 Sep	Carmel 371 26 Sep	Monterey 371 26 Sep	Monterey 374 30 Sep	Monterey 394 6 Oct
Untreated	7.3±0.70	14±1.0	22±1.9	0.70±0.117	3.9±0.29
Periph. Puffers	4.3±0.58	13±0.5	21±1.2	1.00±0.243	2.6±0.55
CheckMate	5.4±0.51	15±1.2	25±1.0	1.54±0.317	3.5±0.35
Gridded Puffers	6.2±0.46	9±0.8	18±1.5	1.03±0.209	2.7±0.27
Guthion	5.9±0.77	4±0.5	9±0.7	1.69±0.523	2.2±0.35

Mean±standard error, n = 16.

Table 12. Goodness of fit and significance of regression of trap capture on navel orangeworm damage to Nonpareil almonds on three spatial scales

	256 ha		16 ha		4 ha	
Week of	r^2	P	r^2	P	r^2	P
28 April	0.05	0.78	0.12	0.65	0.06	0.34
2 June	0.22	0.53	0.09	0.70	0.09	0.26
23 June	0.76	0.13	0.96	0.02	0.39	0.01
14 July	0.94	0.03	0.98	0.01	0.39	0.01
28 July	0.64	0.20	0.88	0.06	0.56	0.00
11 August	0.43	0.35	0.60	0.22	0.55	0.00

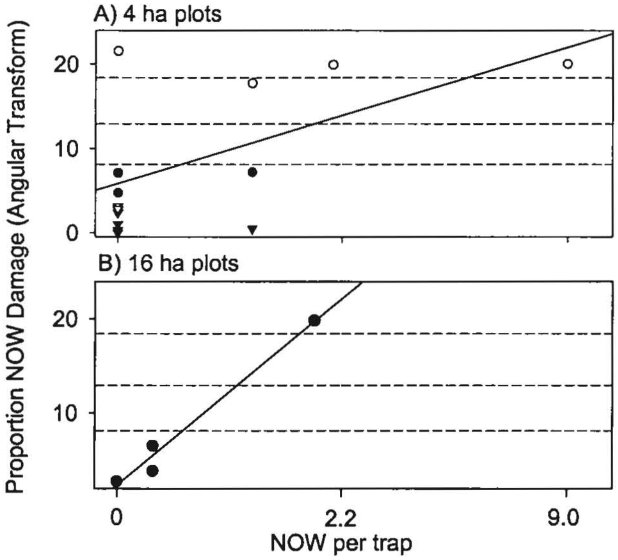

Figure 6. Regression of flight trap from 23 June on subsequent damage to Nonpareil almonds harvested between 17 and 28 August. The horizontal dashed reference lines represent 2, 5, and 10% navel orangeworm damage. A) Data from traps centered in 4 ha quadrants of the 16 ha plots regressed on 4 nut samples from that quadrant; and B) data from 4 traps in a 16 ha plot regressed on 16 samples pooled from that plot.

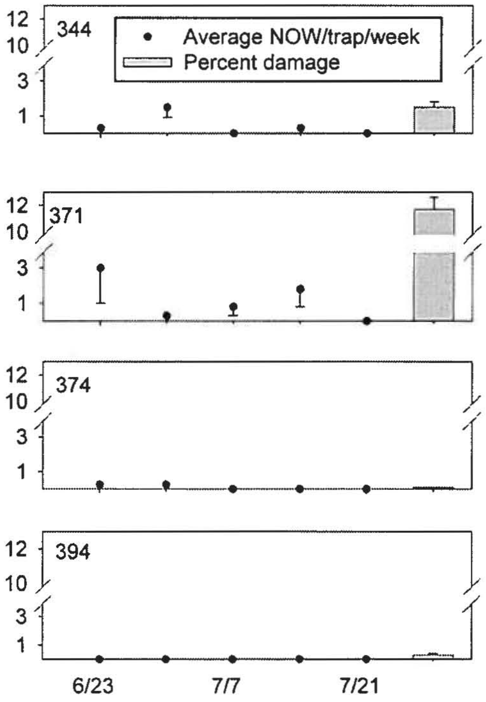

Figure 7. Comparison of weekly trap captures and subsequent damage to Nonpareil almonds.

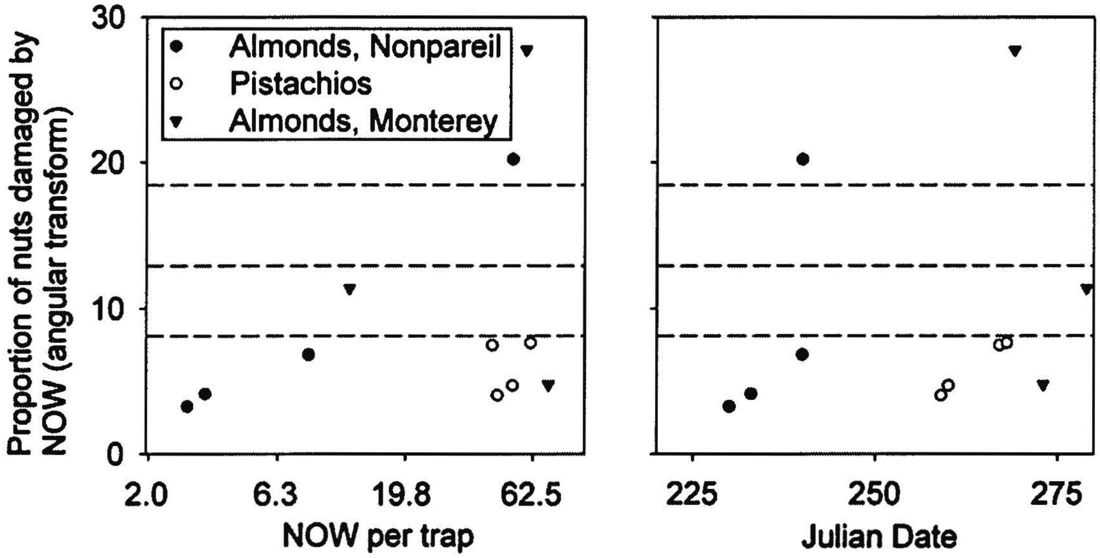

Figure 8. Regression of adult navel orangeworm abundance and harvest date on damage to nuts in the untreated plots. The horizontal dashed reference lines represent 2, 5, and 10% navel orangeworm damage. A) Regression damage on moths captured in flight traps 1-4 weeks prior to harvest. B) Regression of damage on the Julian date of harvest.