Mating Disruption for Suppression of Navel Orangeworm Damage in Almonds

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Executive Summary

1) We compared the effect of mating disruption targeted against navel orangeworm (NOW) using a previously-used single-component and two new multi-component sex pheromone blends, referred to hereafter as “Single”, “Multi A”, and “Multi B”, respectively. We compared the effect of these materials on the ability of males to locate females mating status in sentinel females in almonds and pistachios, and on navel orangeworm damage to almonds. Mating disruption treatments were applied to 40 acre treatment plots in almonds and 20 acre plots in pistachios, and compared to untreated control plots which were not protected with either mating disruption or a residual insecticide.
2) Compared to untreated controls, all mating disruption treatments significantly reduced males captured in female-baited flight traps and mating in sentinel females. In almonds, shutdown of flight traps and mating assays was almost complete, and there were no differences between the mating disruption treatments. NOW was more abundant in pistachios, and in this crop there were significantly fewer males and less mating in Multi B compared to Single treatment plots, whereas differences were not significant between males captured and females mated in Multi A and Multi B treatment plots.
3) In Nonpareil almonds, one or more of the mating disruption treatments had significantly less damage than the untreated control in each of the four ranches examined. In one ranch with heavy NOW pressure (19% damage in untreated control nuts) the plot receiving the Single treatment had significantly less damage than those receiving the Multi A and Multi B treatments, and all three had significantly less damage than untreated controls. In three ranches with light NOW pressure (<2% NOW damage in untreated controls), Multi A had less damage than Single in two of the three, and Multi B had less damage than single in all three. In harvest samples from pollinator varieties Multi B had numerically less damage than the control in the ranches with less NOW pressure; this difference was significant in two of these three ranches.
4) Therefore the moth reproductive biology data show that the multi-component formulations disrupt mate finding more effectively under conditions of high abundance, and the damage data suggest that this greater effectiveness on NOW reproductive biology may result in less damage to almonds in Nonpareil under low NOW pressure, and also in pollinator varieties harvested later in the season.

Introduction and Objectives

Much research on mating disruption in moths has focused on mechanisms and efficiency with which candidate formulations disrupt male orientation to females (Cardé and Minks 1995). It seems likely, however, that additional factors also affect the efficacy with which mating disruption reduces crop damage. One of these factors is the effect of reduced or delayed mating on the net reproductive rate of females within a protected area, and this is likely affected by the importance of delayed mating and the importance of polyandry for the target species (Vickers 1997), and also by the probability of immigration of mated females from outside the protected area (Schumacher et al. 1997, Hughes et al. 2002).

The other factor is the relationship between density of adult females and damage to the target crop. For example, we have previously examined effects of mating disruption on behavior of the navel orangeworm *Amyelois transitella* (NOW) in almonds and pistachios, and on relative abundance in and damage to these crops by this species (Burks et al. 2003, 2004). We found that NOW abundance was consistently greater in the pistachios than in the almonds that we examined. The flight trap data indicated that, compared to Nonpareil almonds, in which harvest damage of 5 to 12% was associated with an average of 20-40 moths per trap per week over the prior month, in pistachios ≤2% damage was associated with an average of 40-90 moths per trap during the same preharvest interval. We demonstrated differences between males captured in pheromone-baited flight traps and number of moths mated in assays in pistachios, but not in almonds. In contrast, we were able to demonstrate treatment effects on crop damage in almonds but not in pistachios. Thus behavioral assays in pistachios were more useful in predicting damage responses to mating disruption in almonds than were the same assays in almonds.

In these studies (Burks et al. 2003, 2004) we found that the principal component of the NOW sex pheromone, (*Z,Z*)-11,13-hexadecadienal (Coffelt et al. 1979), emitted from a time release system (Puffers)(Shorey and Gerber 1996) at 19.2 mg/acre/per night over 40 acre treatment blocks, reduced damage in Nonpareil almonds under conditions of high NOW pressure but not when NOW pressure is low. When NOW damage in untreated control blocks was in the range of 6-12%, mating disruption consistently reduced damage by around 50%, similar to efficacy obtained with residual insecticides. When NOW damage in untreated control blocks was low (<3%) neither mating disruption nor residual insecticides had a statistically significant effect on damage.

Unlike some moths, NOW males are not efficiently attracted to a point source with only the principal component of the sex pheromone. For this reason, we have used unmated females as a pheromone source in our studies on the effects of crop and season on relative abundance of this species, and on effects of mating disruption treatments. Last year the discovery of additional NOW pheromone components was published (Leal et al. 2005). It is expected that a more authentic blend of sex pheromone components will disrupt orientation of males at a lower dose than a less complete blend (Cardé and Minks 1995), but whether this results in more efficacious crop protection depends on the target species and perhaps other circumstances, such as the scale examined (Ryne et al. 2001).

The objective of the current study was to compare the effects of two novel multi-component mating disruption formulations with the single-component formulation used previously. We examined the effects of these formulations on NOW mating success in both pistachios and almonds, and the effects on NOW damage in Nonpareil almonds.

Materials and Methods

This study was conducted in 640-acre blocks on four almond and three pistachio ranches, all owned by Paramount Farming and located in Kern County. The almond ranches are designated by their owner as 336, 344, 345, and 370; and three pistachio ranches are designated 401, 446, and 451. The pistachio test areas were square, with one 20 acre test plot centered in each quarter of the test area. The pistachio test plots were erroneously described as 40 acres in the preliminary report. The almond test plots were 40 acre squares separated by 440 yards from other test plots. In both crops untreated control was placed upwind, with respect to the prevailing wind, of the other plots.

Mating disruption was performed using timed-release aerosol dispensers, hereafter referred to as Puffers (Shorey and Gerber 1996, Burks and Brandl 2004). Three formulations were tested, labeled here as Single, Multi A, and Multi B. The Single formulation, used in this and the previous studies, consists of (*Z,Z*)-11,13-hexadecadienal (11Z13Z:16al)(90% optical purity per HPLC) placed in an organic solvent and released at 15-30 minute intervals from 6PM to 6AM PDT at a rate equivalent to 19.2 mg/acre/night. Puffers were placed in trees at two-thirds canopy height at even intervals throughout the treatment plot and a density of two devices per acre. Multi A and Multi B were novel mixtures containing unspecified ratios of 11Z13Z:16al and other components similar to those described by Leal et al. (2005). Products were released at a similar dose and at the same intervals as the old formulation. Puffers were activated during the week of April 25, 2005, and continued until harvest.

Efficacy of the mating disruption treatments in reducing the ability of NOW males to find and mate with females was monitored throughout the season using a combination of flight traps, oviposition traps, and mating assays placed in each treatment plot. Six female-baited wing traps (Suterra LLC, Bend, OR) were placed at three positions along a southwest to northeast transect across each treatment plot, 170 yards apart in almonds and 120 yards apart in pistachios. At each of these positions one trap was hung at 5 feet and another at two-thirds canopy height. Three unmated female NOW, prepared and placed as described in Burks and Brandl (2004), were used as a pheromone source. Mating assays and oviposition traps (NOW Traps, Suterra LLC, Bend OR) were placed on the opposite side of trees at nine positions 67 m apart within each plot (Fig. 1C). Mating assays were performed using modifications of the mating assay of Curtis and Clark (1984). A 473-ml round polypropylene cup was suspended from the top of a wing trap by clips, and contained a second 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 mating assays were hung from tree branches 1.5 m high, and arranged in a three by three grid with traps 120 yards apart in almonds and 85 yards apart in pistachios. The following week, females were again placed in plastic vials for transport to the laboratory where the bursa was dissected to determine presence or absence of a spermatophore. Flight traps, oviposition traps, and mating assays were serviced and evaluated on a weekly basis. Mating assays in almonds (but not pistachios) were suspended during the interval of June 13 to July 25.

Harvest samples (ca. 700) of almonds taken in Nonpareil and pollinator rows close to the 9 mating assay/oviposition sites within the treatment plots. Nonpareil harvest samples for ranches 345, 336, 344, and 370 were taken on August 8, 15, 19, and 22, respectively. Data for pollinator varieties are presented where the same variety was represented in each treatment plot within one ranch. This was the case for Butte, harvested on September 5 at Ranch 370; Monterrey, harvested on September 30 at Ranch 336 and on October 6 at Ranch 345; and for Fritz, harvested on October 7 at Ranch 344. Samples were held in cold storage and analyzed for NOW damage by Paramount Farming Company research personnel.

Data collected included the number of males captured in pheromone traps, the proportion of mated females recovered from mating assays, and NOW harvest damage to almonds. Alternatives to Analysis of Variance (ANOVA) were used because the data was inherently binomial in the case of the mating assays, or because assumptions of homogeneity were violated even after transformation in the case of flight traps and harvest data. Flight trap data were analyzed by Poisson regression of treatment, ranch, and their interaction on counts of males in traps. Mating assay data were analyzed by logistic regression of treatment, ranch, and their interaction on the proportion of females mated. Poisson and logistic regression were performed using the GENMOD procedure of the SAS System (SAS Institute, Cary, NC). Contingency tables and χ^2 analysis was also used to compare trap counts and proportions of females mated in almonds. NOW damage among almonds from harvest samples were compared within ranches using 2 x 2 and/or 2 x n contingency tables and χ^2 analysis (Stokes et al. 1995).

The preliminary report also included data examining effect of mating disruption treatments on egg counts in oviposition traps and comparing NOW damage to harvest samples in mating disruption plots and surrounding areas treated with methoxyfenozide. These data are omitted from the final report because both are ambiguous, and the egg trap data neither support nor contradict those presented here.

Results

All three mating disruption treatments significantly reduced, in both crops, the number of males captured in female-baited flight traps and mating in females (χ^2, $P < 0.0001$) (Tables 1-3). The males captured in female-baited flight traps in almonds represent a reduction of ≥98.5% at Ranch 344 and ≥99.7% at the other three ranches. Of the 406 females recovered from assays in control plots in all four almond ranches over the season, 250 (59%) were mated. In the almond treatment plots, 3 out of 400 females recovered were mated in the Single treatment, and 0 out of 406 and 1 out of 414 were recovered from almond plots that received the Multi A and Multi B treatments. Differences between numbers of males in flight traps or proportions of females mated were not statistically different between the three mating disruption treatments in almonds.

In pistachios, however, significant differences were observed between mating disruption treatments. Poisson regression of predictor variables on males in flight traps found significant effects due to treatment (F = 1081.84.14, df = 3, 1408; $P < 0.0001$), ranch (F = 18.00, df = 2, 1408; $P < 0.0001$), week (F = 23.36, df = 6, 1408; $P < 0.0001$), and the interaction of treatment and ranch (F = 23.33, df = 6, 1408; $P < 0.0001$). Across the three ranches, significantly more males were captured in female-baited flight traps in plots treated with Single compared to those treated with either Multi A ($\chi^2 = 4.00$, $P = 0.0454$) or Multi B ($\chi^2 = 8.96$, $P = 0.0028$). There was, however, no significant difference in the number of males captured in female-baited flight traps in plots treated with Multi A v. Multi B ($\chi^2 = 1.24$, $P = 0.2662$). Logistic regression of predictor variables on proportion of female mating showed similar trends. Effects of treatment ($\chi^2 = 986.72$, df = 3, $P < 0.0001$), ranch ($\chi^2 = 18.75$, df = 2, $P < 0.0001$), week ($\chi^2 = 207.39$, df = 17, $P < 0.0001$), and the interaction of ranch and treatment ($\chi^2 = 20.68$, df = 6, 1427; $P = 0.0021$) were all significant. There were significantly more females mated in the plots treated with Single than in treated with either Multi A ($\chi^2 = 8.18$, $P = 0.0042$) or Multi B ($\chi^2 = 20.69$, $P < 0.0001$), but the there was no significant difference in the proportion of females mated between Multi A and Multi B ($\chi^2 = 3.18$, $P = 0.0745$). Plots of weekly flight trap and mating assay activity in pistachio treatment plots show that, in both late June and early August, peaks of flight trap activity occurred a week ahead of peaks in proportion of females mated (Fig. 1).

The NOW damage data from Nonpareil harvest samples demonstrate reduction by 50% or more of NOW damage by all three mating disruption treatments in Ranch 370, where NOW pressure was greatest (Table 4). In two of the three almond ranches with lower NOW pressure (336, 344, and 345), NOW damage was numerically less in all mating disruption treatments. In all three of these ranches almonds from the Multi B treatment plot had significantly less damage than those from the untreated control plot.

Among pollinator varieties, almonds from all three of the mating disruption treatment blocks at Ranch 370 had significantly less damage than the controls (Table 5). At this ranch and at Ranch 336, damage trends in Butte and Monterrey, respectively, were consistent with those seen in Nonpareil. While that is not true of harvest samples of Fritz and Monterrey from Ranches 344 and 345, pollinator almond samples from Multi B had significantly less damage than those from control plots in three of the four ranches, and numerically less damage than the controls in all four ranches.

Discussion

While all mating disruption treatments significantly reduced capture of males in female-baited traps and mating in sentinel females in both crops, differentiation between the mating disruption treatments was seen only in pistachios, where NOW was more abundant. While differentiation between mating disruption treatments was not seen in biological data from almonds, it was in almond harvest data. Therefore the data from this year, as well as that from 2003 (Burks et al. 2003), demonstrate that biological assays in pistachios predict efficacy of mating disruption treatments in reducing navel orangeworm damage in almonds better than biological assays in almonds.

The trapping and mating assay data from pistachios suggest an order of efficacy of these materials of Single < Multi A < Multi B. While there was no significant difference between Multi A and Multi B in the analysis of either flight trap or mating assay data, the numerical order of counts of males in flight traps from ranches 401 and 451 and of proportion of females mated from ranches 446 and 451 support this rating.

The damage data from Nonpareil almonds were generally consistent with this proposed rating. While χ^2 statistics indicated significantly less damage in Nonpareil almonds from the Single than from the Multi A and Multi B plots in Ranch 370, the more striking comparison is that all three mating disruption treatments had half or less of the damage of the almonds from the untreated control plot in this area of high NOW pressure. The numerical order of percent Nonpareil damage in two of the three other ranches are consistent with the efficacy ratings above, and in all three of these ranches Multi B had significantly less damage than some or all of the other treatment plots. There is therefore evidence that mating disruption with Multi B had benefit under low NOW pressure, something not seen previously with Single.

Damage in the pollinator harvest samples followed similar general trends to those from the Nonpareil data. In Butte at Ranch 370, harvested 14 days after the Nonpareil harvest at that ranch, all mating disruption treatments had 50% or less of the damage in the untreated control plot. The order of damage among mating disruption treatments was different from that predicted based on pistachio flight trap and mating assay data, and from that from the Nonpareil data at that ranch. These observations suggest that the differences between damage in samples from the mating disruption treatment plots at 370 (as opposed to the larger difference between mating disruption and untreated control plots) were due to factors other than the treatments themselves. The Fritz and Monterrey almonds sampled at the other three ranches were harvested much later, 49 to 56 days following the Nonpareil harvest. At Ranch 336, where Monterrey almonds were harvested 49 days later than Nonpareil, the numerical and statistical damage comparisons were consistent with the prediction based on biological data from pistachios and the harvest data from Nonpareil almonds. Among pollinator samples from ranches 344 and 345, harvested in October, greater NOW damage occurred in plots treated with Single and Multi A formulations than in the untreated control plots. But, in ranches 336, 344, and 345, almonds from the Multi B treatment plot had the numerically lowest proportion of damage and significantly less damage than almonds from some or all other treatment plots. The damage data therefore suggest that the greater biological activity of the multi-component blends, particularly of Multi B, may result in less damage to almonds under conditions of low pressure in Nonpareil, or in pollinator varieties harvest much later in the season.

Analysis of damage data would more conventionally be done with ANOVA. ANOVA is inappropriate for the Nonpareil data, in particular, because, even with transformation and/or the use of mixed models, the great difference in mean and variation between 370 and the other ranches result in violations of the assumption that the variance of the error is randomly distributed. ANOVA would not detect significant differences among the treatments, in part because the large treatment plots required for efficacy necessarily result in few replicates and therefore few degrees of freedom. Increasing true replication would require more 40 acre plots and therefore more sections, and there were not sufficient sex pheromone components available to allow this. The statistical approach that was adopted here is less robust in ability to infer to other locations compared to a necessarily larger scale study more amenable to analysis by ANOVA. Nonetheless, the damage and the biological results presented here support each other, and the approach used was the best possible within the constraints of practical limitations.

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Table 1. Effect of mating disruption treatments on sums of NOW males captured in 40 acre plots at four almond ranches between May 1 and September 15, 2005

	Ranch 336		Ranch 344		Ranch 345		Ranch 370	
Treatment	Males	Traps	Males	Traps	Males	Traps	Males	Traps
Untreated	1755	120*	275	120	1759	120	5045	119
Single	0	120	4	120	2	120	3	119
Multi A	1	120	4	120	2	120	13	120
Multi B	3	120	0	120	5	120	8	120

*Totals are for 6 traps per plot per week over 20 weeks

Table 2. Effect of mating disruption treatments on sums of NOW males captured in 20 acre plots at three pistachio ranches between May 1 and September 15, 2005

	Ranch 401		Ranch 446		Ranch 451	
Treatment	Males	Traps	Males	Traps	Males	Traps
Untreated	9684	120*	8550	119	3549	120
Single	89	120	177	120	502	120
Multi A	15	120	233	120	267	120
Multi B	10	120	142	120	121	120

*Totals are for 6 traps per plot per week over 20 weeks

Table 3. Effect of mating disruption treatments on mating status of sentinel NOW females in 20 acre plots at three pistachio ranches between May 1 and September 15, 2005

	Ranch 401		Ranch 446		Ranch 451	
Treatment	Trials	%Mated	Trials	%Mated	Trials	%Mated
Untreated	113	92.0	147	91.2	146	72.6
Single	148	10.1	155	19.4	127	15.8
Multi A	122	5.7	142	16.2	137	7.7
Multi B	130	6.1	152	7.9	136	4.2

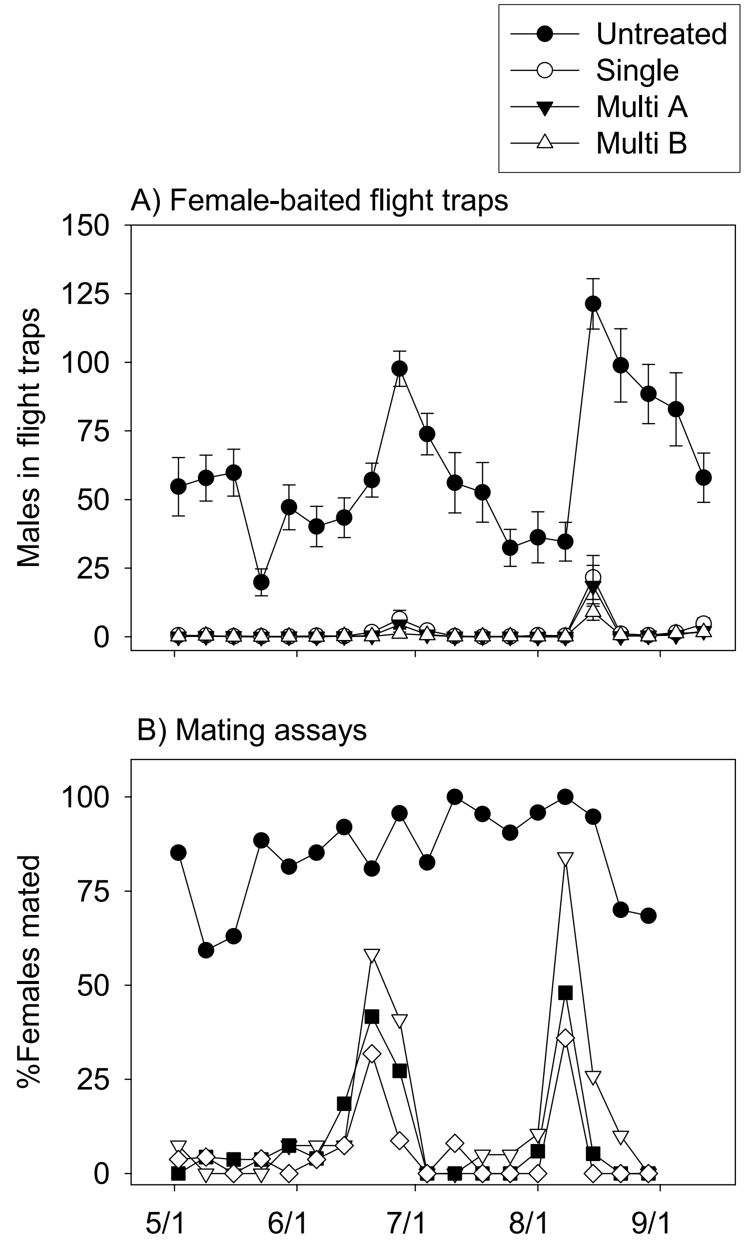

Figure 1. Weekly variation in the impact of mating disruption treatments in pistachios on the ability of NOW males to find females, and on mating in females. A) Males captured in flight traps baited with unmated females. Points and error bars indicate mean and standard error of moths captured per trap in three ranches (n = 18). B) Percent mated among females placed in the orchard and recovered a week later (≤27 assays per treatment per week).

Table 4. Effect of mating disruption treatments on NOW damage in Nonpareil almonds sampled from 16 ha plots at 4 ranches

	Ranch 336		Ranch 344		Ranch 345		Ranch 370	
Treatment	**Nuts**	**%Dmg**	**Nuts**	**%Dmg**	**Nuts**	**%Dmg**	**Nuts**	**%Dmg**
Untreated	7208	0.75a[1]	5751	1.81a	5485	1.31a	7634	18.60a
Single	6563	0.56ab	6562	1.97a	6243	0.80b	6835	6.86c
Multi A	5110	0.31bc	6523	2.18a	5022	0.70bc	5765	9.05b
Multi B	7007	0.26c	7409	0.96b	5935	0.49c	6702	9.77b

[1] Percentages within columns followed by different letters are significantly different (2 x 2 Pearson's χ^2, $P < 0.05$)

Table 5. Effect of mating disruption treatments on NOW damage in pollinator variety almonds sampled from 16 ha plots at 4 ranches

	Ranch 336[1]		Ranch 344		Ranch 345		Ranch 370	
Treatment	**Nuts**	**%Dmg**	**Nuts**	**%Dmg**	**Nuts**	**%Dmg**	**Nuts**	**%Dmg**
Untreated	6737	2.00a[2]	5893	2.48b	5823	5.72b	6912	9.16a
Single	5436	0.90b	6430	3.47a	6727	8.22a	7008	3.20c
Multi A	5298	0.53c	5367	3.02a	7249	6.35b	7070	2.79d
Multi B	7499	0.40c	6713	1.04c	5443	4.98b	7225	4.57b

[1] Varieties examined were: Ranch 336, Monterrey; Ranch 344, Fritz; Ranch 345, Monterrey; and Ranch 370, Butte.

[2] Percentages within columns followed by different letters are significantly different (2 x 2 Pearson's χ^2, $P < 0.05$)