Insect and Mite Research

Project No.: 14-ENTO7-Zalom

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Objectives:

- 1. Determine treatment timing of bifenthrin, methoxyfenozide, spinetoram, chlornitraniliprone, and flubendiamide for navel orangeworm (NOW) control in spring based on comparison of male trap captures using the Suterra NOW pheromone lure and egg-laying using the traditional black egg traps baited with almond presscake.
- 2. Evaluate residual efficacy of bifenthrin, methoxyfenozide, chlornitraniliprone, and flubendiamide.
- 3. Determine if low temperatures delay mating or oviposition by NOW females.

Interpretive Summary:

This report presents results of studies conducted In 2014 that focused on evaluating efficacy and treatment timing for the navel orangeworm (NOW) at the 'May spray' timing and to relate these to navel orangeworm phenology as indicated from monitoring with navel orangeworm egg traps and pheromone traps that record male navel orangeworm flight. Additional studies on navel orangeworm control included estimating the residual efficacy of four registered insecticides applied during the spring, and a small study of insecticide efficacy at the 'May spray' timing. A laboratory study was also initiated in early 2015 to determine temperature effects, if any, on mating and oviposition by navel orangeworm females. Results of the field efficacy and treatment timing study using bifenthrin (Brigade), methoxyfenozide (Intrepid), chlornitraniliprone (Altacor), and flubendiamide (Belt) showed that all of these products provided significantly better control resulting in less damage at each of the 6 weekly treatment timings when compared to the untreated control. Efficacy of Brigade, a pyrethroid insecticide that is not recommended for use as a 'May spray' due to potential negative effects on biological control, differed between treatment timings to a greater extent than the other products possibly due to a shorter residual effectiveness. Results of the field residue experiment conducted in 2013 suggested that Brigade residual activity sufficient to prevent infestation was about 2 weeks, Intrepid about 4 weeks, Altacor about 3 weeks, and Belt about 3 weeks. An identical study was conducted in 2014, but results were not conclusive due to very low navel orangeworm presence in the experimental orchard. The laboratory study of lower temperature effects on navel orangeworm mating and oviposition was conducted with

Individuals from both a laboratory colony and wild collected from infested nuts, and compared mating and oviposition at constant temperatures as low as 48.2 $\mathrm{^oF}$ and variable daily temperatures as low as 60.8 $\mathrm{^{\circ}F}$ day and 41.0 $\mathrm{^{\circ}F}$ night. In general, number of fertile eggs laid was fewer at the lowest temperatures, but the females laid viable eggs at all of the experimental temperatures.

Materials and Methods:

Objective 1. *Treatment timing and efficacy of 'May sprays' as related to male NOW pheromone trap captures and eggs laid in NOW egg traps.* The 'May spray' timing offers the potential to obtain some level of control of both NOW and peach twig borer (PTB) as these insects have flights that overlap somewhat in many years. The May spray controls the first generation of NOW following spring moth emergence. Females of the first flight lay their eggs on the mummy nuts that remain in the orchards, so the infestation of mummy nuts can be quite high. The current May spray timing recommendation for NOW is 100 degree-days after the first eggs are laid for 2 consecutive sampling periods on egg traps (Engle and Barnes 1983, Zalom et al. 1998), but this may be modified when the relationship between males flights as recorded using the navel orangeworm pheromone is better understood relative to egg hatch as monitored with egg traps. The recommended PTB treatment timing is at 400 degree-days after the first males are captured in pheromone traps (Rice et al. 1982).

The site of the spring 2014 navel orangeworm control study was a mature 20 acre almond orchard on near Ripon, but in San Joaquin Co. The block had not been dormant treated by the grower and no spring insecticide sprays were applied. Peach twig borer male flights were monitored using 4 wing type traps baited with PTB Biolures from Suterra LLC., and navel orangeworm activity was monitored with 3 wing type traps baited with NOW Biolures also from Suterra (for males moths) and 12 black NOW egg traps (one trap in each tree surrounding a pheromone trap) baited with almond presscake plus almond oil. All traps were place in the orchard on March 3 and checked twice weekly until April 28 after which the traps were checked weekly. Using our 'mummy strand' protocol, twenty uninfested Nonpareil nuts saved from the 2013 harvest were hot glued to strands of vegetable mesh mid-March, 2014. The mummy strands were all deployed at mid-canopy in Nonpareil trees on March 27 which was earlier than the start of NOW egg trap captures but several weeks after male navel orangeworm moths were first captured in pheromone traps and before the PTB biofix April 18 (**Figure 1**). Eight replicates of each product were treated at weekly intervals for 7 weeks starting on March 27. There were 36 treatment x date combinations in all plus a water only control, with 8 mummy strands allocated for each treatment including 20 for the controls. The number of strands deployed totaled 300 (6000 mummies) representing 5 products X 8 reps X 7 weeks, plus 16 reps of untreated control strands. The rates of the insecticides applied were Altacor (4 oz.), Belt (4 oz.), Intrepid (16 oz.), Brigade (16 oz.), and Delegate (7 oz.). All were mixed into the equivalent of 100 gal per acre, and included the nonionic surfactant, Dyne-amic, at 0.25% v/v. The strands were removed from the trees at 615 NOW degree-days from the date they were deployed and returned to UC Davis where they were hand-cracked to determine infestation (nuts with larvae or pupae present) and damage (nuts with larvae, pupae or damage present). Data were analyzed by analysis of variance following arcsin transformation, with individual treatments and treatment timing compared to the untreated

control and means for treatment timings for each product compared to one another by Students t-test.

Figure 1. Navel orangeworm pheromone and egg trap captures in the study orchard during 2014 indicating application dates and navel orangeworm degree-days from January 1, March 3, and egg trap biofix (May 2).

Objective 2. *Residual efficacy of 'May sprays'.* The second experiment was conducted using the same almond strand approach, but was intended to provide a better estimate of residual activity as well. The site of the study was an almond orchard located at Delta College near Manteca in San Joaquin Co. The block had not been dormant treated by the grower, and no insecticide sprays were applied. A total of 176 strands of almond mummies were used for this experiment. Forty strands were designated for each of 4 insecticides, and 16 strands for the untreated control. Each week starting April 15, 8 of the 40 strands designated for each chemical treatment were treated and hung within the tree canopy of isolated roadside olive trees, a non-host for NOW, with no obvious source trees nearby. The Rates of the insecticides applied were Altacor (4 oz.), Belt (4 oz.), Intrepid (16 oz.), and Brigade (16 oz.). All were mixed into the equivalent of 100 gal per acre, with the nonionic surfactant, Dyne-amic, included at 0.25% v/v. When the last set of 8 strands was treated 0n May 14, all of the strands were transferred to the Manteca/Ripon almond orchard, along with the 16 untreated strands. The strands were left in the almond orchard for 2 weeks (May 29), then returned to the laboratory and held separately by treatment and date until about 600 NOW DD were accumulated to determine infestation. The nuts were hand-cracked to determine infestation at that time. Analysis of variance following arcsin transformation was conducted to determine differences in infestation between treatments (including untreated) on each date. This design effectively provides 5 two-week duration treatment residue periods following each application.

Objective 3. *Low temperature effects on mating or oviposition by NOW females.* We have observed in the more northern almond production areas that spring NOW egg trap captures can occur several weeks after consistent male captures begin in pheromone traps. **Figure 1** presents such an example in 2014. The generally lower NOW population in these areas levels

relative to the central and southern production areas is likely a factor as is the greater attraction of males to the NOW pheromone traps relative to female attraction to egg traps for oviposition. To test the hypothesis that lower temperatures may also play a factor, perhaps in mating success, female and male NOW from a laboratory colony and from F1 offspring of wild NOW collected from mummies nuts were exposed to a series of constant temperatures just below the lower developmental temperature for NOW, and to a series of variable temperatures representative of those that commonly occur in April in the southern Sacramento Valley. Newly eclosed adults were collected and the sexes separated until sufficient moths that had emerged during a 48 hour period were obtained to initiate a trial. Twenty 20 pairs of virgin females and males were used in every trial of both NOW sources and each temperature regime. The adults were collected and the sexes held separately for 24 hours at 71.6°F and 12:12 day length before all individuals were transferred to growth chambers at the designated experimental temperature to acclimate for an additional 24 hours. Twenty of the females and males were then transferred to one gallon containers lined on top and sides with 68.5 sq. in. of paper towels for egg laying and provisioned with two 10% honey soaked dental wicks, and the containers transferred to growth chambers set to designated constant or variable temperatures. The moths and any eggs that had been laid were removed from each container after 72 hours with the females being transferred to two 12 oz. glass jars (10 females per jar) each lined with 21.8 sq. in. of paper towels and provisioned with one 10% honey soaked dental wick, then held for 72 hours at 71.6 $^{\circ}$ F (22.0 $^{\circ}$ C) after which time the eggs were removed. The eggs removed following both egg-laying periods were counted when first removed and held for 48 hrs. at 71.6 °F when the number of eggs tinged with the characteristic orange/red color indicating fertility was determined. Constant temperatures at which moths from the lab colony were held ranged from 48.2 to 57.2 $\mathrm{^oF}$ (9 to 14 $\mathrm{^oC}$) and number of replicates ranged from 1 to 3 at each temperature. The results of the constant temperature study with individuals from the laboratory colony informed an similar study using F1 moths from a colony started from wild larvae obtained from mummy nuts and established on standard NOW wheat bran diet (Tebbets et al. 1978) that was conducted at 50.0, 51.8 and 53.6 $\mathrm{^{\circ}F}$ (10, 11 and 12 $\mathrm{^{\circ}C}$). There were 3 replicates at each temperature with 1 replicate conducted in each of the 3 growth chambers set to a specific temperature. The identical methods were used for studies using moths from the laboratory colony and the 'wild' colony conducted at variable daily temperatures (maximum and minimum) of 60.8/41.0, 64.4/44.6 and 68.0/48.2 °F (16/5, 18/7 and 20/9 $\mathrm{^{\circ}C}$). Data for the 3 replicated studies were analyzed by analysis of variance following arcsin transformation, with individual treatments and treatment timing compared to the untreated control and means for treatment timings for each product compared to one another by Students t-test.

Results and Discussion:

It was interesting to note that male NOW were captured in the pheromone traps as soon as they were deployed in late March while the first eggs were detected in the egg traps about a month later. However, the peak of male moth capture in the pheromone trap occurred on April 30 while the peak number of eggs recorded on the egg traps was on May 7, or only a week apart. Degree-day timing of the 'May spray' would have been May 4 if based on PTB biofix and on May 12 if based on NOW egg trap biofix. Only the last 2 application dates in our study (May 5 and May 13) bracket these recommended dates.

Objective 1. *Treatment timing and efficacy of 'May sprays' as related to male NOW pheromone trap captures and eggs laid in NOW egg traps.* The first study provides an estimate of treatment success with either Altacor, Belt, Intrepid, Brigade, or Delegate by timing treatments at weekly intervals starting the week following the beginning of egg-laying and we hope that these results can be used to start to address treatment timing of "May sprays' using the new NOW Biolure pheromone lure by comparison to the traditional NOW egg trap. Results (**Table 1**) indicated that all treatment timings of all products resulted in less navel orangeworm infestation (*F*=5.0621, df=37,317, *P*<0.0001) and damage (*F*=5.3717, df=37,317, *P*<0.0001)

Table 1. Infestation and damage of almond mummies treated with different registered insecticides at weekly intervals starting at the initiation of oviposition of the overwintering flight of navel orangeworm at Ripon, 2014.

ANOVA statistics, *F*=5.0621, df=35,317, *P*<0.0001. Means followed by the same letter do not differ significantly at *P*=0.05 by Student's t-test following arcsine transformation.
² ANOVA statistics, *F*=5.3717, df=35,317, *P*<0.0001. Means followed by the same letter do not differ significantly at

P=0.05 by Student's t-test following arcsine transformation.

when compared to the untreated control. However, in general, the earlier treatment timings had more damage than the later (May 5 and May 13) treatment timings (**Figure 2**). This was notably true for the Brigade and Delegate treatments.

Figure 2. Percent damage of almond mummies treated with different registered insecticides at weekly intervals starting at the initiation of oviposition of the overwintering flight of navel orangeworm at Ripon, 2014.

Figure 3. Average percent navel orangeworm damage of nuts pre-treated weekly over a five week period and then simultaneously exposed to navel orangeworm oviposition for a two week period in in May, 2014.

Objective 2. *Residual efficacy of 'May sprays'.* The results of this experiment for resulting navel orangeworm damage are provided on **Figure 3**. Unfortunately, because of very low NOW damage at the Delta College site (mean $+$ SD = 1.1 $+$ 3.0), these results were not statistically different by analysis of variance (*F*=1.0579, df=20,162, *P*<0.4005). In 2013, our study suggested that Brigade residual activity sufficient to avoid infestation was about 2 weeks, Intrepid 4 weeks, Altacor 3 weeks, and Belt 3 weeks and there was a statistically significant difference between the insecticides and dates (*F*=2.3483, df=20,162, *P*=0.002).

Objective 3. *Low temperature effects on mating or oviposition by NOW females.* The initial study of NOW moth exposure from our lab colony to a range of relatively low constant temperatures that are similar to the minimums that might be recorded in the field during late winter revealed that there is definitely a trend with greater egg laying and fertility as temperatures increase (Figure 4). No eggs were laid by females at 48.2°F (9°C) and fewer than 0.20 eggs per female were laid at 51.8°F (11°C), and none of the eggs laid at the latter temperature were fertile. When the females held at 48.2° F were transferred to a constant temperature of 71.6°F (22.0°C) for 72 hours, the females did lay eggs but none of the eggs hatched as opposed to 100 percent hatch of eggs produced by females held at 57.2 \degree F (14 \degree C) and then at 71.6^oF for 72 hours. Table 2 presents the results of a similar experiment conducted with F1 offspring of field collected NOW larvae and conducted at 3 constant temperatures. Relatively few eggs were laid by these females at any of the low constant temperatures to which they were exposed, although there were significantly more eggs laid at the highest of these temperatures (53.6 °F). None of the eggs that were laid changed color indicating development at any of these temperatures. When these females were transferred to 71.6 $\mathrm{^oF}$, all laid a comparable number of eggs but very few developed. It is possible that successful mating was restricted at all of these temperatures. When the male and female moths from the lab colony were exposed to variable temperatures representative of daily maximum and minimum regimes that might occur in late winter, the total number of eggs laid at the highest of the three regimes evaluated (68.0/48.2 \degree F) was almost twice that of the other two temperature regimes evaluated (**Table 3**). The number of eggs that changed color indicating development was significantly greater at this temperature regime than observed for the other two. These data support the possibility of a temperature effect on successful mating, but the resolution of the study is insufficient to suggest a threshold since it was not possible to control temperatures at the precise time of mating. When male and female moths from the F1 offspring of wild collected NOW were exposed to the same variable temperatures, differences in total eggs laid and percent of eggs that changed color were less apparent between the three temperature regimes, although the pattern of greater number of eggs laid and greater percent eggs turning color was seen (**Table 4**). Overall, more eggs were laid by the 'wild' NOW females than the lab colony perhaps indicating better adaptation of that population to cooler temperatures.

Figure 4. Total eggs laid during the first 72 hours after placing 20 NOW virgin females and males from a laboratory colony in the same container at various constant temperatures (degrees C) and fertility of those eggs, and total eggs laid during the next 72 hours after the females were transferred to 22.0°C (71.6°F) and percent fertility of those eggs measured after an additional 48 hours.

¹ Means followed by the same letter do not differ significantly at $P=0.05$ by Student's t-test 2 F=17.5361, df=2, 8, P<0.0031
 3 F=0.9333, df=2, 8, P<0.4437
 4 F=0.508, df=2, 8, P<0.625

Table 3. Total eggs laid during the first 72 hours after placing 20 virgin females and males from our lab colony in the same container at three variable temperature regimes, and percent fertility of those eggs measured after an additional 48 hours at 71.6° F.

Max./Min. Temp $(^{\circ}F)$	Mean Total Eggs \pm SD ^{1,2}		Mean % Fertility \pm SD ^{1,3}	
60.8/41.0	171.3 ± 107.2	A	$15.1 + 10.2$	
64.4/44.6	173.0 ± 74.6		32.6 ± 14.5	A
68.0/48.2	$347.3 + 41.2$		$62.6 + 12.5$	В

¹ Means followed by the same letter do not differ significantly at $P=0.05$ by Student's t-test 2 $F=4.9098$, df=2, 8, $P<0.0546$
 3 $F=11.0423$, df=2, 8, $P<0.0098$

Table 4. Total eggs laid during the first 72 hours after placing 20 virgin females and males from F1 offspring of wild collected NOW larvae in the same container at three variable temperature regimes, and percent fertility of those eggs measured after an additional 48 hours at 71.6° F.

Max./Min. Temp (°F)	Mean Total Eggs \pm SD ^{1,2}				Mean % Fertility \pm SD ^{1,3}				
60.8/41.0	489.0 ± 198.5		A		$33.7 + 9.5$				
64.4/44.6	535.0 ± 125.0		А		41.1 ± 20.8	А			
68.0/48.2	764.3 ± 250.1			54.4	\pm 7.4	А			
\pm Means followed by the same letter do not differ significantly of D, \overline{DE} by \overline{C} tydentia that									

Means followed by the same letter do not differ significantly at $P=0.05$ by Student's t-test 2 F=1.6653, df=2, 8, P<0.2659
 3 F=1.719, df=2, 8, P<0.2659

Additional almond NOW research conducted this year but not reported. A study sponsored by the California Department of Food and Agriculture (CDFA) Office of Pesticide Consultation and Analysis (OPCA) was conducted in conjunction with the Coalition for Urban/Rural Environmental Stewardship (CURES) compared the effectiveness of two proposed spray application techniques to reduce drift in California orchards to the conventional standard. The study was conducted in a 60 acre commercial almond orchard near Arbuckle using Brigade at the hullsplit timing in 2014. The two methods evaluated include an Inward Only application, where the outside rows of the airblast sprayer are turned off when treating the outside two or three rows of the orchard, and a Gear Up, Throttle Down (GUTD) application, where the tractor engine speed and power take-off (PTO) output are reduced and the ground speed increased. As a result, larger nozzle sizes are used to keep the application rates the same (larger droplet sizes, reduced drift). Drift, deposition and efficacy of each of the three application methods were measured. For drift and deposition, passive samplers (Kimbie sheets) were used and residues measures. For efficacy, NOW egg strips were place at 8 and 16 feet in the tree canopy just prior to the treatment and the eggs returned to the laboratory where larvae emerging were reared on NOW diet. In addition, almonds were removed at 1 day and 14 days following the application from both the 8 and 16 foot heights and the inner and outer tree canopy, and egg strips pinned to the card to determine larval survival. Both methods demonstrated lower drift as compared to the conventional treatment. For all methods 92-94% of the drift was found in the first 50 feet downwind of the orchard. From the outside row, the conventional treatment showed the most drift (15.6 percent of the total bifenthrin residues measured) while the GUTD and Inward Only treatments showed drift of 7.6 percent and 9.7 percent of the total residue, respectively. In examining the efficacy of the treatments, the

GUTD treatments were comparable to the conventional treatments at 0, 1 and 14 days after treatment. For the Inward Only treatment, the control of NOW was poor (21.0 percent survival) immediately following application and was statistically different than the other two methods. Efficacy was best at the 8 foot relative to the 16 foot height in all treatments. A detailed report on this study was prepared by Jim Markle of CURES and submitted to CDFA-OPCA in March 2015. An additional study of the efficacy of Proclaim, Volium Express and two unregistered premixes for NOW control was conducted at the "May spray' timing with all products providing significant (*P*<0.05) control relative to the untreated control.

Research Effort Recent Publications:

- Zalom, F.G., and N.L. Nicola. 2014. Controlling the first generation of navel orangeworm in almonds. Acta Horticulturae. 1028:185-200.
- Hamby, K.A., N.L. Nicola, F.J.A. Niederholzer, and F.G. Zalom. 2015. Timing spring insecticide applications to target both *Amyelois transitella* and *Anarsia lineatella* in almond orchards. J. Econ. Entomol. 108: 683-693. doi: 10.1093/jee/tov021

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- Engle, C.E. and M.M. Barnes. 1983. Developmental threshold temperature and heat unit accumulation required for egg hatch of navel orangeworm (Lepidoptera: Pyralidae. Environ. Entomol. 12: 1215-1217.
- Rice, R.E., F.G. Zalom and J.F. Brunner. 1982. Using degree-days in a peach twig borer monitoring program. Almond Facts 47(2): 60-62.
- Tebbets, J.S., C.E. Curtis, and R.D. Fries.1978. *Mortality of immature stages of the navel orangeworm stored at 3.5°C*. J. Econ. Entomol. 71: 875–876.
- Zalom, F.G., J.H. Connell and W.J. Bentley. 1998. Validation of phenology models for predicting development of the navel orangeworm *Ameylois transitella* (Walker) in California almond orchards. Acta Horticulturae 470: 525-533.