Insect and Mite Research

Project No.: **15-ENT07-Zalom**

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Objectives:

1. Determine treatment timing of bifenthrin, methoxyfenozide, spinetoram, chlornitraniliprone, and flubendiamide for 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.
4. Confirm that mummy nuts that were previously infested in fall are more likely to become reinfested in spring.

Interpretive Summary:

This report presents results of studies by my lab conducted. In 2015 and the spring of 2016 that in one sense or another focused on flight and oviposition of the navel orangeworm (NOW) during spring in the more northernly almond production areas. Although we have recently conducted other studies evaluating efficacy and spring treatment timing for NOW (e.g., Zalom and Nicola 2014; Hamby et al. 2015), our monitoring and treatment timing was based on NOW egg traps (Rice et al. 1976) and a degree-day phenology model to indicate the insect’s activity as has been the case since the original studies using this approach were conducted in the 1980s (Engle and Barnes 1983; Sanderson et al. 1989). Sex pheromone traps developed as a monitoring tool for male NOW became commercially available in 2013. Initial field observations clearly showed that male moth captures in pheromone traps was consistently greater than the number of eggs in corresponding egg traps. Before the pheromone lures were commercially available, Burks et al. (2011) reported that male moth captures in pheromone traps was correlated to proportion of egg traps with eggs present, and that proportion of egg traps with eggs present also correlated to number of eggs per trap. Observations of the relationship of female egg trap captures to male pheromone trap captures first began in 2013 in areas other than the central and southern San Joaquin Valley where the initial studies were conducted. As noted in our proposal, we noted that oviposition as recorded in egg traps began considerably

later than male moth flight was seen in pheromone trap captures at our Ripon, Manteca and Arbuckle sites, and speculated that this could be due to the relatively low numbers recorded in egg traps as opposed to pheromone traps, or to lower temperatures in spring in the more northernly almond production areas than in the warmer areas to the south.

In both spring 2015 and 2016, we deployed pheromone traps, egg traps and for the first time sentinel mummy strands (that measures actual mummy nut infestation) before the anticipated start of egg-laying for comparison. Initial male NOW moth captures and the start of NOW egg-laying as indicated by egg traps differed by several weeks to a month in more northernly growing areas in both 2013 and 2014 whereas this difference was seldom more than a week in the central and southern San Joaquin Valley. The use of almond strands in 2015 and 2016 to indicate successful NOW oviposition and development showed surprising results. In both years, males were captured in pheromone traps when the traps were deployed in mid-March and continued at fairly constant levels for the 6 weeks through early May. Infested mummy strands varied considerably on a weekly basis through this period, with a period of low infestation of strands occurring on both years in late March and early April in 2015 and in early April in 2016. Average daily minimum temperature was 38.6ºF during the period from April 3-9, 2015 and 40.7ºF during the period from March 29-April 3, 2016, the coolest week of each spring during our study, suggesting that while low egg trap captures probably play a role in the observed delay in captures relative to male moths in pheromone traps, periods of low temperature are significant and deter successful infestation of nuts during spring even when male moths are present and captured in pheromone traps.

An associated study with implications for NOW control included estimating the efficacy of four registered insecticides applied during the spring, and is intended to establish spring treatment timing guidelines for these commonly used products using pheromone trap counts in addition to egg traps which has been the established practice recommended to this time. Results indicated that all treatment timings of all products resulted in reduced NOW infestation when compared to the untreated control in 2015. For all but Brigade, the earlier NOW treatment timing had lower infestation than the later (April 21) timing. Results for 2016 are pending.

A laboratory study of lower temperature effects on NOW mating and oviposition was initiated in 2015 with Individuals from both a laboratory colony and wild collected from infested nuts, and compared mating and oviposition at constant temperatures as low as 48.2ºF and variable daily temperatures as low as 57.2ºF (day) and 37.4ºF (night). The total number of eggs and the number of fertile eggs laid were fewer at the lowest temperatures, but at least some viable eggs were produced at each of the experimental temperatures.

Hamby and Zalom (2013) showed that there is a positive relationship between NOW infestation level and any damage, even mechanical damage, to the surface of the kernel. That NOW preferentially infest almonds that have previously been attacked by another insect has long been suggested, but there is little data to support that this is the case. To test this hypothesis, we hung strands of previously field-infested almonds with the larvae killed and uninfested almonds collected from the same orchard in another almond orchard after egg traps detected first eggs were laid in spring. Number of nuts infested with live larvae were significantly greater in previously infested nuts than in previously uninfested nuts in each of 4 repetitions of this study confirming the hypothesis.

Materials and Methods:

Objective 1. *Determine treatment timing of bifenthrin, methoxyfenozide, spinetoram, chlornitraniliprone, and flubendiamide for 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.* The ‘May spray’ timing offers the potential to obtain some level of control of both NOW and PTB as these insects have flights that overlap somewhat in many years. This spring spray controls the first generation of NOW following spring moth emergence, and can also control PTB when flights overlap as is the case in most years and when a product that controls both species is used. 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 NOW 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 females are captured in pheromone traps (Rice et al. 1982).

Two orchards in San Joaquin County were monitored during the entire 2015 season, a mature 20-acre almond orchard on near Ripon and a block at Delta College near Manteca. Neither block was dormant-treated and no spring insecticide sprays were applied in the previous year. Peach twig borer male flights were monitored using 4 wing type traps baited with PTB Biolures from Suterra LLC. and NOW activity was monitored with 3 wing type traps baited with NOW Biolures also from Suterra (for male 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 orchards on March 10 and checked twice weekly until April 14 after which the traps were checked weekly. Using our ‘mummy strand’ protocol, twenty uninfested Nonpareil nuts saved from the 2014 harvest were hot glued to strands of vegetable mesh mid-March, 2015, and these served as surrogate mummies for field studies. In 2015, 400 strands were hung in the Ripon orchard at mid-canopy in Nonpareil trees on March 27 which was also the first of the 6 weekly treatment dates (noted on **Figure 1**).

The start of NOW egg trap captures in both orchards on March 23 (**Figures 1 and 2**) was earlier than I had ever seen in my 35 years of prior NOW monitoring. This was followed by a period of no or low egg trap capture before the numbers began to increase again on April 10. Being unsure of whether the earlier or later increase corresponded to the biofix date based on egg trap captures, cumulative degree-days (DD) were calculated from both dates and these are presented on both figures along with DD from January 1 and March 10 (when the NOW pheromone traps were deployed. Male moth captures were already well underway at that time.

Eight strands each were treated with either Altacor, Belt, Intrepid, Brigade, or Delegate on each of these dates. Sixteen strands remained untreated as controls to establish the damage level in the absence of treatment. The number of strands deployed totaled 336 (6720 mummies) representing 5 products X 8 reps X 6 weeks, plus the 16 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 of water, and included the nonionic surfactant, Dyne-amic, at 0.25% v/v. The strands were removed from the trees once eggs were no longer laid on the egg traps, and returned to the lab 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 ANOV following arcsine 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.

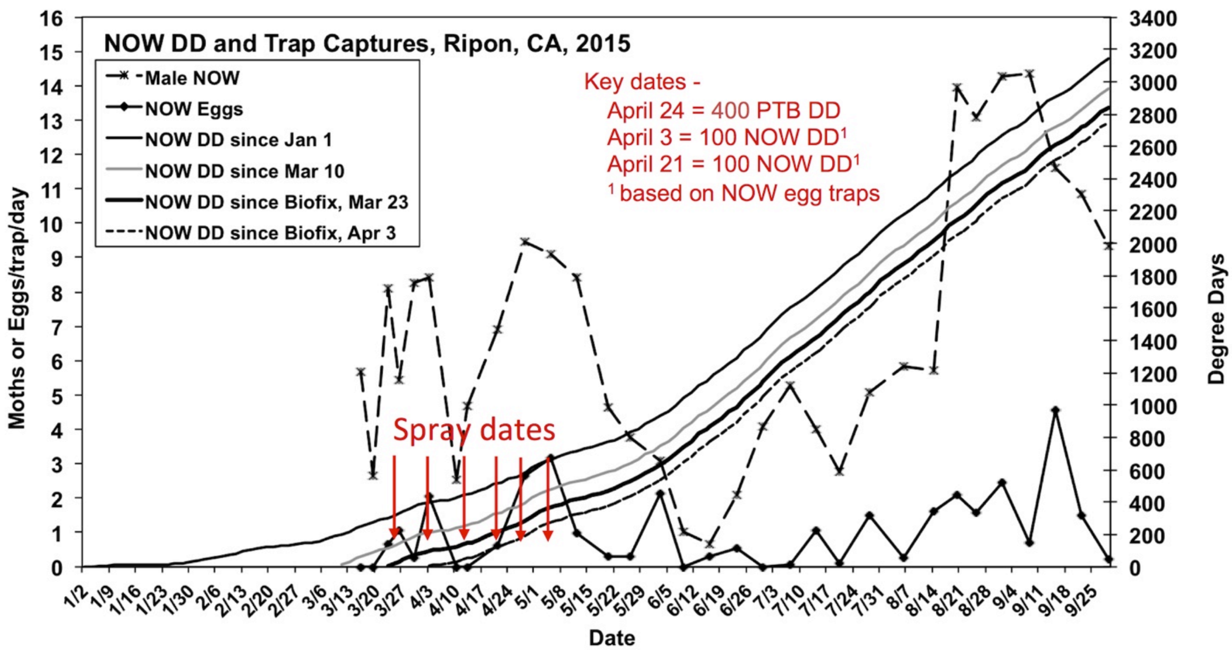

Figure 1. Navel orangeworm pheromone and egg trap captures in the Ripon study orchards during 2015 indicating application dates (arrows) and NOW degree-days from January 1, March 10 (date pheromone traps were hung), first egg trap biofix (March 23) and second egg trap biofix (April 3).

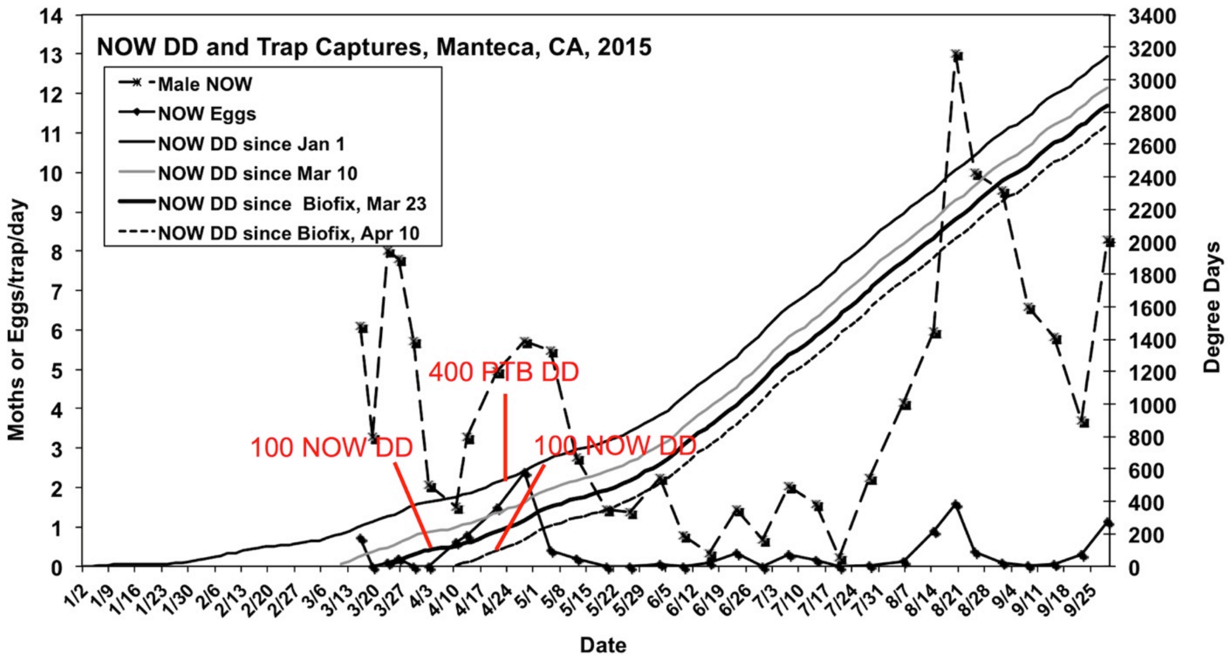

Figure 2. Navel orangeworm pheromone and egg trap captures in the Manteca study orchard during 2015 indicating NOW degree-days from January 1, March 10 (date pheromone traps were hung), first egg trap biofix (March 23) and second egg trap biofix (April 10).

Objective 2. *Evaluate residual efficacy of bifenthrin, methoxyfenozide, chlornitraniliprone, and flubendiamide.* The second experiment was conducted using the same almond strand approach, but was intended to provide a better estimate of residual activity as well. We had completed a successful study in 2013, and were not successful in repeating the study in 2014 because of a lack of NOW infestation at the study site. The site of the 2015 study was a commercial almond orchard located near Ripon in San Joaquin Co. The block had not been dormant treated by the grower, and no insecticide sprays were applied at the spring timing in previous years. A total of 176 strands of almond mummies were prepared for this experiment. Forty strands were designated for each of 4 insecticides, and 16 strands for the untreated control. Each week starting March 24, 8 of the 40 strands designated for each chemical treatment was 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 were treated 0n April 21, all of the strands were transferred to the almond orchard, along with the 16 untreated strands, and hung in the trees. This design effectively provides 5 two-week duration treatment residue periods following each application. Unfortunately, the orchard was unexpectedly sprayed with Altacor on April 30. The strands were retrieved on May 5 and the almonds hand-cracked to determine infestation levels.

Objective 3. *Determine if low temperatures delay 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. 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, but lower late winter/early spring temperatures also occur in this area and may impact NOW mating and oviposition as well.

A field study and laboratory studies were conducted in 2015 and are continuing in 2016 to determine how male NOW moth captures in pheromone traps relate to NOW egg trap captures in a site with modest NOW populations present, and test the hypothesis that lower temperatures may affect mating or oviposition. In the field study conducted S. Clinton Ave. in San Joaquin County, 4 Suterra NOW pheromone traps and ten NOW egg traps were deployed on March 10, 2015 and March 15, 2016, and checked twice weekly. Ten sentinel mummy strands were deployed weekly for 6 weeks starting March 10, 2015, and for 6 weeks starting March 15, 2016 with a 2-week gap between the April 11 and April 26 deployment dates. The mummy strands were exchanged after each week for new strands and returned to the lab where they were examined for eggs and held for 6 weeks before hand-cracking to determine presence of larvae. The egg traps will also be checked weekly, and any eggs present will be recorded and removed. Temperatures were recorded daily within the tree canopy using two data loggers, and averaged.

Female and male NOW from a laboratory colony were exposed to a series of constant temperatures ranging from 48.2ºF (9ºC) to 57.2ºF (14ºC) in growth chambers, and to a series of variable daily temperatures (maximum and minimum) representative of those that commonly occur in April in the southern Sacramento Valley ranging from high/low temperatures of 57.2ºF/ 37.4ºF (14ºC/ 3ºC) to 68.0ºF/ 48.2ºF (20ºC/ 9ºC). 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 71.6ºF (20ºC) and 12:12 (light:dark) 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 the 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. The females were then 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 a constant temperature of 71.6ºF (22.0ºC) after which time the eggs were removed. The eggs removed following both egg-laying periods were counted when first removed and held for 48 hours at 71.6ºF when the number of eggs tinged with the characteristic orange/red color indicating fertility was determined. Three trials were conducted at the 48.2, 50.0, 51.8 and 53.6ºF (9, 10, 11 and 12ºC) constant temperatures and one trial at the 55.4 and 57.2ºF (13 and 14ºC) constant temperatures. Four trials were conducted at each variable temperature with each trial being considered a replicate. Data for constant temperatures and variable temperatures were subjected to ANOV with mean total eggs and percent fertility recorded for each treatment further analyzed by Student's t-test.

Similar studies used F1 offspring of wild NOW collected from mummy nuts and established on standard NOW wheat bran diet (Tebbets et al. 1978) were conducted at constant temperatures of 50.0, 51.8 and 53.6ºF (10, 11 and 12ºC) and variable temperatures of 60.8/41.0, 64.4/44.6 and 68.0/48.2ºF (16/5, 18/7 and 20/9ºC), with results reported in our 2014-15 annual report. Studies of additional temperatures corresponding to those for the laboratory colony are underway.

Objective 4. *Confirm that mummy nuts that were previously infested in fall are more likely to become reinfested in spring.* We conducted a study in 2013 and 2014 to test the hypothesis that almonds that have previously been attacked by another insect are preferentially infested by NOW. We attempted to obtain a third year of data in 2015, but there was an insufficient NOW populations present at the study site and insufficient data were obtained. However, the experiment was successfully repeated in 2016. We field-collected almonds from a highly infested orchard following harvest in fall 2015, separated infested from uninfested nuts leaving hulls intact, and placed them into a cold room at -20C for 6 weeks to kill the larvae. Twenty nut strands were made with uninfested or infested nuts from this collection, and 14 previously infested strands and 14 uninfested strands were hung on May 3 in both an almond orchard at Delta College near Manteca in San Joaquin County and in an untreated commercial orchard on S. Clinton Ave. in San Joaquin Co. There were a total of 560 almond mummies hung at each site. The strands remained in the orchards for 4 weeks, then were collected and returned to our laboratory where they were held for approximately 600 degree-days to allow eggs to hatch and larvae to mature for ease of identification. NOW infestation results for each orchard were compared by ANOV.

Results and Discussion:

Objective 1. *Determine treatment timing of bifenthrin, methoxyfenozide, spinetoram, chlornitraniliprone, and flubendiamide for 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.* As noted previously, the start of NOW egg trap captures in the area was earlier than I had ever seen in my 35 years of prior NOW monitoring. The first increase in egg trap captures was followed by a period of no or low egg trap captures before the numbers began to increase again after April 10 (**Figure 2**). **Figure 3** presents the percent NOW damage corresponding to treatments of the 5 products at each of the 6 dates, and its relation to the recommended 100 DD treatment timing based on the earlier (left arrow of each pair) or later biofix (right arrow of each pair) date. For all but the Brigade treatments, application based on the earlier biofix resulted in less damage than the later biofix. This is useful to consider should a similar situation occur in future years.

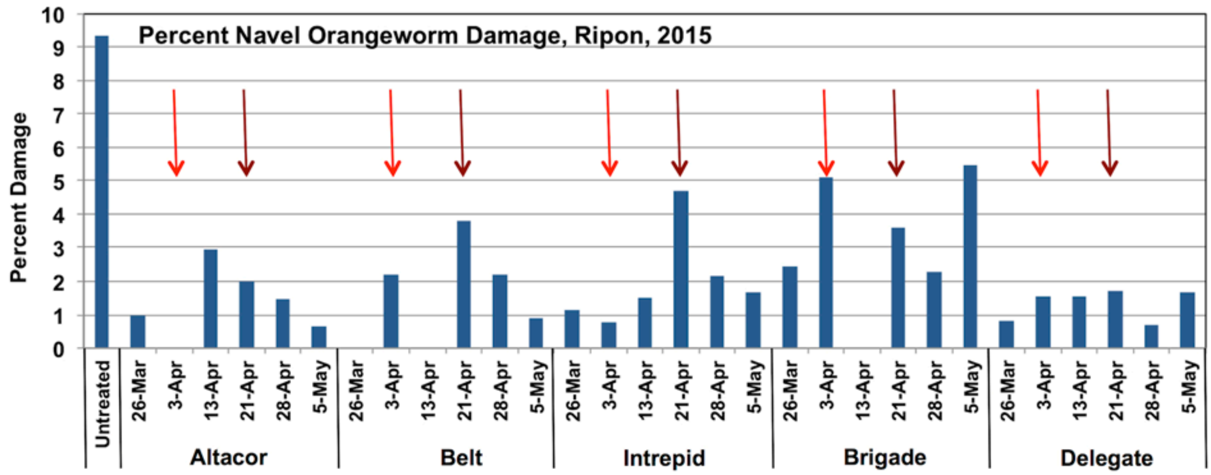

Figure 3. Percent damage of almond mummies treated with different registered insecticides at weekly intervals starting at the initiation of oviposition of the overwintering flight of navel orangeworm at Ripon, 2015. Arrows represent the recommended100 degree-day treatment timing based on a March 23 (left arrow of each pair) and April 10 (right arrow of each pair) biofix date.

Analysis of these results (**Table 1**) indicated that all treatment timings of all products resulted in significantly reduced NOW damage (Includes nuts with larva/pupa and nuts with some frass or webbing but no larva; F=2.6559, df=30,265 P<0.0001) when compared to the untreated control. Unfortunately, the entire orchard was unexpectedly sprayed with Altacor two days after the April 28 treatment date, complicating data interpretation and probably reduced the overall infestation levels. Amount of damage observed was probably greater in relation to infestation (only 2.01 $\pm$ 3.39 percent) because of the April 30 Altacor spray which would have had less effect on larvae already present in nuts that had been infested prior to the application, and the resulting test of significance for infestation showed only a weak effect of product and timing F=1.4936, df=30,265 P<0.0541.

Table 1. Infestation and damage of almond mummies treated with different registered insecticides at weekly intervals starting March 26 at /Ripon, 2015.

Treatment	Spray date	Rate/ac	Mean + SD[1,2] % infestation				Mean + SD[1,3] % damage			
Control	n/a	n/a	2.01	±	3.39	A	9.35	±	10.72	A
Altacor	3/26	4 oz.	0.00	±	0.00	B	0.96	±	2.72	BCD
Altacor	4/3	4 oz.	0.00	±	0.00	B	0.00	±	0.00	D
Altacor	4/13	4 oz.	1.48	±	2.74	AB	2.95	±	5.48	BCD
Altacor	4/21	4 oz.	0.63	±	1.77	AB	2.02	±	2.80	BCD
Altacor	4/28	4 oz.	0.00	±	0.00	B	1.48	±	2.74	BCD
Altacor	5/5	4 oz.	0.00	±	0.00	B	0.66	±	1.86	CD
Belt	3/26	4 oz.	0.00	±	0.00	B	0.00	±	0.00	D
Belt	4/3	4 oz.	0.00	±	0.00	B	2.22	±	4.54	BCD
Belt	4/13	4 oz.	0.00	±	0.00	B	0.00	±	0.00	D
Belt	4/21	4 oz.	0.83	±	2.36	AB	3.81	±	3.26	BCD
Belt	4/28	4 oz.	0.00	±	0.00	B	2.22	±	3.08	BCD
Belt	5/5	4 oz.	0.00	±	0.00	B	0.89	±	2.53	BCD
Intrepid	3/26	16 oz.	0.00	±	0.00	B	1.14	±	3.21	BCD
Intrepid	4/3	16 oz.	0.00	±	0.00	B	0.78	±	2.21	BCD
Intrepid	4/13	16 oz.	0.00	±	0.00	B	1.52	±	2.81	BCD
Intrepid	4/21	16 oz.	0.00	±	0.00	B	4.71	±	5.27	BCD
Intrepid	4/28	16 oz.	0.00	±	0.00	B	2.17	±	4.34	BCD
Intrepid	5/5	16 oz.	0.83	±	2.36	AB	1.67	±	4.71	BCD
Brigade	3/26	16 oz.	1.67	±	3.11	AB	2.46	±	3.40	BCD
Brigade	4/3	16 oz.	1.25	±	3.54	AB	5.10	±	5.25	BC
Brigade	4/13	16 oz.	0.00	±	0.00	B	0.00	±	0.00	D
Brigade	4/21	16 oz.	1.32	±	2.44	AB	3.59	±	5.04	BCD
Brigade	4/28	16 oz.	1.59	±	2.97	AB	2.28	±	3.19	BCD
Brigade	5/5	16 oz.	2.30	±	4.48	A	5.46	±	5.27	B
Delegate	3/26	17 oz.	0.00	±	0.00	B	0.83	±	2.36	BCD
Delegate	4/3	17 oz.	0.71	±	1.89	AB	1.55	±	2.67	BCD
Delegate	4/13	17 oz.	0.00	±	0.00	B	1.56	±	2.89	BCD
Delegate	4/21	17 oz.	0.00	±	0.00	B	1.73	±	3.20	BCD
Delegate	4/28	17 oz.	0.00	±	0.00	B	0.69	±	1.96	CD
Delegate	5/5	17 oz.	0.00	±	0.00	B	1.67	±	4.71	BCD

Objective 2. *Evaluate residual efficacy of bifenthrin, methoxyfenozide, chlornitraniliprone, and flubendiamide.* As mentioned in the methods section, the seven-week setup period for this study was completed and all of the 64 strands (1280 nuts) transferred to the study orchard where they were hung for two weeks. However, the orchard was treated with Altacor while the previously treated strands were in the orchard compromising the study. Although the strands were retrieved on May 5 and the almonds hand-cracked to determine infestation levels, data

obtained were insufficient to make any conclusions. The study is being repeated in 2016, and is currently in progress.

Objective 3. *Determine if low temperatures delay mating or oviposition by NOW females.* **Figure 4** presents pheromone and egg trap captures from 2015 together with average number of infested mummy nuts from 10 strands hung during 6 consecutive periods between March 10 and April 21 with associated daily minimum temperatures and average low temperature for each period. Although the pheromone traps at our Ripon site indicated male moth flight was already occurring during the week of March 10 when the traps were first deployed and on each week thereafter averaging 2.5-8.4 moths per trap per night during this period, no eggs were recorded and no nut infestation was observed during the period from April 3-9 when the average daily minimum temperature was 38.6°F. Percent nut infestation recorded using the almond strands was considerably greater than eggs trapped on the egg traps, likely because there were, in effect, 200 volatile sources (10 strands x 20 nuts per strand) in comparison to 10 egg traps. The pattern of egg trap captures appeared to follow that of percent infestation of the strands, but the strands were clearly a more sensitive way of measuring female reproductive success during each period.

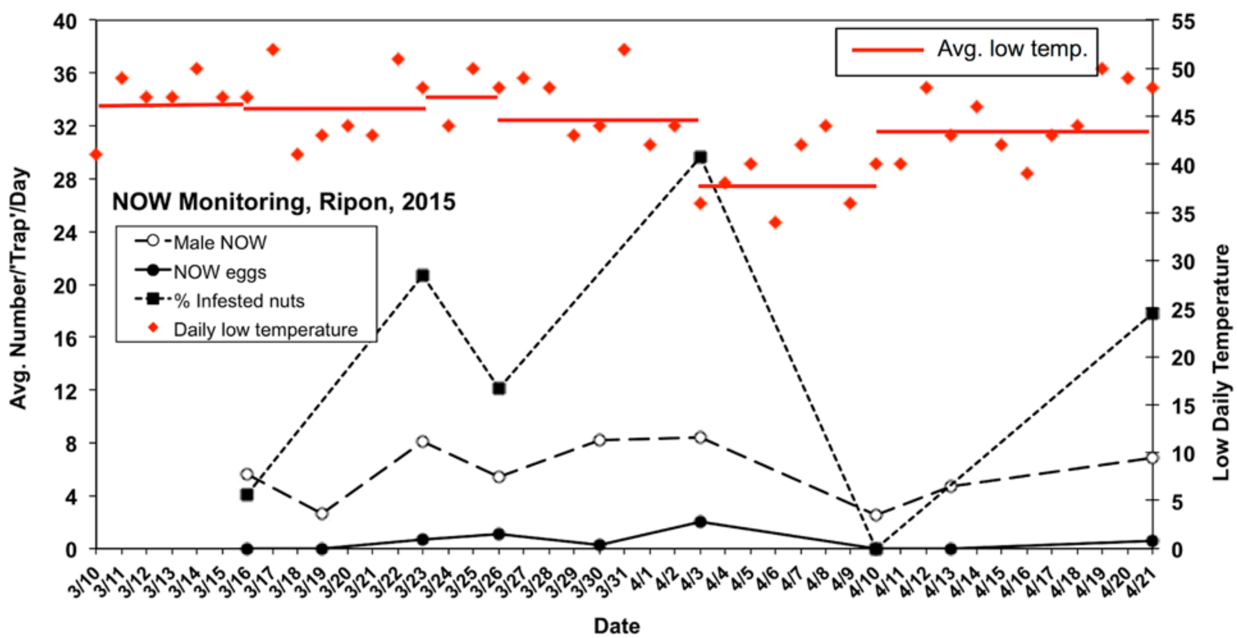

Figure 4. Navel orangeworm pheromone, egg trap and almond mummy infestation during successive spring periods at Ripon, 2015, indicating daily minimum temperatures and average low temperatures for each period of mummy deployment.

The study was repeated in 2016 at the same site near Ripon (**Figure 5**). As in 2015, the pheromone traps at our Ripon site indicated male moth flight was already occurring during the week of March 15 when the traps were first deployed and on each week thereafter averaging 1.7-7.7 moths per trap per night during this period, no eggs were recorded and no nut infestation was observed during the initial period when the average daily minimum temperature was 41.7°F and was greatly reduced again during the period from March 29 through April 10. The average daily minimum temperature was 40.7°F for the period March 29 through April 2.

Percent nut infestation recorded using the almond strands was again considerably greater than eggs trapped on the egg traps, and in this year the egg trap biofix did not occur until May 3. Using this biofix date would have missed the successful nut infestation that occurred during the week of March 22-28. The actual peak egg trap capture was recorded on the May 9 sample date and averaged 1.85 eggs per trap per night.

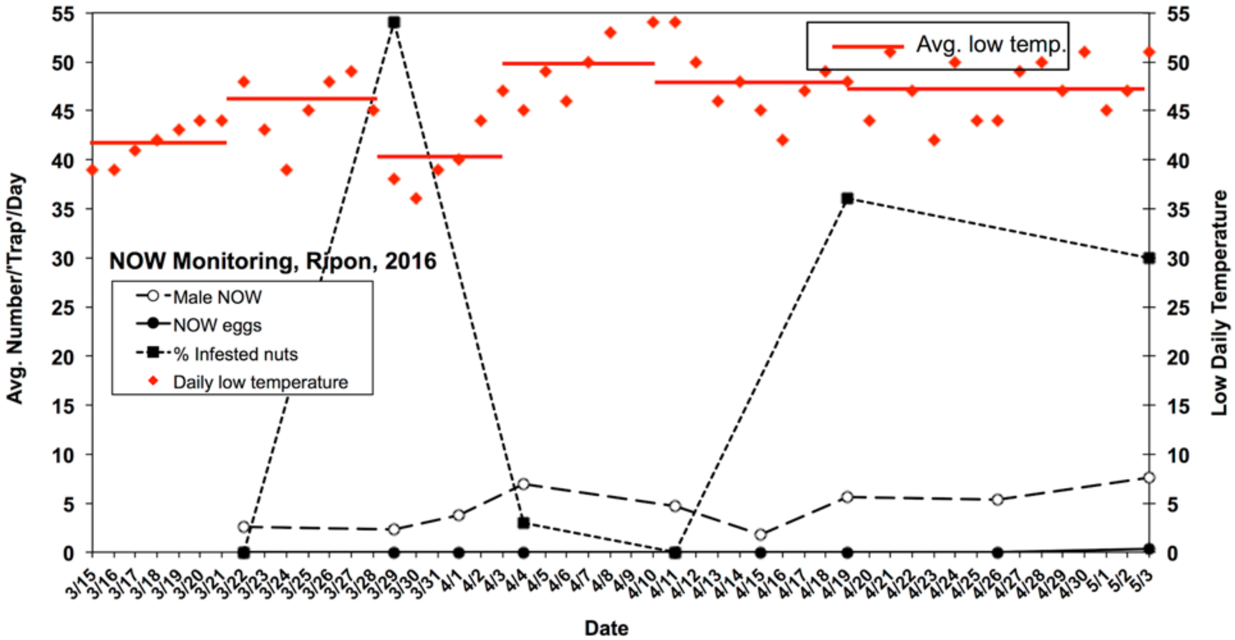

Figure 5. Navel orangeworm pheromone, egg trap and almond mummy infestation during successive spring periods at Ripon, 2016, indicating daily minimum temperatures and average low temperatures for each period of mummy deployment.

The results of these field studies suggest that it is difficult to set a spring biofix date for degree-day accumulations in orchards where NOW populations are very low using up to NOW eggs traps and also using pheromone traps because of the seemingly consistent trap captures even well before nut successful mummy nut infestation occurs. The almond strands themselves are a good indicator of infestation, but they are a post-hoc evaluation method. However, these results also suggest that hanging many more sources of mummy nut volatiles like egg traps, while impractical, might improve sensitivity. Cooler periods where low temperatures average below about 41ºF appeared to eliminate nut infestation. An exact mechanism for this observation, whether lack of mating, lack of oviposition, or lack of female flight (males were still captured in pheromone traps) cannot be determined from these data.

Virgin female and male NOW from our laboratory colony were exposed to a series of constant temperatures ranging from 48.2ºF (9ºC) to 57.2ºF (14ºC) in growth chambers, and to a series of variable daily temperatures (maximum and minimum) representative of those that commonly occur in April in the northern San Joaquin Valley and the southern Sacramento Valley ranging from high/low temperatures of 57.2ºF/37.4ºF (14ºC/3ºC) to 68.0ºF/48.2ºF (20ºC/9ºC). 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 6**). Only 0.7 eggs were laid per female on average at 48.2ºF (9ºC), increasing to as high as 190.8 eggs per female at 55.4ºF (13ºC). None of the eggs laid at either 48.2ºF or 50.0ºF (9ºC and 10ºC, respectively) were fertile, but egg fertility increased to over 85 percent at study temperatures of 55.4ºF (13ºC) or higher. When the females were removed from the constant temperatures and transferred to a constant temperature of 71.6ºF (22.0ºC) for 72 hours, the females held at all of the constant temperatures did lay eggs but the fertility of those eggs ranged considerably from only 2.7 percent at 48.2ºF (9ºC) to 97 percent at 57.2ºF (14ºC). The likely explanation for this is that mating did not occur at the lower temperatures, but those females still laid infertile eggs. These results have important implications for NOW reproductive success. **Figure 7** presents the number of fertile eggs laid by females during the 72-hour mating period when temperatures were held constant (left graph) and the 48 hours following the mating period when temperatures were raised to a constant 71.6ºF (20ºC) (right graph). Reproductive success was calculated as mean total eggs laid during each period divided by the proportion of those eggs that were fertile. The number of fertile eggs increased substantially from practically none at 48.2ºF (9ºC) to almost 80 and 800 at 55.4ºF (13ºC) or higher for the period of constant low temperature and post mating period, respectively.

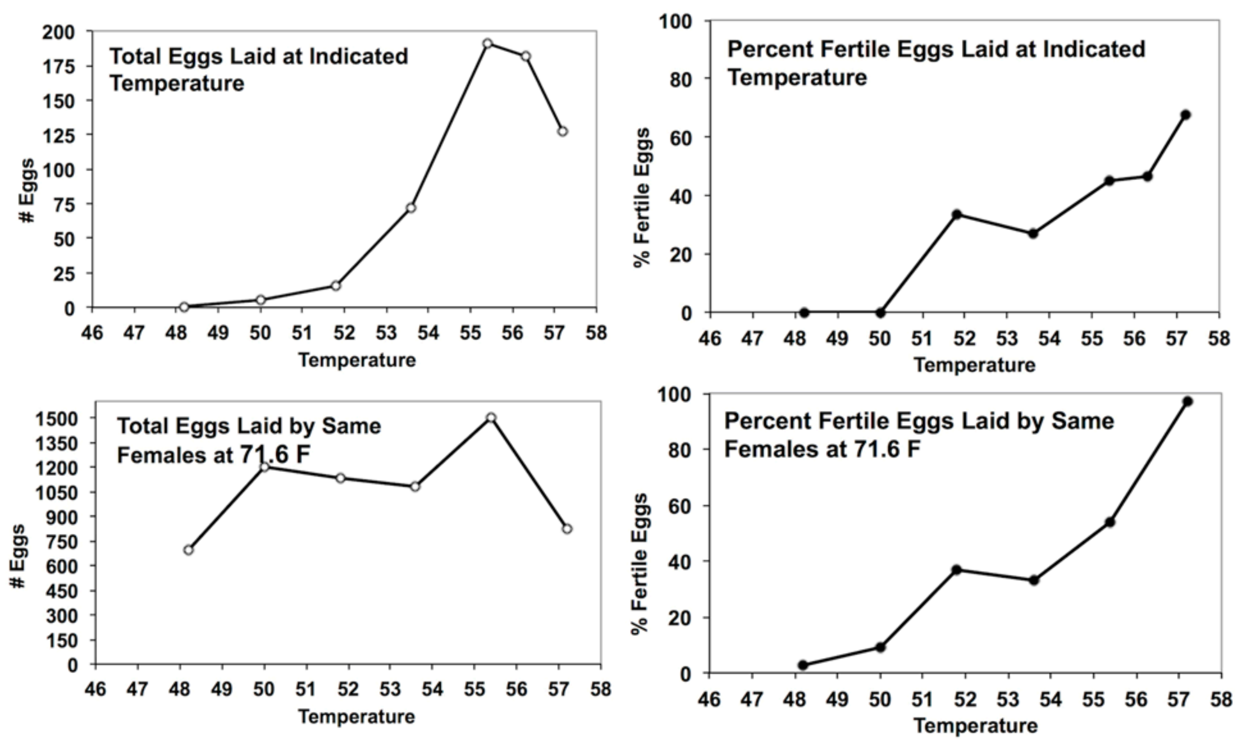

Figure 6. Total eggs laid during the first 72 hours after placing 20 NOW virgin females and males from a laboratory colony in the same container at various constant temperatures and fertility of those eggs, and total eggs laid for the 48 hours after the mating period when the females were transferred to 71.6ºF (22.0ºC) constant temperature. Percent fertility of those eggs was measured after an additional 48 hours.

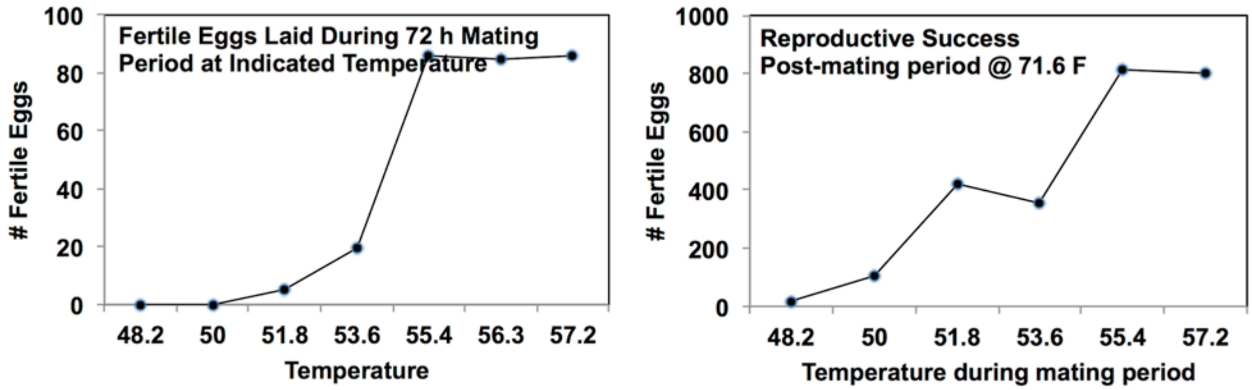

Figure 7. Number of fertile eggs laid to each temperature during the 72-hour constant temperature period and during the 48-hour post mating period when temperature was held at 71.6ºF (20ºC).

Table 2 presents the total eggs laid during the first 72 hours after placing 20 virgin females and males from our lab colony in the same container at four variable temperature regimes ranging from high/low temperatures of 57.2ºF/37.4ºF (14ºC/3ºC) to 68.0ºF/48.2ºF (20ºC/9ºC) and a 12:12 (day:night) photoperiod (to approximate a late winter and early spring light regime). The percent fertility of those eggs were measured after an additional 48 hours following the mating period when the eggs were transferred to 71.6ºF (22.0ºC) constant temperature. Eggs were laid at all of the variable temperature regimes, but the number eggs laid almost double at each succeeding regime, with total eggs laid ranging from 86.8 to 593.3, and there was a significant difference ($P<0.05$) in number of eggs laid between these values. Mean percent fertility similarly increased. Reproductive success calculated as mean total eggs laid during the 72 hours at each variable temperature regime divided by the proportion of those eggs that were fertile indicated that the number of fertile eggs increased significantly from the lowest variable temperature regime to the highest (F=4.825, df=3,11, $P<0.0334$), ranging from 10.3 to 262.8. Variable temperatures in our growth chamber are only a crude approximation of actual conditions since they do not precisely reflect actual temperatures during diurnal periods when mating and oviposition maybe occurring. However, these results are consistent with our observations of reduced nut infestation of our mummy strands in the field during colder spring periods.

Table 2. Total eggs laid during the first 72 hours after placing 20 virgin females and males from our lab colony in the same container at four variable temperature regimes, percent fertility of those eggs measured after an additional 48 hours following the mating period when they transferred to 71.6ºF (22.0ºC) constant temperature, and reproductive success.

Max./Min.Temp (ºF)	Mean Total Eggs ± SD[1,2]		Mean % Fertility ± SD[1,3]		Reproductive Success
57.2/37.4	86.8 ± 44.6	B	11.9 ± 8.9	B	10.3
60.8/41.0	171.3 ± 107.2	B	16.4 ± 6.4	B	29.2
64.4/44.6	173.0 ± 74.6	B	24.8 ± 8.5	AB	73.7
68.0/48.2	347.3 ± 41.2	A	44.3 ± 17.9	A	262.6

[1] Means followed by the same letter do not differ significantly at P=0.05 by Student's t-test
[2] F=10.0372, df=3,11, $P<0.0044$
[3] F=4.8250, df=3,11, $P<0.0334$

Studies using F1 offspring of wild NOW collected from mummy nuts to expand the number of constant and variable temperature regimes previously reported in our 2014-15 annual report are underway.

Objective 4. *Confirm that mummy nuts that were previously infested in fall are more likely to become reinfested in spring*. Although it has long been speculated that previously insect-infested almonds are preferentially infested by NOW, experimental evidence has been lacking. We initiated a three-year study in 2013 to test this, successfully obtaining results in 2013 and 2014. After insufficient NOW populations at the 2015 study site, we were successful obtaining data at 2 sites in 2016. Results from 2013 and 2014 (not included in prior annual reports) and 2016 are presented in **Table 3.** In both study orchards and in each year, highly significant differences in NOW infestation were found, with percent active NOW infestation of previously infested exceeding the infestation levels of previously uninfested nuts between 22.3 percent and 45.8 percent. These results are important as they indicate that mummies remaining in orchards with high levels of NOW damage the previous season are more highly susceptible to infestation the following spring, a factor that is likely important to winter mummy removal. These results may also indicate presence of a cue for how females select mummy nuts in which to oviposit.

Table 3. Percent larval infestation of previously infested and previously uninfested Nonpareil mummy nuts collected the previous fall and held at -20C for 6 weeks prior to using for strands that were then hung in an untreated orchard near Ripon or at Delta College (San Joaquin Co.) in spring 2013, 2014 and 2016.

Site	Treatment	Year	n=	Dates of NOW exposure	Mean ± SD percent infestation
Ripon	Uninfested	2013	20	April 16-June 5	14.4 ± 12.4
Ripon	Preinfested	2013	9	April 16-June 5	36.7 ± 15.5[1]
Delta College	Uninfested	2014	14	April 4-May 28	9.9 ± 16.1
Delta College	Preinfested	2014	14	April 4-May 28	54.6 ± 12.6[2]
Ripon	Uninfested	2016	14	May 3-May 31	19.6 ± 12.9
Ripon	Preinfested	2016	14	May 3-May 31	48.5 ± 15.0[3]
Delta College	Uninfested	2016	14	May 3-May 31	9.1 ± 12.7
Delta College	Preinfested	2016	14	May 3-May 31	54.9 ± 12.1[4]

1 ANOV statistics, F=17.2634, df=1,28, P=<0.0003
2 ANOV statistics, F=60.2221, df=1,27, P=<0.0001
3 ANOV statistics, F=29.8127, df=1,27, P=<0.0001
4 ANOV statistics, F=95.1113, df=1,27, P=<0.0001

Research Effort Recent Publications:

Hamby, K.A., J.D. Henderson, H.B. Scher, and F.G. Zalom. 2015. Organophosphate insecticide activity reduced when mixed with copper(II) hydroxide in peach dormant sprays. J. Entomological Sci. 50(4): 284-294.

Markle, J., F. Niederholzer, and F. Zalom. 2016. Evaluation of spray application methods for navel orangeworm control in almonds. Pest Manage. Sci. http://dx.DO.orgI/10.1002/ps.4279

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