Statewide Monitoring Study to Determine Relationship between Navel Orangeworm (NOW) Egg and Male Moth Capture

Project No.: **15-ENTO13-Tollerup**

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Objectives:

Continue work begun in 2014

1. Evaluate NOW population dynamics over the almond-production region of California from the mid San Joaquin Valley (Fresno County) to the Sacramento Valley region (Glenn / Tehama counties).
    a. Determine biofix dates for male-moth capture and egg-laying at several sites throughout the almond-producing regions.
    b. Evaluate relationship between intra-season male-moth and egg-laying data.
    c. Evaluate the relationship between egg-capture and male-moth capture biofixes.
    d. Evaluate applicability of the UC IPM navel orangeworm degree-day model using a male-moth capture biofix.

Data analyses

2. Analyze NOW capture and environmental data from 2014 and 2015 to determine if factors such as solar radiation, day length, and/or minimum day or nighttime temperature play a role in initiation of egg-laying.

Interpretive Summary:

The use of female synthetic sex pheromone to lure male navel orangeworm (NOW) holds promise in simplifying our ability to monitor NOW flights and in predicting important phenological events of this pest. However, we have a limited understanding of how male moth capture data relate to egg capture, the most common tool used to establish biofix for the NOW phenological model. A primary objective of this 2-year study was to determine how egg and male moth capture relate; and if and how male moth capture information can be utilized for making NOW management decisions. At orchards located from the mid to upper San Joaquin Valley, to the upper Sacramento Valley Region, we deployed egg and pheromone traps at 17 and 14 sites in 2014 and 2015 respectively. We monitored the traps at least weekly from early March to October.

Seasonal navel orangeworm population levels varied among sites and years. Pooling the data across sites and years could not established a relationship between male catch and eggs laid using regression analysis of cumulative egg and male moth capture data.

No observable trend in egg biofix dates occurred among the sites located in southern or northern regions. At all sites during both years, pheromone traps began capturing male moths within the first few days of deployment, consequently we could not establish a biofix base on those data.

Using logistic regression analysis of pooled data over the first flight determined that a probability of 0.67 exists for the initiation of egg laying if male moth capture is at or above 10 moths per trap per week. The model predicts that when male moth capture is at or above 10 per trap per week, there is a probability of 0.67 of egg deposition is occurring. The practical application of such a model would be to predict the occurrence of an egg biofix in the absence of egg traps.

We initiated work on objective 2. To date, temperature and solar radiation data were collected for seven site locations during 2015. We are working to analyze those data as well as other environmental data to determine if and how they can be utilized in developing a more robust logistic model.

Materials and Methods:

We selected 17 and 15 orchard sites in different growing regions, southern, mid, and northern San Joaquin Valley (Kern, Fresno, Madera, Merced, San Joaquin, and Stanislaus counties); and Sacramento Valley Region (Glenn and Butte counties) in 2014 (**Table 1**) and 2015 (**Table 2**). Because egg biofix dates were not established at all sites, some sites are not shown in **Tables 1 and 2**.

Monitoring NOW was conducted using egg traps (ET) and pheromone traps (PT). Egg traps consisted of Trécé (Adair, Oklahoma) black NOW egg traps filled with food-grade whole almond meal purchased from Wonderful Orchards (Bakersfield, California). Pheromone traps were Suterra LLC (Bend, Oregon) or Trécé white-top wing traps baited with a Suterra NOW sex pheromone lure.

A monitoring set consisted of four ET and a single PT deployed in an (ET ET PT ET ET) configuration. Within-set trap spacing was approximately 100 ft. between traps hung approximately 5 to 6 ft. above the soil surface and within the same tree-row. Trap sets were not placed immediately on orchard edges and approximately 500 ft. was established between traps sets.

We deployed trap sets by mid-March and monitored them twice weekly to an egg biofix date. White-top wing trap bottoms were replaced as needed and almond meal and pheromone lures were replaced every four weeks. At eight orchard sites, a HOBO (Onset Corp. Bourne Massachusetts) weather data logger was placed within a monitoring set. Temperatures at times 18:00, 19:00, 20:00, 21:00, 22:00, and 23:00 were averaged on four days prior to egg biofix at each site where the HOBO data logger was deployed.

Also for the same sites, total daily solar radiation data were collected for each of the same four days. Solar radiation data are collected via the National Oceanic and Atmospheric Administration for the California Irrigation Management Information System (Data). Solar radiation data were downloaded from CIMIS stations located in Fresno (CIMIS, no. 80, Fresno State), Merced (CIMIS, no. 0148), San Joaquin (CIMIS, no. 70, Manteca), Stanislaus (CIMIS no. 71, Modesto), Yolo (CIMIS no. 6, Davis), Colusa (CIMIS, no 32, Colusa), and Butte (CIMIS no. 12, Durham) through the University of California IPM Statewide Program web site.

Statistical Analyses.
Seasonal navel orangeworm population levels for egg and male moth capture each year were estimated by calculating the cumulative mean as the sum of trap means across weeks.

A logistic regression analysis was used to evaluate the relationship between egg and male moth capture. Logistic regression, models dichotomous outcome variable and allows for explanatory variables to be categorical, discrete and / or continuous (UCLA Institute for digital research and education 2014). In this case, the outcome variable represents the yes / no occurrence of eggs and the predictor variable, the yes / no exceedance of a male moth capture threshold.

The outcome of the regression is the log of the odds and modeled as a linear combination of the predictor variables: $\log(p(x) / 1 - p(x)) = \beta_0 + x\beta_1$. Where p is the probability of a positive outcome, and log is the natural log. The β_0 is the log (odds of a positive outcome) and β_1 is the log (odds of a positive outcome / odds of a negative outcome). To obtain the probability of the outcome, the β values are back converted to odds and then to probability.

Logistic regression analysis was conducted on seasonal capture data over the first flight period. Flight periods were demarcated in to three periods: flight 1 (March – June), flight 2 (commencing at ~ 956 DD from biofix), and flight 3 (commencing at ~ 2012 DD from biofix). From the point of egg biofix, we calculated the approximate point at which adults begin to emerge and fly by subtracting 100 DD. Data were pooled between years and among all sites. To establish a male moth capture threshold, we analyzed yearly pooled data at 2, 5, 10, 12, and 15 moths per trap per week.

Descriptive statistics were used to analyze minimum temperature and solar radiation data.

Results and Discussion:

Objective 1. Evaluate Population Dynamics. The population level of navel orangeworm differed considerably among the sites in 2014 and 2015. Mean cumulative egg capture in 2014 varied between 0.75 (Fresno (Sel)), and 118.53 (San Joaquin (Fd). Mean cumulative moths per trap in 2014 ranged between a low of 155.67 (Fresno (Sel)) to a high of 1265.17 (San Joaquin (Fd)) (**Table 3**). In 2015 mean cumulative egg capture varied between 0.67 and 500 at Fresno (Parl), and Glenn (West Orl) respectively (**Table 4**). Mean cumulative male moth capture ranged between ~200 (Stanislaus (Gar)) to 1519 (Madera (21)) (**Table 4**). Regression analysis indicates that no relationship exists between the 2014 and 2015 cumulative egg and male moths capture data ($P < 0.05$, $R^2 = 0.04$).

Weekly mean egg capture in 2015 tended to remain below 20 over all three flight periods with the exception of three sites, Butte (West Chi), Glenn (West Orl), and Glenn (East Orl), in which egg capture peaked above 100, 75 and 35 eggs per trap respectively (**Figure 1**). At the sites in Madera, and San Joaquin counties, male moth capture tended to be greater than 20 moths per week over the duration of the first flight, and third flight periods (**Figure 1**). At only four sites: Madera (19), San Joaquin (Laubertie, et al.), Stanislaus (Gnz), and Stanislaus (Rdn) did weekly mean moth capture tend to be lower during the second flight period than first and third flight periods (**Figure 1**).

Navel orangeworm population dynamics described by (Rice 1975) Rice (1975) indicates that egg-laying and male moth activity follow a pattern of lower activity during the second flight period relative to the first and third periods. In this study, we found that this pattern occurred only at sites: in Merced and San Joaquin counties during.2014, and in Madera (19), Merced (Lg), Merced (Win), and Stanislaus (Laubertie, et al.) in 2015. Albeit, egg and male moth capture activity varied considerably among sites and between years, activity within sites tended to follow a similar pattern both in 2014 (data not show) and 2015 (**Figure 1**). One unusual activity pattern that we observed at the three sites in the Sacramento Valley region, was that egg capture peaked during the second flight period relative to the first and third periods (**Figure 1**).

In 2015, pheromone and egg traps consistently demarcated the commencement of the second and third flights (**Figure 1**). At nearly all sites, the 956 and 2012 DD points were preceded by a decreasing trend then followed by an increasing trend in male moth capture (**Figure 1**). And although additional degree-day analyses need to be conducted, initial analyses indicate that similar results occurred in 2014. This was an unexpected result. Conventional thought is that at low population levels, egg traps do not attract females sufficiently enough to demarcate the tail end of the first flight and subsequent commencement of the second flight period.

Since data in both years were highly variable, drawing a generalized conclusion regarding the pattern of egg and / or male moth capture is difficult. Other variables in NOW population dynamics that should be considered are the level of mummy sanitation within an orchard and the role neighboring orchards as a source of NOW. These sources of moths likely acted as a major factor contributing to the intra-seasonal flight dynamics observed in this study.

<u>Objective 1.a. Egg and Male Moth Biofix</u>. Biofix dates occurred over a narrow range in 2014 occurring from 10 Apr to 21 Apr; 50% of biofix dates were between 14 Apr and 17 Apr (**Table 1**). One thousand and fifty-six DD were reached over an 11-day period, from 24 Jun to 5 Jul (**Table 1**). In 2015 egg biofix occurred earlier and over a wider range of dates between 16 Mar and 24 Apr with the median date occurring on 23 Mar (**Table 2**). Fifty percent of the dates occurred between 15 Mar and 31 Mar. The point at which 1056 DD accumulated ranged between about a 16-day period from 16 Jun to 2 Jul (**Table 2**). One reason for the earlier biofix dates was that during Mar of 2015 warmer temperatures occurred and a (mean ± SE) of 6.4 ± 3.6 DD per day accumulated in contrast to a mean of 4.8 ± 2.2 DD per day accumulating in Mar of 2014.

In both years of the experiment, male moth biofix was not established. Unlike some other lepidopterous pests, NOW does not have a natural resting period, or diapause (Michelbacher, et al. 1961). Male, as well as female moths fly year-round given adequately warm temperatures. However, despite the absence of a diapause, NOW does have a period when moths are not flying if temperatures are low enough. Other researchers have found that the first flight of moths begin in late winter after a short hiatus during cold winter temperatures (Higbee, personal contact).

One likely reason that we did not observe a period in which males were not captured relates to the mild winters that have occurred over the past few years. Higbee (personal communication) also observed that male moths were consistently captured in Feb and Mar of 2014 and 2015. Based on our data collected during these years, it seems that establishing a male moth biofix is may not be practical.

Objective 1. b. Relationship Between Intra-Season Male Moth and Egg Laying Data.
Within the first flight period of 2014 and 2015 pooled data, 628 observations were used in the analysis. Of these 260 observations had no eggs observed and pheromone traps were below the threshold of 10 moths per trap per week. On 83 of the observations, the pheromone trap threshold was met, although no eggs were detected on the traps. Eggs were detected on 114 observations without the pheromone threshold being reached. On 170 observations both the detection of eggs and the pheromone trap threshold were met.

Analyzing the data using logistic regression resulted in a highly significant model: $\log (p(x) / 1 - p(x)) = -0.82 + 1.55x$ ($P < 0.001$). The model predicts that when male moth capture is at or above 10 per trap per week, there is a probability of 0.67 of egg deposition occurring.
On a practical basis, this model could be used to detect the initiation of egg-laying during the first flight period in the absence of egg traps i.e. establish a biofix using pheromone traps. During the second and third flight periods, the model provides a higher level of certainty, and additional analyses are being conducted to determine how the model can be used to predict the initiation of the second and third flight periods.

At this stage, the model does not provide a high enough degree of certainty to replace egg traps with pheromone traps. However, the addition of other explanatory variables such as temperature at sunset, daily solar radiation, geographical region, level of sanitation, and proximity to pistachio could improve its predictive power.

Objective 1. c. and d. Because male moth biofix data were not obtained, these objectives were not addressed.

Objective 2. This objective is still under investigation and we are continuing work in this line of research. The goal was to incorporate environmental data into the logistic regression analysis, however this has proven more challenging than originally understood. To date, we have conducted basic descriptive analyses on temperature and solar radiation data from seven sites in 2015. Our results indicate that mean temperatures on four days prior to biofix ranged between ~58 and 78°F with 75% of the mean temperatures falling above 60°F (**Figure 2**). Our data are in close agreement with those of (1980) Andrews et al. (1980) who found that the female flight threshold was just below 64.2°F. Daily solar radiation over the four days prior to the initiation of egg deposition ranged between 435 and 675 Watts / m^2 (**Figure 3**). Our data suggest that a minimum threshold lies at approximately 435 Watts / m^2 (**Figure 3**).

In future work we will continue our efforts in this line of research by working more closely with a statistician to determine how and what environmental data to incorporate into a logistic model.

Table 1. Navel orangeworm 2014 egg biofix date and date at which 1056 degree-day heat units was reach for 13 sites located in six counties

County	Biofix (egg)	Week of year	Mean male capture (± SE) at egg biofix	Date (1056 DD from biofix)
Madera (19)	18-Apr	17	14 (5.9)	28-Jun
Madera (21)	14-Apr	16	26.3 (3.7)	26-Jun
Merced (Atwr)	14-Apr	16	27.3 (8.5)	24-Jun
Merced (Bel)	14-Apr	16	46 (8.5)	24-Jun
Merced (LeG)	14-Apr	16	63.3 (8)	24-Jun
Stanislaus (Gar)	14-Apr	16	15 (4.0)	24-Jun
Stanislaus (Gnz)	17-Apr	16	11.7 (2.7)	25-Jun
Stanislaus Rdn	14-Apr	16	11 (1.7)	24-Jun
San Joaquin (Fd)	18-Apr	16	34 (11.1)	4-Jul
San Joaquin (Dc)	21-Apr	17	18 (1.5)	5-Jul
Colusa (Ma)	10-Apr	15	6.3 (2.8)	25-Jun
Glenn (Ht)	14-Apr	16	35 (5.4)	26-Jun
Glenn (Vg)	10-Apr	15	13.3 (2.6)	26-Jun

Table 2. Navel orangeworm 2015 egg biofix date and date at which 1056 degree-day heat units was reach for 15 sites located in eight counties

County	Biofix (egg)	Week of year	Mean male capture (± SE) at egg biofix	Date (1056 DD from biofix)
Fresno	24-Apr	17	26 (4.0)	26-Jun
Madera (19)	31-Mar	14	29 (4.7)	22-Jun
Madera (21)	31-Mar	14	18.3 (2.9)	22-Jun
Merced (LeG)	16-Mar	12	4.7 (1.5)	16-Jun
Merced (Win)	19-Mar	14	17.7 (2.2)	17-Jun
Stanislaus (Gar)	23-Mar	13	6.7 (2.7)	27-Jun
Stanislaus (Rdn)	16-Mar	12	35 (13.2)	25-Jun
Stanislaus (Gnz)	30-Mar	14	19.3 (2.7)	30-Jun
San Joaquin (Fd)	23-Mar	13	32.3 (6.9)	26-Jun
San Joaquin (Dc)	16-Mar	12	36.3 (2.3)	24-Jun
Colusa (Gb)	24-Mar	13	43.3 (10.1)	23-Jun
Colusa (Ma)	8-Apr	15	39.7 (12.3)	28-Jun
Glenn (West Orl)	20-Mar	12	62 (7.5)	21-Jun
Glenn (East Orl)	27-Mar	13	24.3 (0.3)	2-Jul
Butte (West Chi)	18-Mar	12	6.3 (2.3)	18-Jun

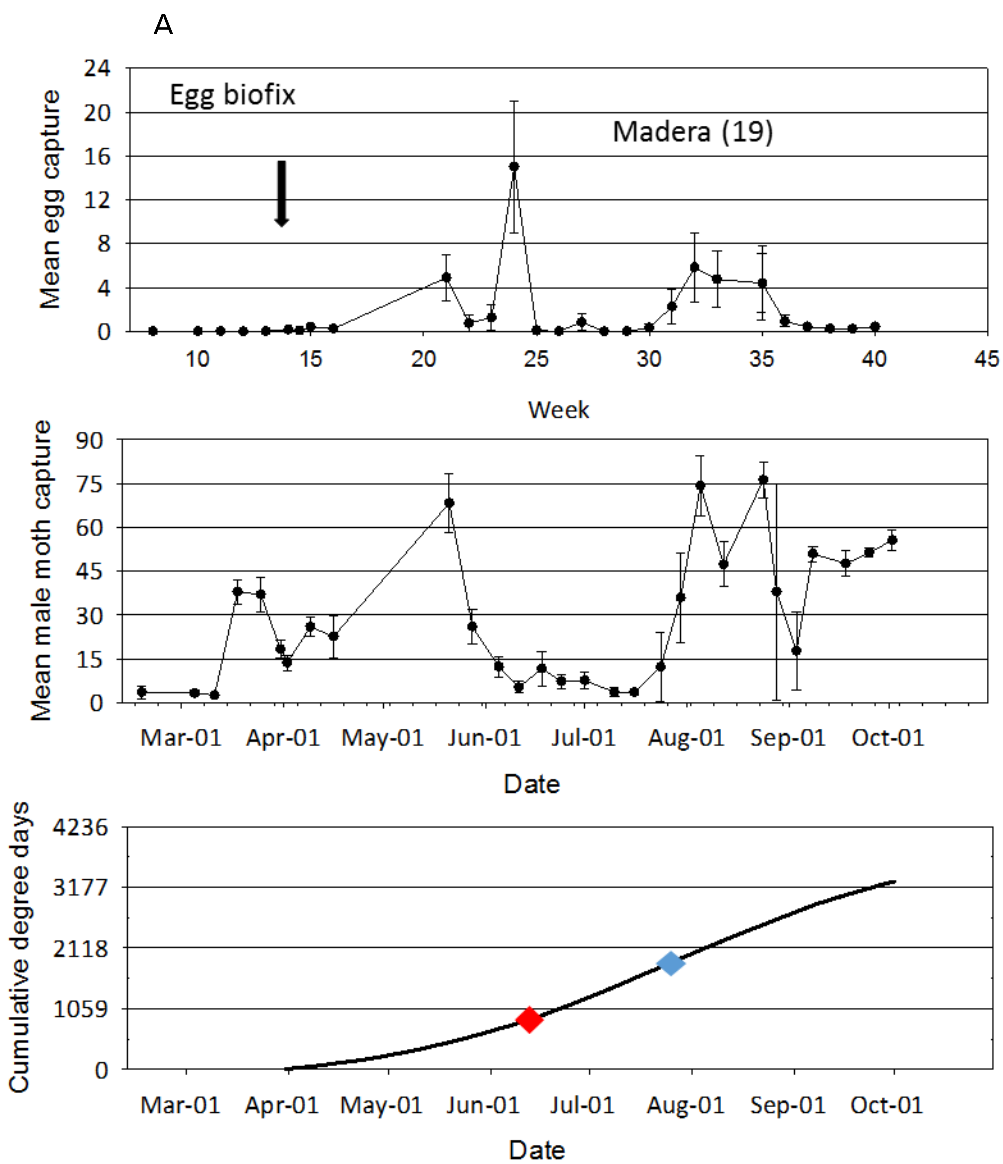
Table 3. Cumulative mean egg and male moth capture data from 2014

County (site)	Cumulative weekly (mean ± SE) capture		N
Kern (45)	4.59 ± 0.09	418.5 ± 4.91	31
Kern (48)	1.66 ± 0.05	342.5 ± 2.69	32
Fresno (Sel)	0.75 ± 0.02	155.67 ± 0.7	28
Madera (19)	36.55 ± 0.52	442.67 ± 1.74	29
Madera (21)	77.25 ± 0.91	679.83 ± 3.32	30
Merced (Atwr)	13.25 ± 0.24	441.17 ± 2.99	31
Merced (Bel)	19.5 ± 0.16	506 ± 3.44	31
Merced (LeG)	88.42 ± 0.57	1086.83 ± 4.5	31
Stanislaus (Gar)	28.8 ± 0.32	236.3 ± 1.39	31
Stanislaus (Gnz)	25.23 ± 0.3	320.83 ± 1.67	31
Stanislaus Rdn	81.84 ± 0.57	696.62 ± 5.2	31
San Joaquin (Fd)	118.53 ± 1.64	1265.17 ± 7.12	27
San Joaquin (Dc)	46.51 ± 0.56	688.5 ± 4.78	27
Colusa (Gb)	108.92 ± 1.42	486.33 ± 2.61	39
Colusa (Ma)	58.45 ± 0.95	511.58 ± 2.64	38
Glenn (Ht)	82.38 ± 0.92	461.17 ± 3.7	23
Glenn (Vg)	17.92 ± 0.2	319.33 ± 2.9	23

Table 4. Cumulative mean egg and male moth capture data from 2015.

County (site)	Cumulative weekly (mean ± SE) capture		N
	egg	male	
Fresno (Parl)	0.67 ± 0.02	307.5 ± 2.33	27
Madera (19)	43.4 ± 0.59	749.83 ± 4.28	28
Madera (21)	116.88 ± 1.2	1519 ± 5.96	28
Merced (LeG)	40.58 ± 0.39	1096 ± 4.58	33
Merced (Win)	30.92 ± 0.32	433.83 ± 2.25	34
Stanislaus (Gar)	66.78 ± 0.71	199.8 ± 0.81	31
Stanislaus (Rdn)	156.49 ± 1.02	614.22 ± 4.25	31
Stanislaus (Gnz)	47.91 ± 0.31	400.5 ± 2.12	31
San Joaquin (Fd)	213.95 ± 1.51	1281.67 ± 5.12	29
San Joaquin (Dc)	79.76 ± 0.76	780.17 ± 3.79	29
Colusa (Gb)	77.38 ± 0.97	748.33 ± 3.75	26
Colusa (Ma)	53.38 ± 0.85	631.83 ± 3.47	26
Glenn (West Orl)	500.23 ± 7.41	598 ± 5.67	22
Glenn (East Orl	283.92 ± 2.53	552.17 ± 2.96	27
Butte (West Chi)	454 ± 6.28	479 ± 3.12	26

Figure 1 consists of multiple figures (A – I); Legend located at bottom of Figure 1-I



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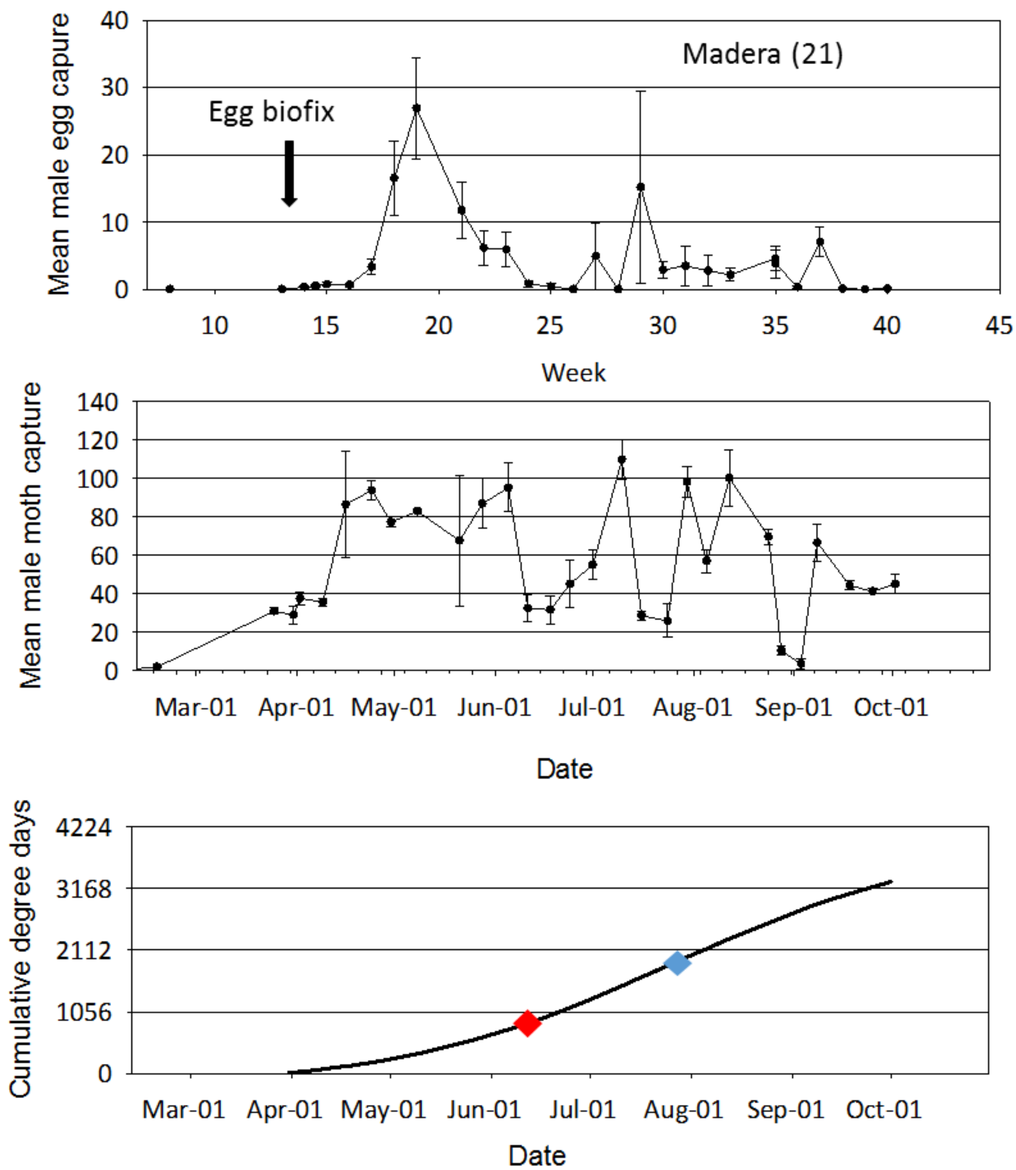
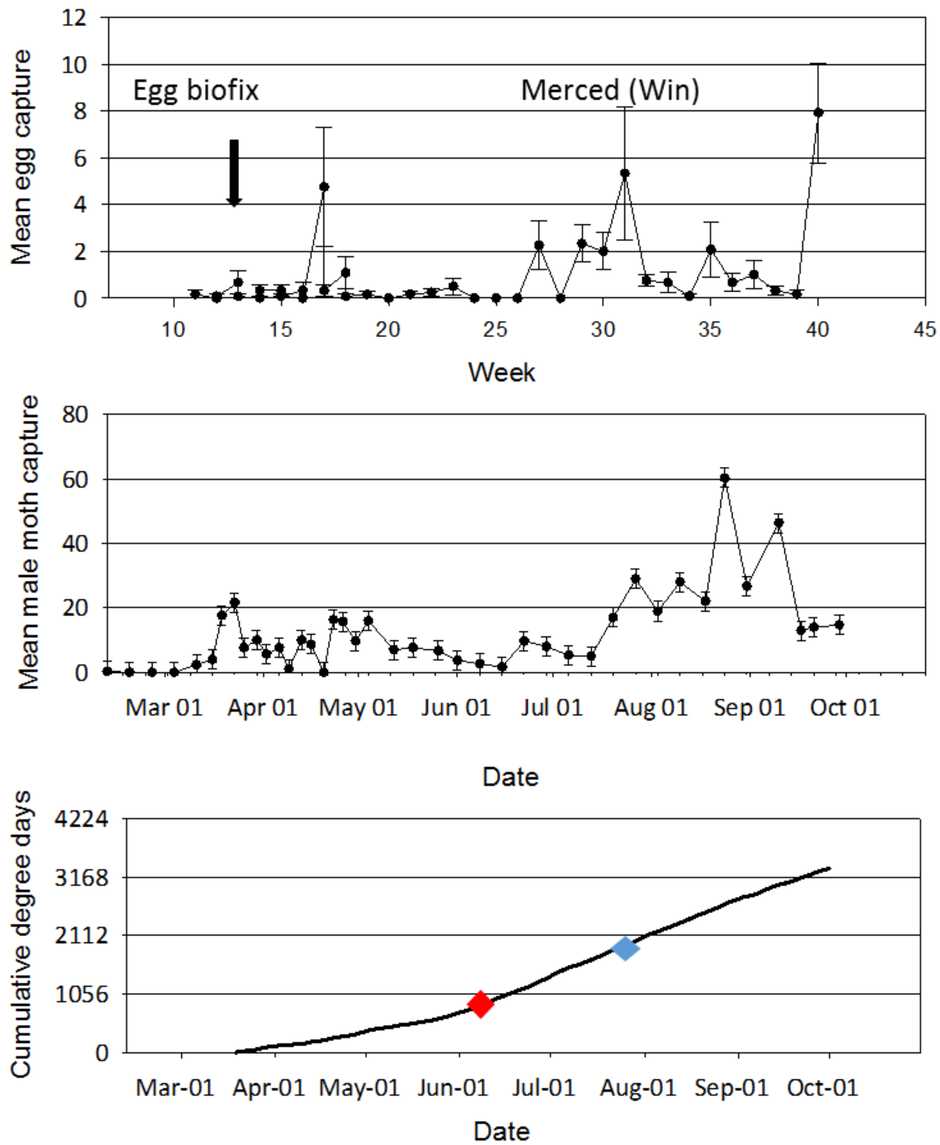
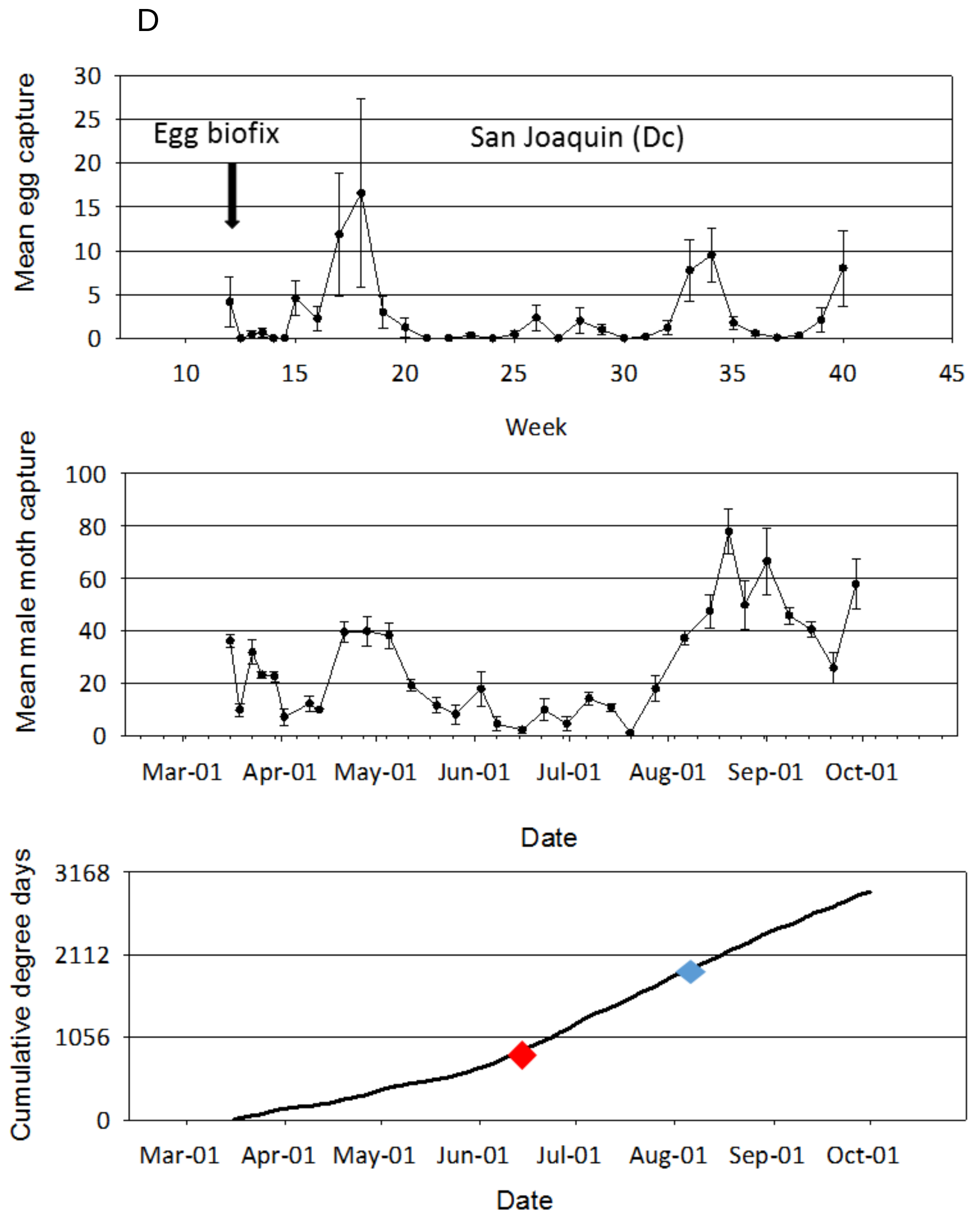


Mean male egg capure
40
30
20
10
0
Madera (21)
Egg biofix
10
15
20
25
30
35
40
45
Week
Mean male moth capture
140
120
100
80
60
40
20
0
Mar-01
Apr-01
May-01
Jun-01
Jul-01
Aug-01
Sep-01
Oct-01
Date
Cumulative degree days
4224
3168
2112
1056
0
Mar-01
Apr-01
May-01
Jun-01
Jul-01
Aug-01
Sep-01
Oct-01
Date

C



Egg biofix
Merced (Win)
Mean egg capture
Week
Mean male moth capture
Date
Cumulative degree days
Date


D
Mean egg capture
Egg biofix
San Joaquin (Dc)
0
5
10
15
20
25
30
10
15
20
25
30
35
40
45
Week
Mean male moth capture
0
20
40
60
80
100
Mar-01
Apr-01
May-01
Jun-01
Jul-01
Aug-01
Sep-01
Oct-01
Date
Cumulative degree days
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Apr-01
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Jul-01
Aug-01
Sep-01
Oct-01
Date

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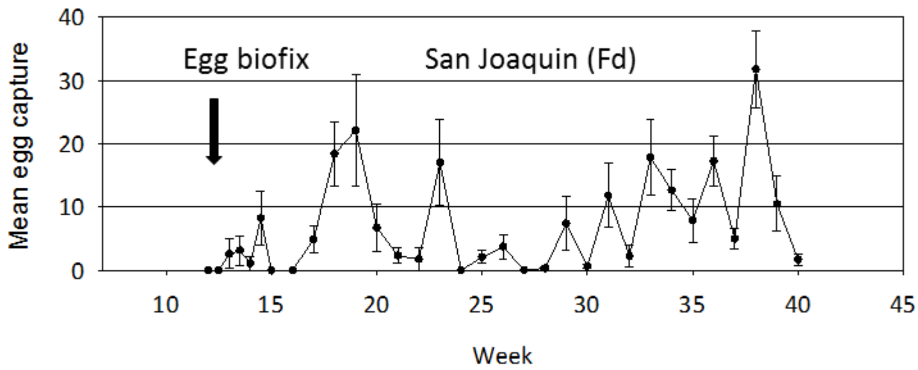
Egg biofix
San Joaquin (Fd)
Mean egg capture
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10
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20
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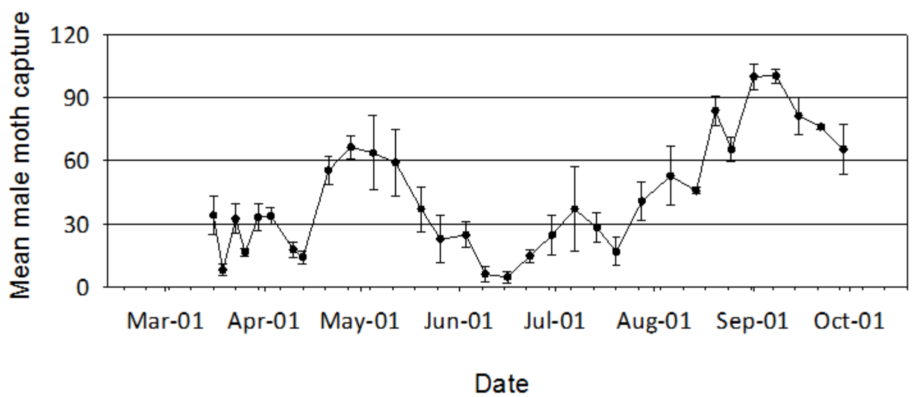
30
35
40
45
Week


Mean male moth capture
120
90
60
30
0
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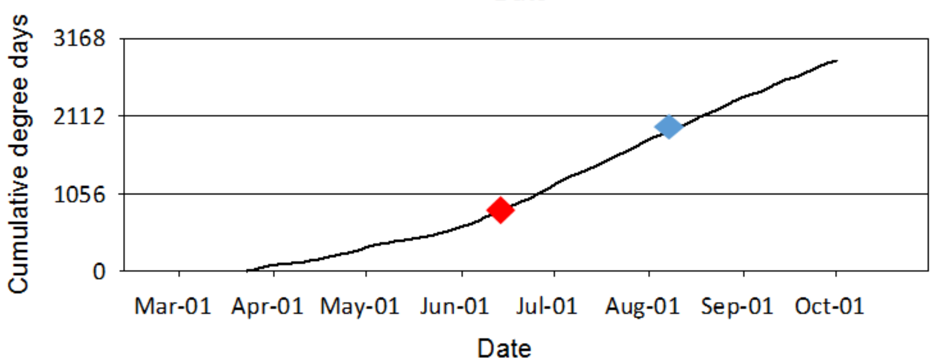
Apr-01
May-01
Jun-01
Jul-01
Aug-01
Sep-01
Oct-01
Date


Cumulative degree days
3168
2112
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Mar-01
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May-01
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Sep-01
Oct-01
Date

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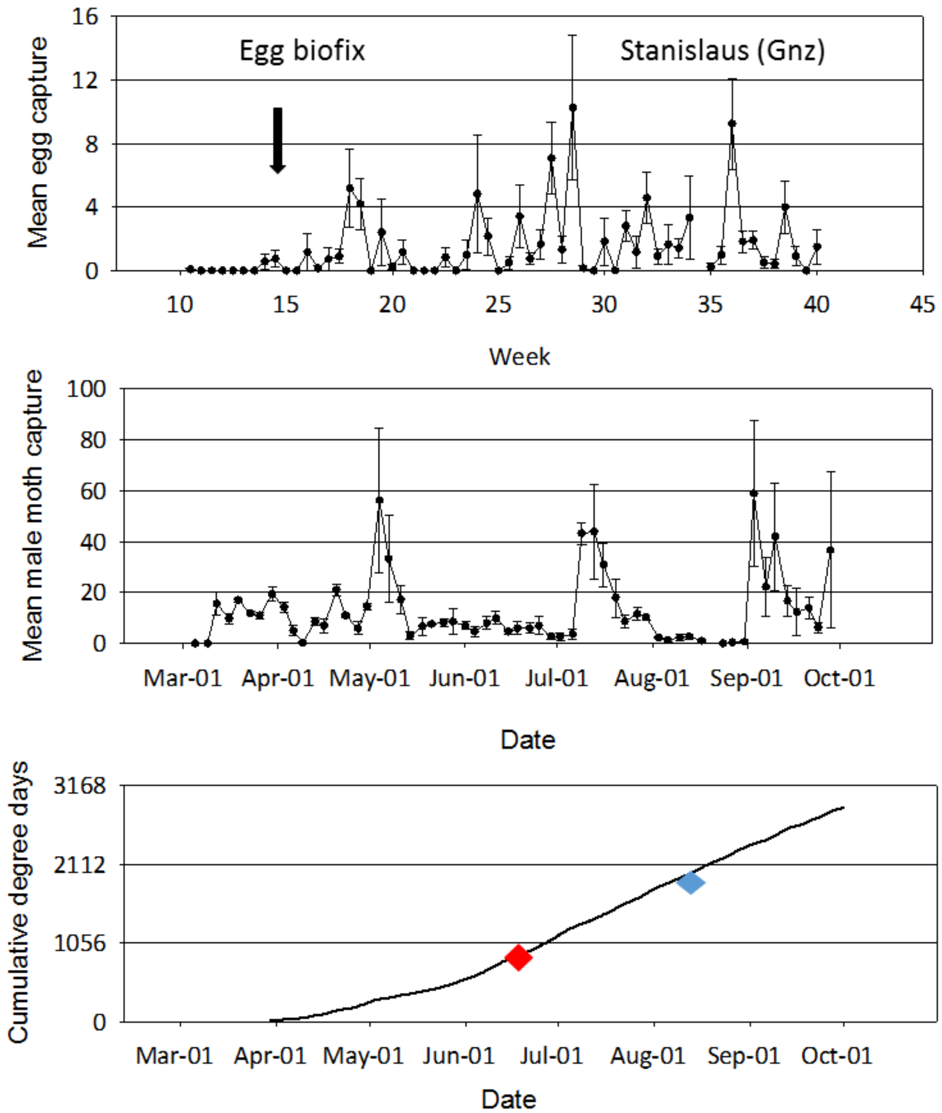
Egg biofix
Stanislaus (Gnz)
Mean egg capture
0
4
8
12
16
10
15
20
25
30
35
40
45
Week
Mean male moth capture
0
20
40
60
80
100
Mar-01
Apr-01
May-01
Jun-01
Jul-01
Aug-01
Sep-01
Oct-01
Date
Cumulative degree days
0
1056
2112
3168
Mar-01
Apr-01
May-01
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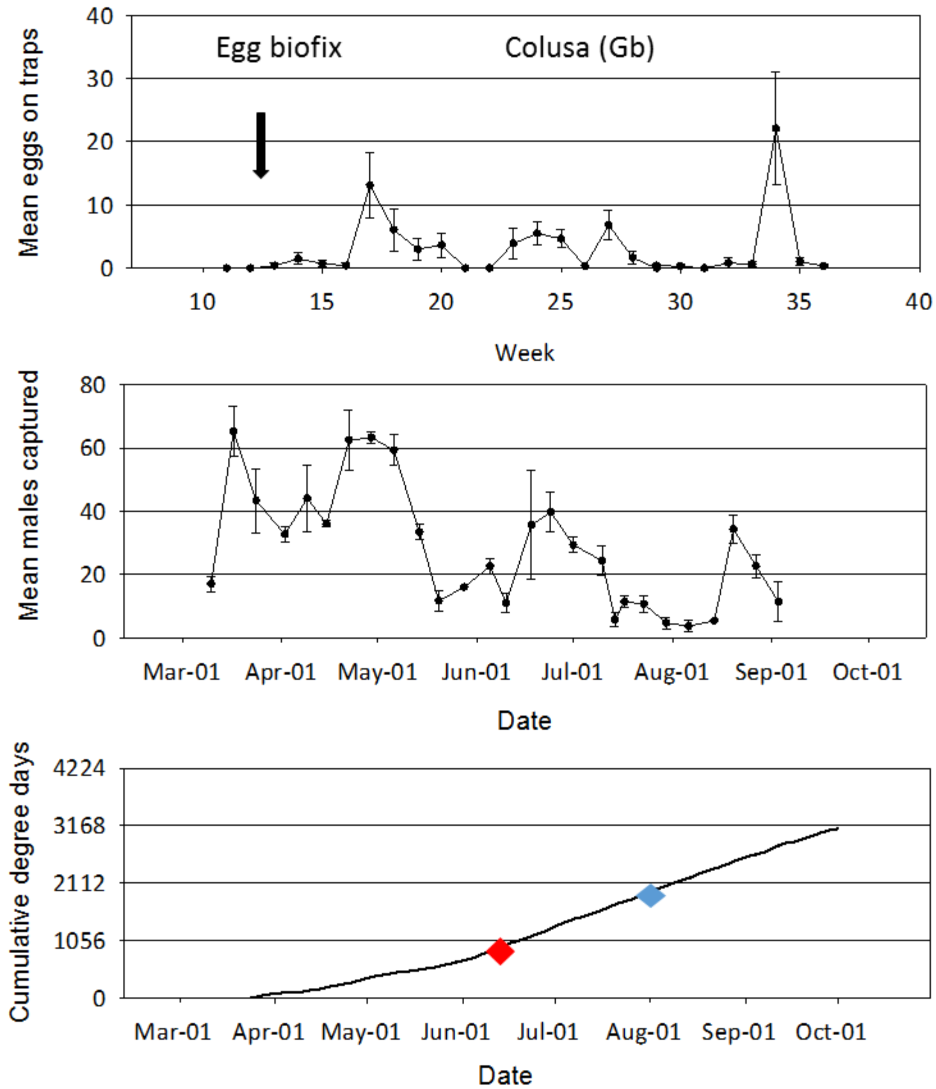
Aug-01
Sep-01
Oct-01
Date

G


Egg biofix
Colusa (Gb)
Mean eggs on traps
0
10
20
30
40
10
15
20
25
30
35
40
Week
Mean males captured
0
20
40
60
80
Mar-01
Apr-01
May-01
Jun-01
Jul-01
Aug-01
Sep-01
Oct-01
Date
Cumulative degree days
0
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Aug-01
Sep-01
Oct-01
Date

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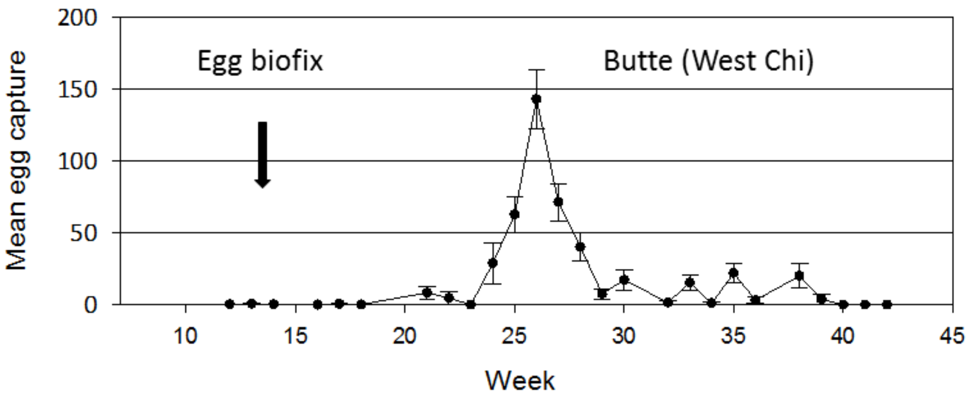
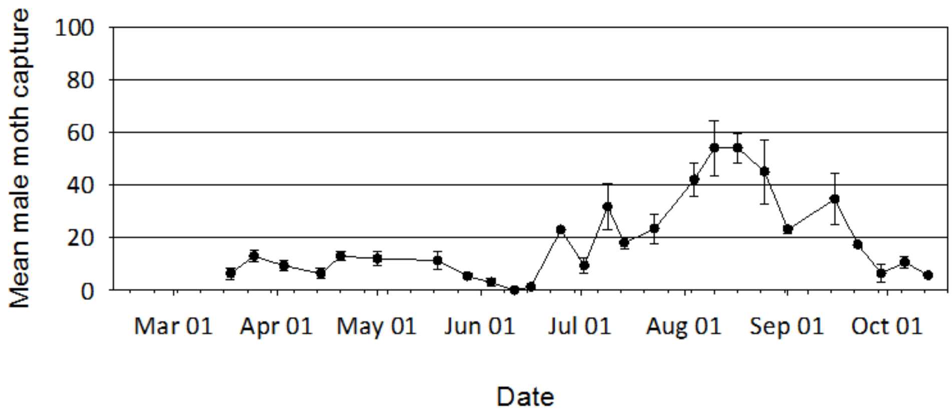
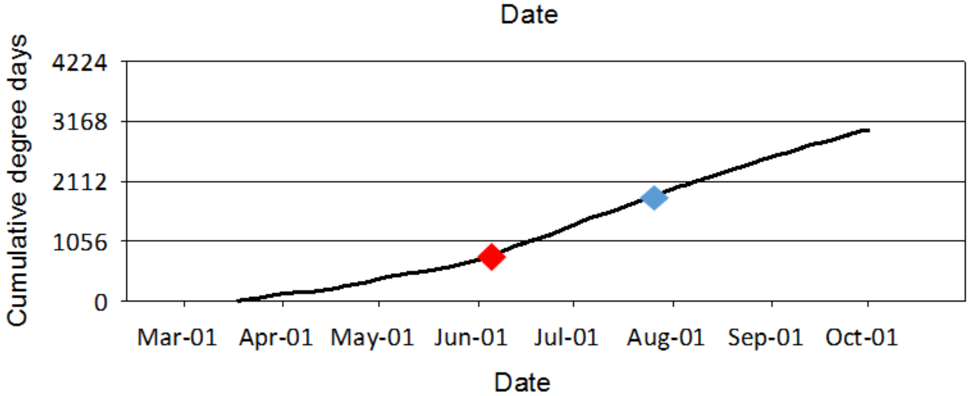


Egg biofix
Butte (West Chi)
Mean egg capture
Week



Mean male moth capture
Date



Cumulative degree days
Date

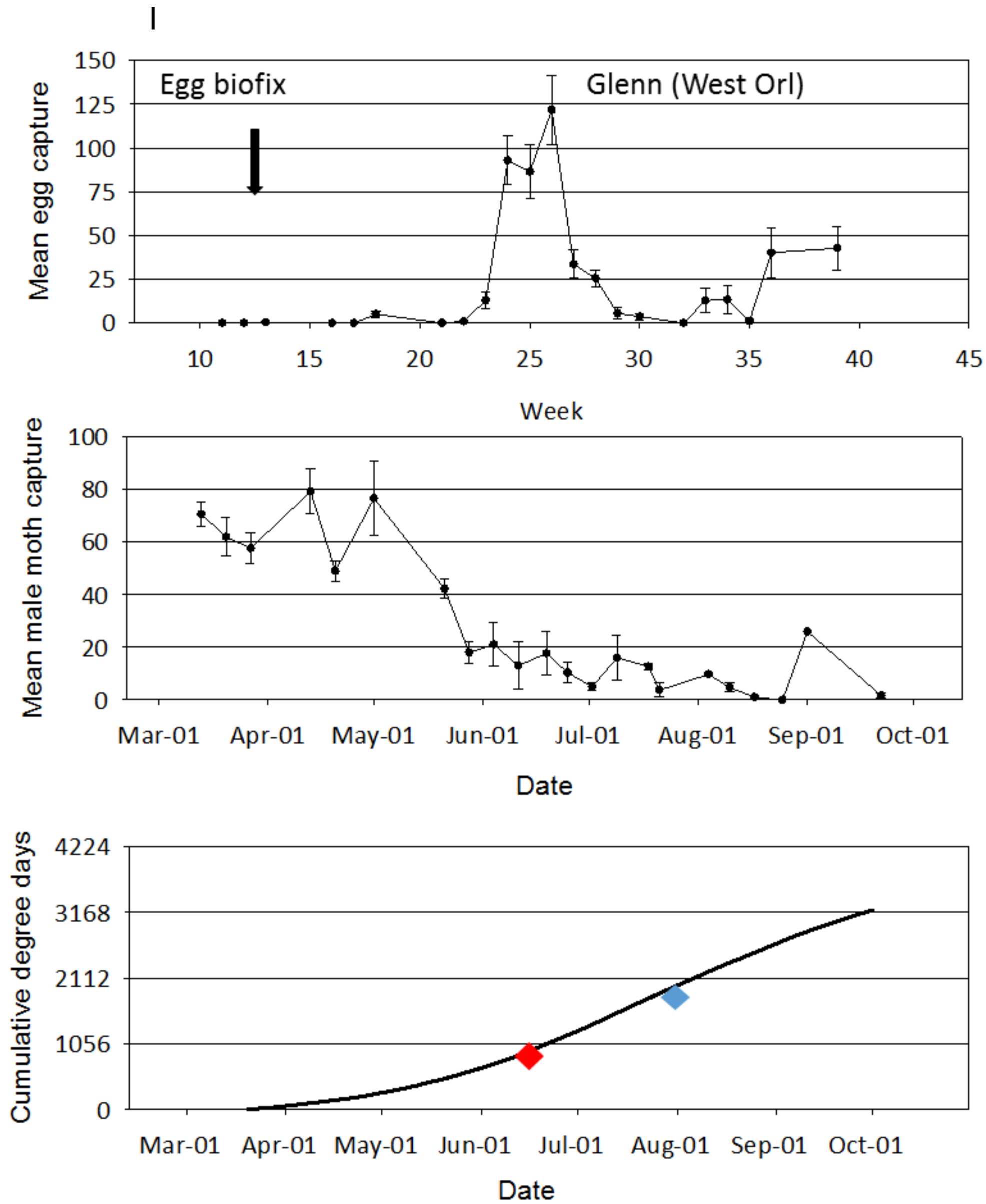


Figure 1. 2015 Mean male and egg capture at nine sites located in A, B (Madera Co.), C (Merced Co.), D, E (San Joaquin Co.), F (Stanislaus), G (Colusa Co), and H (Butte Co.), and I (Glenn) counties. These figures show a representation of total sites sampled. Each figure also shows the accumulated degree days from egg biofix with the red and blue diamond indicating the date at which the second and third flight periods were predicted to begin respectively.

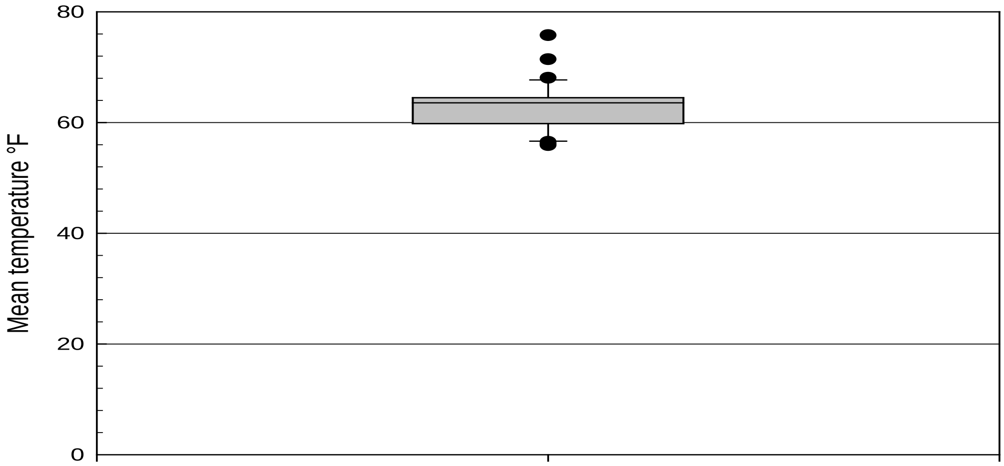


Figure 2. Mean temperatures calculated from four days prior to the occurrence of egg biofix at seven sites in 2015. Means on each day were calculated from temperatures at times 18:00, 19:00, 20:00, 21:00, 22:00, and 23:00. Temperature data collected from HOBO data loggers set in one trap set at each of the seven sites.

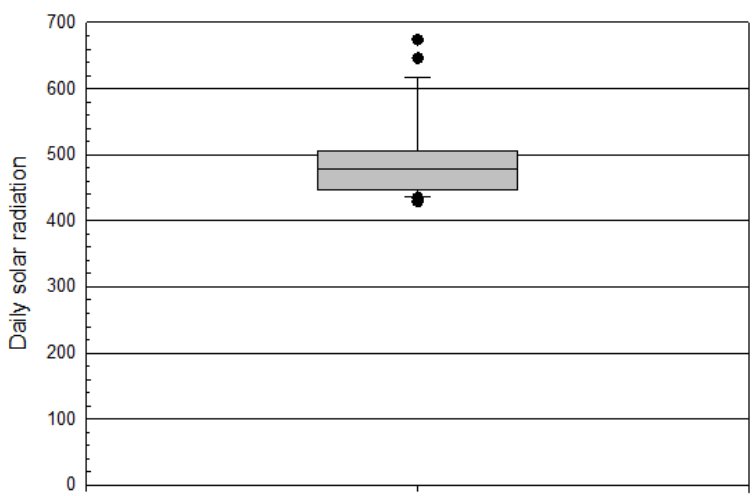


Figure 3. Daily solar radiation values (Watts / m^2) at seven sites located in Fresno, Merced, Stanislaus, San Joaquin, and Glenn counties in 2015. Solar radiation data were down from: (CIMIS, no. 80, Fresno State), Merced (CIMIS, no. 0148), San Joaquin (CIMIS, no. 70, Manteca), Stanislaus (CIMIS no. 71, Modesto), and Colusa (CIMIS, no 32, Colusa).

Research Effort Recent Publications:

Research was presented during a symposium titled: Navel Orangeworm Management for the 21st Century. Moderators and Organizers: Bradley S, Wonderful Orchards, Bakersfield, CA, Higbee and Kristen E. Tollerup UC Statewide IPM Program, Parlier, CA

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