

## Comparison of Trapping for Eggs, Females, and Males of the Navel Orangeworm (*Lepidoptera: Pyralidae*) in Almonds

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**ABSTRACT** The navel orangeworm is the primary insect pest of almonds in California, and egg traps are the primary means of monitoring this pest. A previous study found that the current use of 2–4 traps per 64 ha block usually is not sufficient to provide management information specifically for that block. In this study, we compare data from large grids of egg traps in varied commercial almond orchards with trapping data for females and males, with the objective of finding a more cost-effective monitoring program using currently available attractants. The proportion of egg traps with eggs was highly correlated with mean eggs per egg trap, and with females and males trapped simultaneously at the same location. Almond variety and the type of bait used had little impact on the relationship between the proportion of egg traps with eggs and the number of eggs per traps. Traps in orchards with more unharvested (mummy) almonds had more eggs, suggesting that navel orangeworm abundance affected traps more than competition from mummies. Laboratory experiments comparing age-specific oviposition in two-choice and no-choice situations found that younger, more fecund females laid a greater proportion of eggs on the preferred substrate in a two-choice situation, but that age-specific fecundity was not different between substrates in no-choice tests. These findings indicate that the proportion of egg traps with eggs provides a more stable indication of navel orangeworm phenology than mean eggs per trap. We suggest that similar information could be obtained in a more cost-effective manner with female trapping.

**KEY WORDS** monitoring, egg trap, almonds, *Prunus dulcis*, *Amyelois transitella*

The navel orangeworm *Amyelois transitella* (Walker) is the principal insect pest of California almonds *Prunus dulcis* (Miller) D.A. Webb (Zalom et al. 2009). The navel orangeworm is a highly polyphagous scavenger that has been observed in the fruit or seeds of >20 taxonomically diverse trees and shrubs (Wade 1961). It is, however, generally a poor penetrator and enters the host in later stages of maturity or decay. The navel orangeworm does not enter almonds before hull split, and varieties with tighter seals are less susceptible to damage (Soderstrom 1977). ‘Nonpareil’ is the most widely planted variety in California (Almond Board of California 2009), one of the earliest harvested, and one of the most susceptible to navel orangeworm. Because of poor self-fertility in almonds, Nonpareil is generally planted in alternating rows with other varieties such as ‘Monterey,’ ‘Carmel,’ ‘Sonora,’ and ‘Wood Colony.’ ‘Padre’ has a very hard shell and is very resistant to navel orangeworm; it is often in-

terplanted with ‘Butte,’ which is more resistant to damage than Nonpareil but less so than Padre. Currently, there is no synthetic pheromone lure with sufficient field stability for practical use in monitoring the navel orangeworm. Instead, egg traps (Rice 1976, Rice et al. 1976, Van Steenwyk and Barnett 1985, Kuenen et al. 2008) are used in conjunction with degree-day models to determine the beginning of cohorts and time insecticide applications targeting eggs and/or neonates (Zalom et al. 2009).

The effectiveness of egg traps is known to be affected by crop maturity (Rice 1976), and could plausibly be affected by orchard sanitation and almond variety. Sanitation—removal of leftover (mummy) almonds not harvested with the previous-year crop—is an important part of navel orangeworm pest management (Zalom et al. 2009). A threshold of <2 mummies per tree by 1 February is recommended (Zalom et al. 2009), although some have advocated and implemented much more stringent standards (Higbee and Siegel 2009). Others, however, find that local conditions (e.g., heavy soil with poor drainage) make sanitation more expensive for them and therefore doubt its cost-effectiveness in their particular situation; thus, a wide range of sanitation occurs in commercial practice.

Recent developments may change monitoring and pest management practices for navel orangeworm,

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possibly resulting in new and different roles for trapping with almond meal or other ovipositional attractants. Advances in pheromone chemistry of this species (Kanno et al. 2010, Kuenen et al. 2010) suggest hope for a synthetic pheromone lure practical for commercial monitoring. However, a mating disruption product for this species (Higbee and Burks 2008, Higbee 2010) has become commercially available. In other Lepidoptera controlled with mating disruption, pheromone lures are either less effective (Charmillot 1990, Cardé and Minks 1995) or useless (Roelofs et al. 1973, Kovanci and Walgenbach 2005) for practical monitoring. A possible long-term benefit of mating disruption is reduced use of residual insecticides, but securing this benefit will require improvements in the ability to anticipate damage from this species. We have evidence that eggs on egg traps, over a wide area, are correlated with subsequent damage (C.S.B., unpublished data). However, we have also found that, due in part to the overdispersed distribution of eggs on egg traps, the 2–4 traps per 64 ha management unit typically used in California's southern and central San Joaquin Valley are too few to reliably detect oviposition (Higbee and Burks 2011). A recent study suggested that binomial analysis of traps (counts of either 0 or >0) could provide more consistent results over a variety of different frequency distributions when applied to flying insects of various species in various trap types (Nansen et al. 2008).

In this study, we examine elements with the potential to create a more cost-effective navel orangeworm monitoring program in almonds using the currently available attractants. For this purpose: (1) We compared incidence of eggs (defined here as the proportion of egg traps with >0 eggs) versus the mean using large grids of egg traps at a variety of sites to determine if both are equally informative. (2) We further used these data to examine the influence of bait type and orchard sanitation on egg trap performance. (3) We also compared egg traps with simultaneous captures of males in pheromone traps and females in bucket traps baited with egg traps to determine whether these measures of navel orangeworm activity provide similar information. (4) Finally, we used laboratory experiments to examine how female age, fecundity, and host preference might differently affect incidence and mean in egg traps. Based on findings from these experiments, we argue that incidence of eggs on egg traps should give better information than mean eggs per trap and be less sensitive to factors such as female age structure and orchard phenology. However, we further argue that trapping for females should provide these same benefits in a more cost-effective manner.

### Materials and Methods

**Traps, Baits, and Insect Strains.** Experiments examining the relationship between eggs on egg traps (Pherocon IV NOW, Black, Trécé Inc., Adair, OK) and females captured in white, yellow, and green bucket traps (multi-colored Unitraps, Great Lakes Integrated Pest Management (IPM), Vestaburg, MI) were con-

ducted in Kern County during the 2004 growing season, and an experiment comparing eggs on egg traps with males captured in wing traps with virgin females as a pheromone source was conducted in Fresno County in 2008. Traps were hung in the lower canopy, 1.5–2 m above the ground.

Baits used in the egg traps included commercial almond meal (AM) (Liberty Vegetable Oil Company, Santa Fe Springs, CA) and previous-year almonds and pistachios, ground in a household blender to aggregates of <0.5 cm and mixed in equal ratios (Alm + Pis). In some cases, AM was used with 10% crude almond oil (Liberty Vegetable Oil Company) (AM + CAO) (Van Steenwyk and Barnett 1985, Kuenen et al. 2008). In these experiments alternating sets of egg traps were prepared to allow traps to be exchanged every week and taken to the laboratory so eggs could be counted under a dissecting microscope. In Kern County bait was changed every week, while in Fresno County bait was changed at 4–6 wk intervals because a previous study found that bait age did not influence trap effectiveness within that time period (Kuenen et al. 2008).

To trap females, egg traps were placed on the bottom inside bucket traps with a plastic dichlorvos strip as a killing agent. Orange wing traps (Suterra LLC, Bend, OR) baited with a mesh bag containing three virgin females were used for monitoring males, as described by Burks and Brandl (2004). Navel orangeworm colonies used for virgin female trapping and laboratory experiments were reared on brain diet (Tebbetts et al. 1978) at 16:8 L:D, 26°C, and 60% RH. Using the descriptions of Siegel et al. (2010), laboratory strains SPIRL-1966 and CPQ-2005, respectively, were used to provide virgin females for monitoring the Fresno County sites and for the laboratory experiments described below.

**Comparison of Egg and Adult Trapping.** Grids of egg traps and either bucket traps (Kern County) or wing traps (Fresno County) were used to compare the incidence of eggs on egg traps with mean eggs per egg trap and with females in bucket traps, and to compare whether egg incidence or mean better correlated with males captured in virgin-baited wing traps. Each Kern County site had 20 egg traps and 10 bucket traps, and the Fresno County sites had 16–64 egg traps and 2–4 wing traps each. Weekly means of the traps from each of the sites were used for these comparisons.

Characteristics of the sites used in the study are summarized in Table 1. The most notable difference between the two locations was that sanitation in the Kern County sites was more stringent than the current recommendation of two mummies per tree, whereas in the Fresno County location the number of mummies per tree generally exceeded this amount (Table 2). Trees were also more densely planted at the Fresno County location than at the Kern County location, and the Fresno County location included blocks of Butte and Padre, not found in the Kern County location.

**Kern County Sites.** Twenty egg traps and 10 bucket traps were placed in each of three 8 ha plots within a 25 km radius of Lost Hills, CA. In each of these sites,

**Table 1.** Description of orchard locations and sites

Region	Site	Plot size (ha)	Varieties	Row and tree spacing (m)	Trees per ha
Kern County	1	8	Np,Ca,So <sup>a</sup>	7.3 × 6.4	213
	2	8	Np,Mo,Ca	7.3 × 6.4	213
	3	8	Np,Mo,Wc	7.3 × 6.4	213
Fresno County	1	64	Np,Mo	6.7 × 5.2	288
	2	44	Bu,Pa	7.0 × 5.5	260
	3	56	Np,Mo,Wc	6.7 × 5.2	288
	4	48	Bu,Pa	7.3 × 5.5	249
	5	16	Np,Mo	7.3 × 5.5	249
	6	32	Bu,Pa	7.3 × 5.5	249
	7	32	Np,Mo,Wc	7.0 × 5.5	260
	8	32	Np,Mo	7.3 × 5.5	249

<sup>a</sup> Variety abbreviations: Np, Nonpareil; Ca, Carmel; So, Sonora; Wc, Wood Colony; Bu, Butte; and Pa, Padre.

the trees were 6.4 m apart in rows 7.3 m wide for a density of 213 trees/ha. The orchards at sites 1–3 had been established 15, 7, and 6 yr, respectively, at the time of the study. The 8 ha study plots were parts of larger orchards (32 ha for site 1 and 3, and 64 ha for site 2). Sanitation was assessed in these orchards in January, using samples of eight trees per 4 ha sampling unit as described in Burks et al. (2008).

At site 1, 10 egg traps each were placed in two orchard rows, 10 and 30 rows from the edge of the orchard, with traps hung every five trees. Ten bucket traps were hung in the same pattern 20 rows from the edge of the orchard, so that there was a 73 × 32 m grid of 30 traps. These were part of an experiment on bait formulations described elsewhere (Higbee and Burks 2011), so each position (tree) at this site had three egg traps or three bucket traps. We used the traps baited with AM to compare egg trap means and incidence, and incidence of eggs on egg traps with females in bucket traps. Trapping began 4 March 2004 and continued 39 wk. At sites 2 and 3, five egg traps were hung in every seventh tree at 10 rows intervals, forming a 73 × 47 m grid of 20 traps. Bucket traps were hung in the same manner at 7-tree intervals 10 rows on either side of the egg trap grid, for a total of 10 bucket traps. Egg traps at these sites (including those in bucket

traps) were baited with Alm + Pis. Trapping began 1 March 2004 and continued 26 wk.

**Fresno County Sites.** A total of 324 egg traps and 21 virgin female-baited wing traps were used to compare eggs and males captured in the eight Fresno County sites, which were located in a 5 × 5 km area 18 km from Mendota, CA. Row and tree spacing in these sites was from 6.7 to 7.3 and 5.2–5.5 m, respectively, for planting densities of 249–288 trees/ha (Table 1). These trees were 8–13 yr old at the time of the study. Sanitation assessments were performed in June, using one randomly chosen tree per 4 ha. Egg traps baited with AM + CAO were placed (to the nearest tree) in 100 × 100 m grids (i.e., one trap per ha), with the outermost traps 50 m from the orchard edge. Virgin female-baited wing traps were placed every 16 ha so that they were 200 m apart, 100 m from the nearest orchard edge, and 70 m from the nearest egg trap. The number of virgin-baited traps used was, respectively, 4, 3, and 4 for sites 1–3, and 2 for all remaining sites. Trapping began 21 March 2008 and continued 37 wk. For egg traps from this location there were a total of 11,288 trap-week observations, but irrigation schedules and poor drainage from heavy soils at this location resulted in variation in intervals for changing traps. For regression and correlation, observations were limited to the 7,089 weekly egg trap observations in which the traps were in the field for 7 d.

**Oviposition Substrate and Daily Fecundity.** To examine the effect of female age and fecundity and substrate preference on the number of eggs laid, age-specific fecundity was compared in a two-choice experiment in which females had access to either an egg trap or a filter paper for oviposition, or two no-choice experiments in which one or neither of these substrates were available.

Mated females for these laboratory experiments were obtained by isolating males and females in the last larval instar based on the presence or absence of testes. The lab strain CPQ-2005 had been maintained for 40–45 generations at the time of these experiments. Isolated males and females were placed on bran diet and allowed to complete pupation and eclose to adults. Five males and five females, eclosed within the previous 12 h, were placed in a 0.95 liters glass jar containing a 15 cm diameter filter paper (Whatman one Qualitative, GE Healthcare, Piscataway, NJ) and held overnight under colony conditions. The filter paper was folded in the center and in three equal intervals to each side to provide four pleats, and served to facilitate mating. Under such conditions, the navel orangeworm mates in the last hour of scotophase and mated pairs remain in copulo for 1–2 h (Landolt and Curtis 1982). These mating jars were examined at the beginning of photophase and pairs in copulo were isolated, allowed to uncouple, and the male was then discarded. In all experiments, water was provided *ad libitum* via a damp cotton ball placed on the mesh covering of the experimental container.

A two-choice test was used to examine the proportion of eggs laid on egg traps or filter papers by females of various ages. Filter paper is used as an oviposition

**Table 2.** The relationship between egg trap baits, mummies per tree, and the slope of regression of proportion of egg traps with eggs on mean eggs per trap

County	Site	Egg trap bait	Mummies per tree	Slope	r <sup>2</sup>
Kern	1	AM <sup>a</sup>	0.08 ± 0.02	0.35 ± 0.016	0.93
	2	Alm + Pis	0.02 ± 0.01	0.34 ± 0.028	0.86
	3	Alm + Pis	<0.01	0.34 ± 0.025	0.88
Fresno	1	AM + COA	13 ± 3.4	0.28 ± 0.014	0.94
	2	AM + COA	16 ± 5.2	0.26 ± 0.016	0.93
	3	AM + COA	2 ± 1.1	0.23 ± 0.019	0.88
	4	AM + COA	10 ± 1.6	0.27 ± 0.017	0.92
	5	AM + COA	6 ± 3.5	0.20 ± 0.017	0.81
	6	AM + COA	10 ± 3.3	0.26 ± 0.016	0.93
	7	AM + COA	2 ± 0.8	0.27 ± 0.014	0.95
	8	AM + COA	6 ± 2.2	0.29 ± 0.020	0.93

<sup>a</sup> AM, almond meal; Alm + Pis, equal parts ground almond and pistachio; AM + COA, almond meal with 10% crude almond oil.

substrate in rearing and laboratory tests (Tebbetts et al. 1978). The two-choice experiment examined eggs laid on either 9 cm filter papers or egg traps (as used in the field experiments) and baited with AM + COA, when both were present in 3.8 liters jars. Filter papers were folded as described previously, and the accessible surface area of the egg traps and the filter paper were approximately the same (129 vs. 127 cm<sup>2</sup>). The egg trap and the filter paper were placed on opposite sides of the jar, and a piece of transparent tape was used to attach one edge of the filter paper to the jar such that the top rested against the side of the jar. Jars were maintained under colony conditions and filter papers and egg traps were changed for every 2 d for the life of the female. After removal from the jars, filter papers or egg traps were frozen at -20°C pending evaluation. Females were dissected after death to confirm that mating had occurred. Eighteen replicates were examined in this manner.

Based on preference for oviposition on egg traps observed in the previous experiment, a no-choice experiment was conducted comparing total and age-specific fecundity between females randomly assigned to one of two these two substrates (the egg trap or the filter paper). The substrate and female were held in 0.47 liters glass jars. The jars were maintained under colony conditions filter papers and egg traps were changed for 2-d intervals for the life of the female. Substrates were changed and data collected as described for the two-choice experiment. Fifteen replicates were examined for each substrate.

A second no-choice experiment further examined effects of oviposition substrate and laboratory strain on total and age-specific fecundity. This experiment used adults obtained from eggs collected from egg traps at the Mendota sites and reared in the laboratory as previously described. In this case a third treatment was added: females were placed in 0.47 liters glass jars with no egg trap or filter paper. Jars were maintained and data collected as described for the previous experiment. This experiment was replicated 10 times each for egg traps and jars with no substrate added, and nine times for filter papers.

**Statistical Analysis.** Analysis was performed using the SAS System (Cary, NC) (SAS Institute Inc., 2008). The proportion of egg traps with eggs (incidence) was compared with mean eggs per trap using simple linear regression, with incidence transformed using the arcsine of the square root of the proportion and the mean eggs per trap transformed using the natural log (Zar 1999). Analysis of Covariance (ANCOVA) (Zar 1999, Zarnoch 2009) was used to test for differences in slope and elevation of regression parameters between sites at each location, and between the two locations. Linear regression of mean eggs on egg traps on mean females per bucket traps (untransformed variables) was used to examine the association between these measures of navel orangeworm activity. Pearson (linear) correlation (Zar 1999) was used to test the association of egg traps and males in virgin female-baited traps. The association between the proportion of eggs with egg traps and females trapped concurrently at the

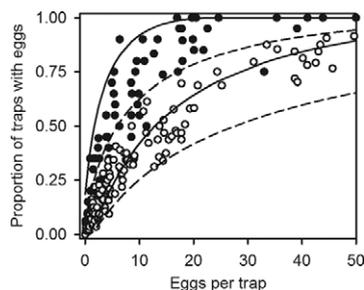


Fig. 1. Comparison of the proportion of traps with eggs (i.e., incidence) with the mean eggs per trap (for the same group of traps). Black dots are from Kern County, 2004, and white circles are from Fresno County, 2008. The solid and dashed lines represent the 95% confidence interval for prediction for Kern and Fresno County, respectively. The horizontal axis includes >95% of the means from Kern County (maximum = 79.6), and >90% of the observations from Fresno County (maximum = 414.1).

Kern County location was nonlinear, so Spearman rank correlation ( $\rho$ ) was used to analyze those data. The experiments examining oviposition substrate and fecundity were analyzed using paired Student's *t*-tests, unpaired Student's *t*-tests, and one-way analysis of variance (ANOVA), respectively. Eggs were transformed as the square root for stabilization of variance. Error is reported as standard error, means, and errors in the figures are based on untransformed data, and 95% confidence intervals for prediction for the regressions of proportion on mean for egg traps were back-transformed.

## Results

**Association of Egg Incidence with Eggs and Mean per Egg Trap.** The egg trap data fit very well to linear regression of the arcsine-transformed proportion of traps with eggs on the log-transformed mean eggs per trap, as indicated by high  $r^2$  values (Table 2) and visual examination (Fig. 1). For the three Kern County sites, the slopes were homogeneous ( $F = 0.19$ ;  $df = 2, 85$ ;  $P = 0.83$ ), as were the intercepts ( $F = 0.52$ ;  $df = 2, 85$ ;  $P = 0.60$ ). For the Fresno County sites there were differences among the slopes ( $F = 2.52$ ;  $df = 7, 160$ ;  $P = 0.0173$ ), but not among intercepts ( $F = 1.66$ ;  $df = 7, 160$ ;  $P = 0.12$ ). Contrast statements revealed a significant difference between the slopes for sites 5 and 8 ( $F = 13.63$ ;  $df = 1, 160$ ;  $P = 0.0003$ ), but no other differences were significant. After removing site 5 as an outlier, ANCOVA comparing the pooled data for the Kern and Fresno County locations found significant differences in both slope (mean and SE of  $0.35 \pm 0.012$  and  $0.27 \pm 0.012$ , respectively) ( $F = 42.32$ ;  $df = 1, 239$ ;  $P < 0.0001$ ) and intercept ( $0.15 \pm 0.027$  and  $0.07 \pm 0.014$ , respectively) ( $F = 9.46$ ;  $df = 1, 239$ ;  $P = 0.0023$ ). Nonetheless, a plot of the data used in this analysis shows great overlap of data points when there are few eggs on traps (e.g.,  $\leq 30\%$  of traps with eggs), and overlap in the 95% confidence interval for prediction even near 100% of traps with eggs (Fig. 1).

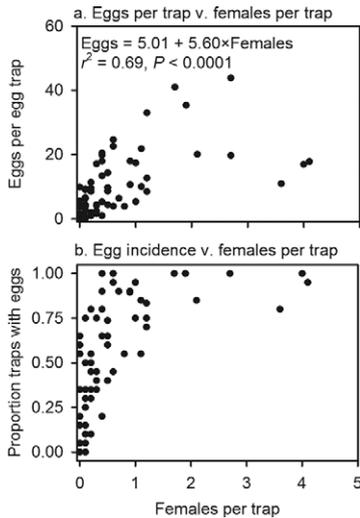


Fig. 2. Comparison of egg traps with concurrent female trapping in Kern County, 2004. The horizontal scale contains >90% of the observations (maximum = 12.2). (a) Mean eggs on egg traps versus mean females in bucket traps. (b) The proportion of egg traps with eggs versus mean females in bucket traps. There was a significant linear association of mean eggs with mean females, and a significant nonlinear association (Spearman  $\rho = 0.84$ ;  $P < 0.0001$ ) of egg incidence with mean females.

**Association of Egg Traps with Concurrent Trapping of Males and Females.** There was a significant linear association of mean eggs on egg traps with mean females per bucket trap with the same almond-based baits in concurrent trapping at the Kern County sites (Fig. 2a), and there was a significant nonlinear association of egg incidence with females captured (Spearman  $\rho = 0.84$ ,  $N = 89$ ,  $P < 0.0001$ ; Fig. 2b). Of the 890 individual weekly traps examined, 68% had zero females, 15% had one female, and 17% had more

than one female. In general, female traps averaged fewer than one female per trap while <50% of egg traps contained eggs, and multiple females per trap were not common unless all egg traps contained eggs (Fig. 2b).

Weekly plots comparing trapping of males and eggs at the Fresno County site revealed earlier activity in the male traps than in egg traps (Fig. 3). Considering only observations after 21 May, the goodness-of-fit for correlation of mean eggs per egg trap with males captured concurrently at the same site ( $N = 113$ ;  $r^2 = 0.09$ ;  $P = 0.0011$ ) is poor compared with that of proportion of egg traps with eggs versus males ( $r^2 = 0.39$ ;  $P < 0.0001$ ), although the goodness-of-fit for mean eggs per trap is improved by log transformation ( $r^2 = 0.35$ ).

**Oviposition Substrate and Daily Fecundity.** The two-choice test showed distinct differences in the number of eggs laid on the two substrates over the life of the female, and in the proportion of eggs laid on paper versus egg trap as the female aged. Over their lifetimes, females laid significantly more eggs on egg traps than on filter paper ( $142 \pm 22$  vs.  $35 \pm 12$ , mean  $\pm$  SE;  $t = 4.05$ ,  $df = 17$ ;  $P = 0.0008$ ). Younger females exhibited a greater preference for oviposition on egg traps compared with older females (Fig. 4a). There was no mortality before day 7 posteclosion, and the mean longevity was  $10.7 \pm 0.54$  d. The difference between total eggs on filter papers and egg traps was significant ( $P < 0.0001$ ) on days 3, 5, and 7 (Fig. 2). Differences between the eggs on the two substrates were not significant on other days ( $P > 0.05$ ).

In the first no-choice test, there was no significant differences between the two substrates in lifetime total egg production per female ( $144 \pm 22$  vs.  $127 \pm 18$ ;  $t = 0.23$ ,  $df = 28$ ,  $P = 0.69$ ). There was also no significant difference in longevity ( $t = 0.53$ ;  $df = 28$ ;  $P = 0.60$ ) between egg traps ( $12.9 \pm 0.74$  d) and paper ( $13.4 \pm 0.69$  d). Moreover, there was no difference between substrates in age-specific fecundity within

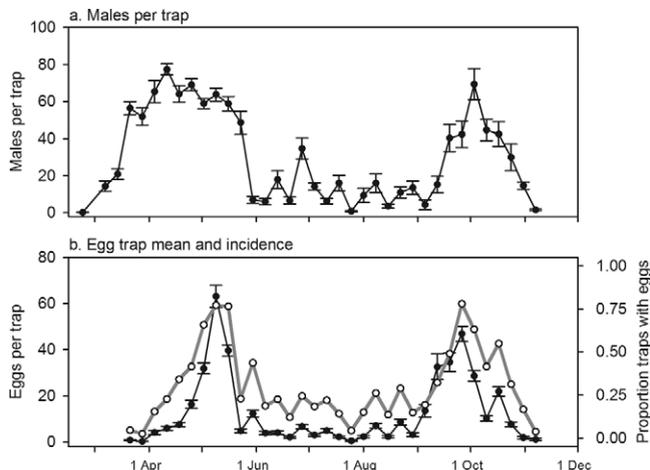
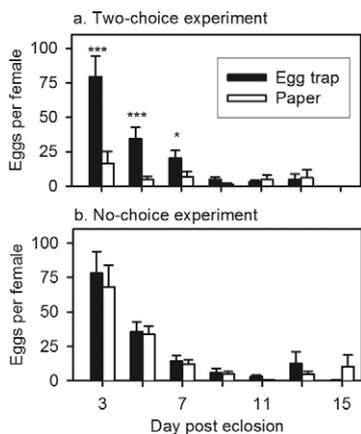


Fig. 3. Comparison of weekly trapping for males and eggs in Fresno County, 2008. (a) Males per pheromone trap (mean  $\pm$  SE). (b) Mean ( $\pm$ SE) eggs per trap (black dots) and incidence of traps with eggs (white circles). The correlation with males per trap after 21 May was higher for egg incidence ( $r^2 = 0.39$ ;  $P < 0.0001$ ) than for egg means ( $r^2 = 0.09$ ;  $P = 0.0011$ ).



**Fig. 4.** Comparison of the proportion of eggs laid on egg traps or filter papers, by age category: (a) Two-choice experiment in which each female had access to both substrates. (b) No-choice experiment, comparing oviposition by females with access to only an egg trap or only a filter paper. While younger, more fecund females in the two-choice test laid significantly more eggs on egg traps than on filter paper (Paired Student's *t*; \*\*\**P* < 0.001, \**P* < 0.01), there was oviposition on both substrates at all ages and less preference as females aged. In the lack of choice, there was equal oviposition on either substrate.

the 2-d increments (Fig. 4b), indicating that the substrate did not significantly affect either the total number of eggs laid or the age at which they were laid.

In the second no-choice test, females reared from eggs obtained at the Fresno County location lived  $9.7 \pm 1.02$ ,  $12.2 \pm 1.16$ , and  $10.0 \pm 0.87$  d when placed with egg traps, filter papers, or in empty jars, respectively. The difference between these means was not significant ( $F = 1.79$ ;  $df = 2, 26$ ;  $P = 0.19$ ). Lifetime fecundity was  $114 \pm 22$ ,  $88 \pm 22$ , and  $131 \pm 34$  eggs per female for these respective treatments. The difference between these means was not significant ( $F = 0.19$ ;  $df = 2, 26$ ;  $P = 0.83$ ). Age-specific fecundity for each of the three treatments was similar to that shown in Fig. 4b for the previous experiment, and the differences were not significant (data not shown).

**Discussion**

The difference between the Kern and Fresno County data sets in slopes of the regression of egg trap incidence on egg trap mean is of interest because of what it reveals concerning the impact of bait type and sanitation on trap effectiveness. While the intercept differed significantly between the two locations, it was in both cases close to zero (i.e., <3% traps with eggs). Therefore, the primary meaning of the slope is that sites with a numerically lower slope had more eggs per traps as that egg traps approach "trap saturation" (defined for this paper as the point at which all egg traps capture eggs) (Fig. 1). In the Kern County data set, the different baits used had no discernable effect on this relationship. The Fresno County data set used the AM bait that was also used in site one of the Kern

County location, with 10% crude almond oil added (Van Steenyk and Barnett 1985). A recent study found no consistent added benefit with the use of 10% crude almond oil in AM, as compared with 3% crude almond oil or none (Kuenen et al. 2008). Therefore, these data indicate that the difference between the baits used in this study was less important to the number of eggs found on traps than orchard factors (e.g., navel orangeworm abundance).

The major difference between the Kern County and Fresno County locations is that the latter had  $\approx 100\times$  as many mummies as the former. The lower slope in the Fresno County site for regression of incidence of means indicates that, at similar levels of trap saturation, these traps had more eggs than the Kern County traps. There was significant variation in slopes between the Fresno County sites, as indicated by a significant site  $\times$  slope term in the ANCOVA. There was, however, little apparent association between the incidence-mean slope and either sanitation level (within the range of the Fresno County site) or variety. For example, the lowest and highest slopes (sites 5 and 8) were both Nonpareil-Monterey plots with relatively few of mummies per tree, and sites 1 and 2, a Nonpareil-Monterey and Butte-Padre plot, respectively, each with many mummies, had slopes similar to each other and the site 8 (Table 2). Some pest management consultants believe that poor sanitation in almond orchards results in fewer eggs on egg traps despite higher navel orangeworm abundance (C.S.B., unpublished data). We have found a similar phenomenon in pistachio sanitation trials, where in blocks containing larger numbers of mummies there were fewer eggs per trap compared with blocks with few mummies. The blocks with more mummies had more navel orangeworm damage, and presumably higher abundance of navel orangeworm (Burks et al. 2005). The comparison presented here between the Kern and Fresno County sites suggest that in almonds, unlike pistachios, any decrease in eggs per trap that might occur because of competition from mummies in more poorly sanitized orchards is more than offset by the accompanying increase in navel orangeworm abundance.

The similarity and difference in the egg traps incidence-mean plot between the Kern and Fresno County locations also illustrates a limitation of using few egg traps per block. At lower points on this curve (smaller values of incidence or mean), the observations from the two data sets are largely overlapping. While there is greater difference between the two data sets as the incidence approaches 1.0, the 95% prediction intervals still overlap (Fig. 1). These observations demonstrate that, even if there is a discernable difference between baits or orchards at periods when there are many eggs per trap, this difference has little relevance during periods when there are few eggs per trap, as is the case in June and July when egg traps are typically used to inform insecticide treatment timing in almonds. In that case, more traps are needed (Higbee and Burks 2011).

The laboratory experiments examining the fecundity and the proportion of eggs placed on preferred and nonpreferred hosts offer a further illustration of why the proportion of traps with eggs might be a more stable index of female abundance than the number of eggs per trap. In the two-choice experiment it was not surprising that egg traps, carefully developed to attract navel orangeworm oviposition, were more attractive than paper. In particular, younger, more fecund females laid more eggs on egg traps than on paper, but eggs were laid on both substrates. As females aged, eggs were placed more evenly on the two substrates. The first no-choice test demonstrated that, in the absence of access to a preferred substrate, total or daily fecundity on a nonpreferred substrate were not reduced. The second no-choice test further supported this point by examining oviposition in an empty jar. The use of adults developed from eggs obtained in the field for this second no-choice experiment indicated that oviposition on paper or glass was not an artifact inadvertently selected for by the laboratory rearing procedures. These experiments are obviously a gross oversimplification of the field situation, in which one egg trap in a tree competes with 0–20 mummies and 5,000–7,000 current-crop almonds (USDA-NASS 2010). Still, these experiments illustrate how the number of eggs laid on the egg traps is affected both by female age and fecundity and by relative attractiveness of surrounding almonds, and why these factors might affect mean (i.e., the number of eggs per trap) more than incidence (i.e., whether a trap as eggs).

The observation of higher correlation of number of males in pheromone traps with egg incidence than with mean eggs per trap further suggests that binomial analysis would be advantageous for egg trap data, as was the case for trap data for various flying insects in a previous study (Nansen et al. 2008). While it is true that the correlation for log-transformed flight trap means was almost as good as that for proportions, it is important to note that these were means of 16–64 traps; far more than the 2–4 traps per block currently used. The primary benefit for binomial analysis would be if it convinced practitioners to increase the number of traps per block. Unfortunately, the amount of time and effort to carefully examine an egg trap and discern the difference between 0 and 1 egg (usually done in the field rather than in the laboratory as in this study) is not much less than the time necessary to count the few eggs found on the vast majority of egg traps. However, the fact that egg trap incidence correlates well with the number of females per trap suggests that trapping females with wing traps baited with almond meal would give information similar to binomial trapping with egg traps. Trapping for females would have the advantage of being amenable to quick visual assessment, similar pheromone traps. If this approach proves useful, it could likely be adopted to other female attractants as they become available in a practical form (Beck et al. 2009, Burks et al. 2009).

One potential objection for going from monitoring eggs to females is that information on population fecundity is given up. For the purpose of making treat-

ment decisions, this loss would probably be offset if the result that it is cost-effective to use more traps in a block. However, another possible use of egg traps is as a research tool, to monitor the effectiveness of mating disruption treatments. That use of egg traps is not an ongoing activity like monitoring for pest management, and has different cost-benefit considerations. In addition, the effect of mating disruption treatment at periods when the both the proportion of traps with eggs and the number of eggs per trap are high is more relevant for assessing the effect of mating disruption treatments. For this purpose egg traps allow direct assessment of effects on fertility, in comparison to inferences based on female captures and spermatophore dissections that have been necessary for other orchard pests (Rice and Kirsch 1990, Vickers 1990, Knight 2006).

In summary, in this study we found that the bait used in egg traps and orchard sanitation had little effect on the number of eggs per egg trap in almond orchards. We also found that egg trap incidence is less variable than mean eggs per trap, and correlates well with males captured in traps with virgin females as a pheromone source. These observations suggest use of binomial analysis for egg trap data. However, that approach would require more egg traps and would not reduce by much the time needed to collect data from egg traps. Therefore, we instead suggest using egg traps in wing traps to trap females, as suggested when these traps were invented (Rice 1976). The data presented in this study demonstrate that females captured in such traps correlate well with the data in egg traps, and it is quicker to assess the number of adult moths in a sticky trap than to count eggs on an egg trap. The reduced time for data collection will allow more traps per block to be examined in the same time, providing better information for individual management blocks.

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