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Research Article

Sampling and Biostatistics

Predicting Nut Damage at Harvest Using Different in-Season Density Estimates of *Amyelois Transitella*: Analysis of Data from Commercial Almond Production

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Abstract

Despite decades of research on management tactics for the navel orangeworm, *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae), on almonds, we still do not have an established means of using in-season pest-density estimates to predict damage to nuts at harvest. As a result, hull-split pesticide applications, although timed carefully to coincide with navel orangeworm oviposition and with crop vulnerability, are not tied to pest densities—thus falling short of our goals under modern pest management. Here we use an ecoinformatics approach, analyzing a pre-existing data set collected in commercial almond production in California, to ask: 1) are navel orangeworm density estimates obtained using different sampling methods in strong agreement with one another? and 2) can we use either single density estimates or combinations of density estimates to explain variation in nutmeat damage at harvest? We find that correlations between density estimates of navel orangeworm made over a single growing season are often weak, and suggest that density estimates taken closer to the time of harvest (catches of adult females between hull split and harvest; infestation of early-split nuts) may be most useful for predicting damage at harvest. Single-density estimates explained ≤39.1% of variation in harvest damage, whereas a combination of predictors explained 51.5% of the total variance in nutmeat damage at harvest. Our results suggest that density estimates taken just prior to harvest may, with refinement, be usable within a predictive framework to guide late-season control decisions.

Key words: ecoinformatics, sampling methods, collinearity, predicting damage, navel orangeworm

Modern agricultural pest management generally strives to incorporate the following elements: a sampling method that provides good estimates of the pest population density; a well-characterized relationship between the estimated pest density and expected economic losses due to crop damage; and effective interventions to suppress the pest population when a damaging density is expected to be reached (Pedigo and Rice 2009). Although such an approach may seem straightforward, in some cases implementation can be quite difficult. For high-value crops attacked by direct pests, tolerance for pest damage is limited, and the associated economic injury levels are quite low. Pest managers may, therefore, be faced with the difficult task of trying to sample a very low-density pest population; yet, sampling methods may not be sufficiently efficient or precise for such applications. It may also be difficult to predict expected levels of crop damage when the pest is sufficiently mobile that insects move readily between fields. In this case, sampling the resident, within-field population may provide only a partial picture of the damage potential of the broader pest population.

The navel orangeworm, *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae), in California almonds exhibits several characteristics that create significant pest-management challenges. Navel orangeworms often feed directly on almond nutmeats. Tolerance for infestation is extremely low (<2% of nuts infested, or lower for some growers), both because of the high value of the almond crop ($6,772/acre, Almond Board of California 2015), and because navel orangeworm vectors *Aspergillus* spp. fungi that produce aflatoxins (Palumbo et al. 2014). Strict limits have been established for aflatoxin B1 contamination of almonds by both the United States (15 ppb) and the European Union (8 ppb), leading to rejection of some almond lots (Schatzki and Ong 2001, Palumbo et al. 2014).

Adult moths are highly mobile, and movement between almond orchards or from pistachio orchards to almond orchards can have substantial influences on infestation at harvest (Andrews and Barnes 1982, Higbee and Siegel 2009, Sappington and Burks 2014). Several sampling methods have been developed, including sampling of overwintering larvae in mummy nuts (Burks et al. 2008), sampling of males with sticky traps baited with virgin females (Curtis and Clark 1984, Burks and Higbee 2015) or synthetic sex pheromone (Higbee et al. 2014), sampling of egg laying ('egg traps') or adult females using traps baited with almond meal (Rice 1976, Higbee and Burks 2011), and sampling of the first nuts to exhibit hull split to assess infestation prior to harvest. Nevertheless, an economic injury level based on in-season monitoring of navel orangeworm has never been developed (University of California 2002, Kuenen and Siegel 2016). Furthermore, some researchers have suggested that standard commercial implementations of navel orangeworm sampling, using low densities of egg traps (2–4 traps per 64 ha management units) may be unreliable because these traps are relatively insensitive (Burks et al. 2011, Higbee and Burks 2011). Perhaps as a consequence, most monitoring has been used only to improve the timing of insecticide applications rather than as a means of estimating population density and deciding which orchards need pesticide applications and which do not (Higbee and Burks 2011). Pesticide resistance has emerged in navel orangeworm populations (Demkovich et al. 2015), threatening effective control. Winter sanitation of mummy nuts and early harvest of mature nuts remain as important elements of integrated control for this pest (University of California 2002).

The goals of the current study were 1) to assess the extent to which different sampling methods for navel orangeworm agree with one another when used in the same almond blocks during the same growing season, and 2) to determine to what extent variation in harvest damage can be explained, either by single navel orangeworm density estimates or by the combined use of multiple, in-season estimates of navel orangeworm density within a focal almond block. We emphasize that we are not seeking to create a predictive model ready for farmer use; to assess predictive accuracy of such a model would require some sort of cross-validation (e.g., a year-forward cross validation, using a candidate model to predict 'test' infestation data that are separate from the 'training data' used to fit the model). Rather, our goal was to identify those metrics of navel orangeworm density that have the highest potential, with future refinement, to serve as the basis for such a predictive model.

The ability to predict navel orangeworm damage based on estimates of pest damage within a focal block depends, in part, on the relative sizes of the resident, within-block pest populations, and the density of immigrating moths coming from neighboring orchard blocks. For this reason, the most useful data should reflect the real spatial scale of commercial almond production. With this in mind, we adopt an ecoinformatics approach, using a pre-existing data set to address ecological processes that occur at spatial scales too large for easy experimental manipulation (Rosenheim and Gratton 2017). Our results underscore both the management challenges and opportunities for this pest, and highlight the need for continued research.

Materials and Methods

Data Source

We studied two large almond ranches located in western Kern County in the San Joaquin Valley, CA (Wonderful Orchards). Data were originally gathered in support of an area-wide trial of navel orangeworm management using mating disruption; as described subsequently, mating disruption was used at the ranches we studied, but not in the surrounding areas. Data were gathered at the Santa Fe Ranch (36.642059, −119.975457), 2500 acres of almonds planted in 1990 and 1993 and organized into 21 blocks, from 2009 to 2012, and at the Lost Hills Ranch (35.551184, −119.651321), 2800 acres of almonds planted in 1996 and 1997 and organized into 30 blocks, from 2009 to 2015. Each block was planted as a mixture of two or three almond varieties; differences between varieties in infestation by navel orangeworm are analyzed separately (Rosenheim et al. in press); here we analyze only variety Nonpareil, which made up nearly half of both plantings, and which is the dominant variety planted in California (Almond Board of California 2015). Trees were topped to maintain heights between 5.5 and 6.7 m. Both ranches were directly adjacent to potential sources of navel orangeworms, including pistachio orchards or almond orchards, where sanitation to remove mummy nuts was either omitted or minimally performed (B.S.H., personal observations).

Almond blocks were rotated across years through two, or in some cases three, different navel orangeworm control treatments: 1) 'conventional' management, which typically included two insecticide applications (usually methoxyfenozide or bifenthrin, rarely other materials) targeting navel orangeworm, the first during the spring and the second at hull split; 2) 'mating disruption', which usually did not include insecticide treatments; and 3) 'conventional + mating disruption,' which received a combination of both treatments. There was, however, some variation between plots and years in the number of pesticide applications made; to capture this variation in our statistical model, we included variables for the number of insecticide applications during the spring (1 April–15 June) and during hull split (16 June–15 August). Mating disruption was applied using high-emission dispensers ('Puffers'; CheckMate Puffer NOW; Suterra LLC, Bend, OR) releasing just the principal component of the female sex pheromone [(11Z,13Z)-hexadecadienal; Coffelt et al. 1979, Leal et al. 2005, Kuenen et al. 2010]. Details of release rates (full vs. half of normal active ingredient), the number of dispensers per acre (one versus two), and the timing of mating disruption initiation (March, or 'very early'; April, or 'early'; June, or 'late') varied across blocks and years; we coded this variation using categorical variables for our statistical model. Failing to find significant differences between the various implementations of the mating disruption treatment, we collapsed all variants into a single category ('mating disruption'). All blocks received rigorous sanitation to remove mummy nuts, including hand-crews using poles to dislodge nuts remaining in the trees after harvest, hand-removal of nuts lodged in the crotches of major branches and the tree trunk, and flail-mowing of harvest residue on the orchard floor to reduce the number of intact mummy nuts. This sanitation was quite effective; mean numbers of remaining mummies per tree were 0.53 ± 0.98 (mean ± SD) on branches, 0.16 ± 0.25 in crotches of tree, and 7.69 ± 7.10 on the ground.

Sampling Navel Orangeworm Populations

Sampling was conducted using several methods. Although one of these methods, using sticky traps baited with virgin female moths, is strictly a research tool, the remaining methods were all used in a way that is now commensurate with commercial practice in these orchards.

Post-Sanitation Samples of Mummy Nuts

After the completion of sanitation activities and before the spring emergence of adult navel orangeworm moths, each block was sampled to quantify the number of mummy nuts remaining. A single sample, covering four trees in each of two adjacent rows (eight trees total) was taken at 8–16 locations per block, and consisted of all mummy nuts found: 1) still attached to the tree; 2) lodged in the crotches of the main tree limbs or trunk; or 3) on the ground. All nuts were returned to the laboratory, opened, and scored for navel orangeworm infestation. Initial analyses showed that >90% of all mummies were found on the ground; we, therefore, merged all counts to create a single variable, total mummies per tree infested by navel orangeworm, as a first measure of resident navel orangeworm population density within each block.

The remaining sampling methods were conducted at 1–4 locations per block.

Adult Male Moths Attracted to Virgin-Female Baited Sticky Traps

Adult male navel orangeworm moths were collected in orange wing traps (Suterra LLC, Bend, OR) modified as described in Kuenen et al. (2005) and baited with three 1–2-night-old unmated female navel orangeworm adults, confined in a mesh bag (Curtis and Clark 1984). Female moths were lab-reared as described in Higbee et al. (2014). Fresh females were added weekly, and male captures counted. Males largely lose the ability to orient to calling females in blocks under mating disruption treatments (Higbee and Burks 2008, Burks et al. 2016). When we attempted to estimate navel orangeworm population densities by averaging male trap catches from hull split to the approach of harvest (18 June–12 August), nearly all blocks that were under mating disruption had averages of zero catch, and the resulting variable was not a significant predictor of almond damage at harvest (data not shown). We, therefore, averaged male captures only from first catches through 25 March, and prior to the start of mating disruption ('early males'). We emphasize, however, that such an early trapping period, which occurs before most of the overwintering population has emerged (Fig. 1), was, a priori, unlikely to be a strong predictor of nut damage at harvest. Additional work will, thus, be required to determine if later-season male catches in orchards not under mating disruption might be a useful predictor of harvest damage.

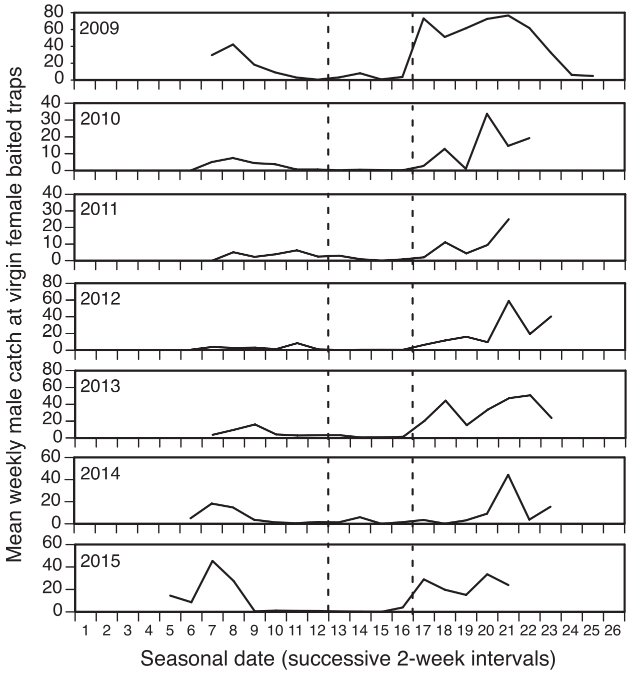

Fig. 1. Navel orangeworm phenology in almond orchards; shown are the mean weekly captures of adult males at sticky traps baited with virgin females across successive 2-wk intervals across all blocks that were managed conventionally (i.e., without mating disruption; there were 3–6 conventionally managed blocks each year) at the Lost Hills Ranch, 2009–2015. Our analyses break the growing season prior to harvest into two periods (break points shown as vertical dashed lines): 1) before hull split (1 January–17 June), and 2) from hull split to harvest (or approaching harvest; 18 June–12 August).

Adult Female Moths Attracted to Almond Meal Baited Sticky Traps

Adult female navel orangeworm moths were collected in Delta sticky traps (Suterra LLC, Bend, OR) baited with commercial almond meal (Liberty Vegetable Oil Company, Santa Fe Springs, CA) held in a standard egg trap (a black plastic cylinder, 8.6 cm long, 1.6 cm in diameter, and fitted with three mesh-covered holes, 1.1 cm in diameter; see Higbee and Burks 2011). Traps were checked weekly to record the number of adult females captured, and baits were changed every 2 wk. Trap catches were averaged over two periods: before hull split (1 March– 17 June) and hull split to the approach of harvest (18 June–12 August; harvest typically occurred from the second week of August through the first week of September; henceforth, we refer to the 18 June–12 August period 'hull split to harvest').

Eggs Deposited on 'Egg Traps'

The same egg traps were also used alone as an oviposition substrate for adult females (Rice 1976, Higbee and Burks 2011). Egg traps were collected weekly, returned to the laboratory, and inspected under a stereomicroscope to count deposited eggs. The numbers of eggs laid per trap were averaged over two periods: before hull split (1 March– 17 June) and hull split to harvest (18 June–12 August).

Pre-Harvest Samples of Early-Split Nuts

Navel orangeworms oviposit on the new crop of almonds when the hulls split as the nuts begin to mature. Samples of the nuts showing the earliest-splitting hulls can be assessed for infestation early enough to inform growers about the potential need for hull-split insecticide applications. Sampling was variable across years, with between 2 and 7 samples taken at roughly weekly intervals beginning between 19 June and 13 July. Samples of up to 30 early-split nuts were taken at each sample; when early split nuts were scarce, as many early-split nuts as could be found in 10 mins of search were collected. Nuts were returned to the laboratory, opened, and scored for infestation by navel orangeworm. Infestation was averaged across samples to produce a single estimate of proportion infestation pre-harvest.

Quantifying Nut Damage at Harvest

Within each block, 1–4 samples of approximately 500 nuts each were taken at harvest each year after the nuts had been shaken to the ground and swept into rows. Sampled nuts were returned to the laboratory, opened, and scored for whether or not the navel orangeworm had damaged the nutmeat. All nut samples taken within a block were combined to create a single sample [mean = 1081 ± 432 (SD) nuts/sample] for statistical analysis. Across all blocks and years, 206,385 nuts were scored.

Statistical Analysis

We performed two analyses to quantify the degree to which the different metrics of navel orangeworm density, generated with different sampling methods or covering different periods of the growing season, were positively correlated with one another. First, we computed simple pairwise correlations between each of the density

Table 1. Matrix of pairwise correlation coefficients for the seven measures of navel orangeworm population densities in almond orchards during the growing season

Variable	1	2	3	4	5	6	7
1. Post-sanitation sample	1.00						
2. Early male catches	0.157	1.00					
3. Egg traps—before hull split	0.182	0.140	1.00				
4. Females—before hull split	0.206	0.224	0.837	1.00			
5. Egg traps—hull split to harvest	0.196	0.134	0.567	0.619	1.00		
6. Females—hull split to harvest	0.089	0.117	0.333	0.309	0.446	1.00	
7. Pre-harvest infestation	0.057	0.118	0.103	0.099	0.143	0.080	1.00
As sole navel orangeworm density metric in model	0.533	0.514	0.591	0.570	0.610	0.625	0.559

metrics. Second, we performed a variance inflation factor analysis, using package 'usdm' in R (Naimi 2015). Variance inflation factor analysis is a method for detecting collinearity, or strong correlations between two or more predictor variables. It calculates whether values of a predictor are strongly correlated with any linear combination of alternate predictors.

We used generalized estimating equations (GEE), implemented in R program geepack, vers. 1.2-1 (Højsgaard 2016), to build a statistical model explaining variation in navel orangeworm damage of almond nutmeats at harvest, while accommodating the repeated observations made on the same almond blocks, which were taken as the basic replicate unit of observation for our analysis (Hardin and Hilbe 2013). Because nut damage (yes/no) is a binary response variable, we used a binomial variance model and a logit link function. We modeled residual variance using the autogressive-1 option, which is appropriate when successive observations are expected to be more similar to each other than observations that are more widely separated in time. All predictors were included as fixed effects (year; the number of pesticide applications made during the spring, or during hull split; navel orangeworm control method; post-sanitation mummy nut infestation; early male moth captures; adult female catches before hull split, or between hull split and harvest; eggs deposited on egg traps before hull split, or between hull split and harvest; and infestation of early-split nuts pre-harvest). Only pesticide applications made with navel orangeworm cited as the target were counted. We fit a full model with all predictors included, and also fit models with each of the navel orangeworm density metrics considered as the sole estimator for pest pressure, to assess our ability to predict nut damage at harvest. Complete cases were required for statistical modeling, and our final data set included observations for 191 block-year combinations.

Results

Catches of adult male navel orangeworms at virgin female baited traps indicated that the period before hull split (1 March–17 June) included the sometimes extended emergence of overwintering moths, whereas the period from hull split to harvest (18 June–12 August) captured the often weak second flight and, in some years, the beginning of the third flight (Fig. 1).

The different methods of sampling navel orangeworm within individual almond blocks produced density estimates that were always positively correlated, but often only weakly so (Table 1). Population density estimates that were contemporaneous (e.g., adult female catches before hull split and egg traps before hull split, $r = 0.837$; adult female catches from hull split to harvest and egg traps from hull split to harvest, $r = 0.446$) were generally more strongly correlated than estimates that were widely separated in time within the growing season (e.g., post-sanitation sample and pre-harvest infestation, $r = 0.057$; Table 1). The variance inflation factor analysis confirmed that collinearity is not strong within this set of predictors (Table 2). Only adult female catches before hull split and egg traps before hull split showed variance inflation factors >2, a result almost entirely explained by the strong pairwise correlation of these two variables (the pairwise correlation alone would produce a variance inflation factor of 3.34, which accounts for almost all of the total collinearity seen for these variables). Sticky traps baited with almond meal attract primarily gravid females (B.S.H., unpublished data); thus is not surprising that these traps and the egg traps generated correlated estimates of navel orangeworm density. Omitting one of these variables produced minimal changes in the full predictive model of nutmeat damage at harvest (data not shown). None of the variables reached a variance inflation factor value that would normally motivate omitting variables from the predictive model (i.e., variance inflation factor > 4, 5, or 10, depending on different rules of thumb; O'Brien 2007), and thus all the variables were retained.

Table 2. Variance inflation factor analysis of the seven measures of navel orangeworm population densities in almond orchards during the growing season

Variable	Variance inflation factor
Post-sanitation sample (infested nuts/tree)	1.05
Early male catches	1.08
Egg traps—before hull split	3.46
Egg traps—hull split to harvest	1.86
Adult female catches—before hull split	3.90
Adult female catches—hull split to harvest	1.27
Pre-harvest infestation	1.04

The full GEE statistical model of navel orangeworm damage of almond nutmeats at harvest incorporated many significant predictors and explained 51.5% of the total observed variation in damage (Table 3, Fig. 2). All significant effects were in the expected directions: increasing the number of hull split insecticide applications was associated with decreasing damage, whereas higher estimates of navel orangeworm density at any stage during the growing season were associated with higher damage at harvest (Table 3). Perhaps not surprisingly, the strongest predictors were density estimates of navel orangeworm taken later in the growing season: adult female catches from hull split to harvest and pre-harvest samples of infestation of nuts exhibiting early hull-split ($P < 0.001$ for each). Statistical models that included single navel orangeworm density estimates, along with variables for year, insecticide applications, and navel orangeworm management treatment generated correlation coefficients for the whole model (predicted versus observed) of ≤0.625, corresponding to 39.1% of total observed variation explained. When information from multiple density estimates was combined within a model, nearly half of the variation in damage remained unexplained. On the other hand, for the roughly three-quarters of the cases (147/191 = 77.0%) where

Table 3. GEE analysis of factors associated with navel orangeworm damage of almond nutmeats at harvest

Variable	Coefficient estimate	SE	Wald statistic	*P*
(Intercept[a])	-3.430	0.660	27.04	<0.0001***
Year				
2010	-0.960	0.494	3.77	0.052
2011	-2.220	0.496	20.03	<0.0001***
2012	-0.630	0.478	1.74	0.187
2013	-1.421	0.516	7.59	0.0059**
2014	-0.570	0.455	1.57	0.211
2015	0.213	0.422	0.25	0.614
Spring insecticide applications[b]	-0.683	0.398	2.94	0.087
Hull-split insecticide applications[c]	-0.204	0.070	8.47	0.0036**
Treatment (mating disruption)	-0.281	0.285	0.97	0.325
Treatment (conventional + mating disruption)	-0.387	0.216	3.22	0.073
Post-sanitation sample (infested nuts/tree)[d]	0.868	0.314	7.62	0.0058**
Early male catches[e]	-0.0002	0.0101	0.00	0.983
Adult female catches, before hull split[f]	0.204	0.563	0.13	0.717
Egg traps, before hull split[g]	0.075	0.026	8.15	0.0043**
Adult female catches, hull split to harvest[h]	3.172	0.876	13.13	0.0003***
Egg traps, hull split to harvest[i]	-0.009	0.041	0.05	0.822
Pre-harvest infestation[j]	4.213	0.897	22.06	<0.0001***

**$P < 0.01$; ***$P < 0.001$.

[a] Conventional navel orangeworm management treatment during 2009 is the baseline for comparison. The predicted proportion of nuts damaged by navel orangeworm at harvest is calculated as:

$$P(damage) = \frac{exp\left(\beta_0 + \beta_1 x_1 + \beta_2 x_2 + \cdots + \beta_q x_q\right)}{1 + exp\left(\beta_0 + \beta_1 x_1 + \beta_2 x_2 + \cdots + \beta_q x_q\right)},$$

where the coefficient estimates (β_0, β_1, etc.) are given above.

[b] Mean ± SD number of spring insecticide applications = 0.393 ± 0.490 (range: 0.0–1.0).

[c] Mean ± SD number of hull split insecticide applications = 0.524 ± 0.739 (range: 0.0–4.0).

[d] Mean ± SD number of infested nuts per tree = 0.101 ± 0.162 (range: 0.0–1.264).

[e] Mean ± SD number of males caught per trap per week = 3.38 ± 6.41 (range: 0.0–47.0).

[f] Mean ± SD number of adult females caught per trap per week before hull split = 0.081 ± 0.188 (range: 0.0 – 1.786).

[g] Mean ± SD number of eggs deposited per egg trap per week before hull split = 2.10 ± 4.15 (range: 0.0–32.93).

[h] Mean ± SD number of adult females caught per trap per week from hull split to harvest = 0.043 ± 0.081 (range: 0.0–0.500).

[i] Mean ± SD number of eggs deposited per egg trap per week from hull split to harvest = 1.137 ± 3.051 (range: 0.0–25.38).

[j] Mean ± SD proportion of early-split almonds infested with navel orangeworms prior to harvest = 0.016 ± 0.042 (range: 0.0–0.333).

the model predicted <2% nutmeat damage, such that growers might conclude that additional suppression of navel orangeworm densities was not needed prior to the harvest of Nonpareil nuts, actual nutmeat damage observed at harvest was generally modest, and we never observed damage levels >3.5% (Fig. 2B).

Discussion

We analyzed data from large-scale commercial almond production in California to assess the degree to which different sampling methods produce estimates of navel orangeworm densities that are in mutual agreement, and to see if these density estimates can be used to predict nutmeat damage at harvest. We find that although some density estimates, and especially those taken during the same periods of the growing season, are in good agreement, many of the estimates, and especially those separated more widely in time, are only very loosely correlated (Tables 1 and 2). Single navel orangeworm sampling methods, used alone, produced density estimates that explained ≤39.1% of observed variation in nutmeat damage, whereas a model incorporating multiple density estimates explained about half (51.5%) of all variation in damage.

Based on these results, we see some potential for building a predictive model for nut damage. The worst-case scenario for a grower using a predictive model would be to refrain from applying insecticides prior to harvest, and then sustain heavy damage. In our initial effort to explain variation in almond damage, such a worst-case scenario was not observed. The model predicted low, and generally below-threshold damage (<2%) for about three-quarters of all almond blocks (147/191 = 77.0%); in these cases, above-threshold damage was sustained only relatively infrequently (15/145 = 10.3% of cases), and in no case did the actual damage exceed 3.5%. Thus, with further improvements, we believe there is potential for predictive models to reduce the number of hull-split pesticide applications, thereby reducing costs, minimizing secondary outbreaks of spider mites (Hamby et al. 2013), and reducing the selection pressure that drives the evolution of pesticide resistance (Demkovich et al. 2015).

Although damage of 2.0–3.5% of nutmeats may be unacceptably high for many growers, we emphasize that our estimates of nut damage, assessed with hand-gathered nuts from the orchard floor after shaking, will generally be higher than damage rates reported for the same almond lots by commercial hulling operations. Nuts that are largely consumed by caterpillars are lighter than whole nuts, and as a result are separated from undamaged nuts during the harvest process. Air streams are used to separate leaves and dirt from intact nuts, and largely consumed nuts are often ejected with the lighter material at this stage. Data collected from 2009 to 2015 at Lost Hills show the magnitude of this effect: for 39 block-year combinations for which we

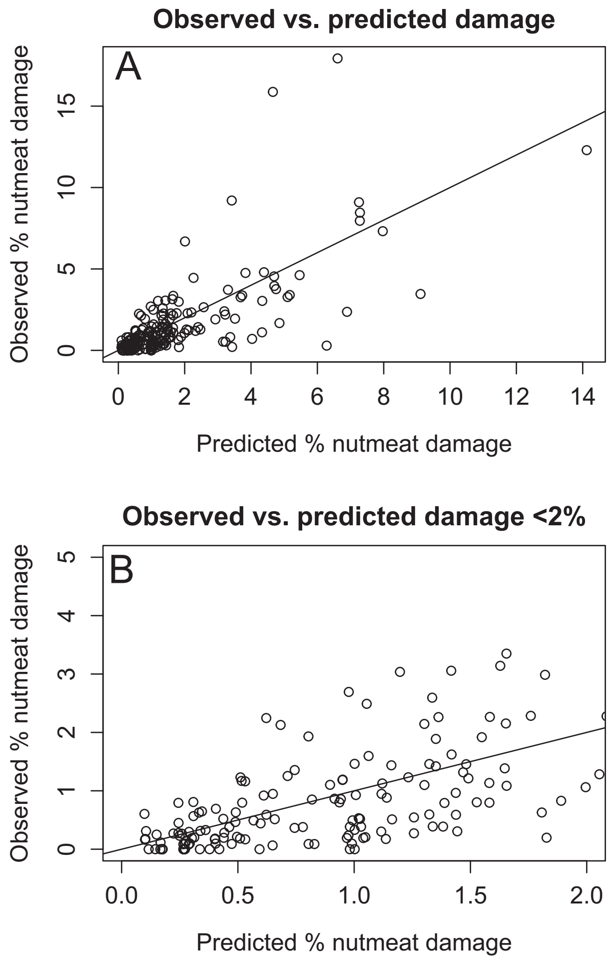

Fig. 2. (A) Observed versus predicted percent of almond nutmeats damaged by navel orangeworm at harvest, where damage was predicted using the full statistical model described in Table 3 (R^2 = 0.515, df = 189, $P < 0.0001$). (B) The same plot, but showing only datapoints where nutmeat damage was predicted to be <2%.

had both types of damage estimates, navel orangeworm nut damage averaged (mean ± SE) 0.75 ± 0.25% for our hand-collected samples versus just 0.43 ± 0.07% for nut samples that were machine harvested and processed as a commercial huller would. Thus, a grower who is accustomed to accepting 1% of nut damage as measured by the huller would not be operating much differently from a grower who accepted 2% nut damage as measured by the methods we use here.

Weakly Correlated Estimates of Navel Orangeworm Density

Several factors could explain our observation that different sampling methods produce estimates of navel orangeworm densities that are often weakly correlated.

First, it is possible that adult female navel orangeworms are sufficiently mobile that their populations are functionally open, with immigration responsible for substantial within-season population build-up within some individual almond blocks. This interpretation is supported by both laboratory studies of adult navel orangeworms showing strong dispersal ability (Sappington and Burks 2014) and field studies demonstrating substantial movement of moths both within and between orchards (Andrews et al. 1980, Andrews and Barnes 1982). When sanitation is applied rigorously in almond orchards, the resident overwintering navel orangeworm population can often be significantly suppressed (University of California 2002). In contrast, pistachio orchards often harbor large overwintering populations of navel orangeworms, and may be important sources of moths for almond orchards (Burks et al. 2008, Higbee and Siegel 2009). If immigration is important, then early-season estimates of the resident navel orangeworm population, taken before most immigration occurs, may be poor predictors of damage sustained at harvest. When almonds are grown in smaller blocks than those studied here, the relative importance of immigration may be still greater.

Second, weakly correlated estimates of navel orangeworm density could reflect other processes that can intervene over the course of a growing season to generate major changes in population density. In addition to immigration, applications of insecticides (e.g., spring applications; Hamby et al. 2015), and variation in weather, host plant resistance, caterpillar decisions to attack the nutmeats versus the hulls of almond fruits (e.g., Rosenheim et al. 2017), and natural enemy impacts could all change navel orangeworm population growth rates. This underscores the utility of using density estimates taken closer in time to harvest, as supported by our finding that the two strongest predictors of damage at harvest (adult female catches from hull split to harvest; pre-harvest samples of early split nuts) were taken during a period that overlaps the early stages of hull split.

Finally, sampling for navel orangeworms is intrinsically difficult, because tolerance for damage from this pest is low, and estimating low-density populations requires quite large sampling efforts. Careful research has demonstrated the limitations of each of the navel orangeworm sampling methods. The use of almond meal attractants in egg traps has long been the standard practice for timing insecticide applications. Burks et al. (2011) and Higbee and Burks (2011) have argued, however, that many traps are needed to obtain a reliable estimate of navel orangeworm oviposition within a given almond block. These authors noted that the standard commercial practice of using 2–4 egg traps per large block, as was done in the data set analyzed here, is often insufficient. Burks et al. (2011) suggested that using the same almond meal baits to capture adult females might be a more efficient sampling method. It is clear, however, that egg traps, trapping of adult females with almond meal, and inspection of early-split nuts will all suffer from the same problem that the density estimates they produce are likely to be distorted, to variable degrees, by competition of the attractive sampling device (i.e., almond meal or an early-split almond nut) with other attractants in the almond orchard, including populations of mummy nuts (Burks et al. 2011) or, especially, the population of newly maturing almonds, which grows rapidly during hull split (Rice 1976). Heavy populations of mummy nuts or growing numbers of newly mature almonds will invariably diminish egg and adult moth recovery in traps. We propose that one possible opportunity for improving the predictive power of adult female catches or observed infestation of early-split nuts will be to tightly standardize the stage of crop phenology (i.e., the proportion of nuts that exhibit hull split) when these sampling methods are used.

Prospects for Predicting Navel Orangeworm Damage

Our analysis suggests that adult female catches from hull split to harvest and infestation of early hull-split nuts may have the best potential for predicting damage at harvest. This is, perhaps, not surprising, as these density estimators are taken just prior to harvest. However, to be useful as an actionable predictor of damage, farmers must still have enough time after estimating navel orangeworm densities and before crop harvest to apply an effective control measure; in the commercial farming operation studied here, some insecticide applications were made as little as 7–14 d before harvest. Indeed, in this study, we chose to evaluate only true predictors, rather than variables that might be correlated with infestation but that were impossible to observe prior to harvest. Harvest date is one such variable that cannot be known with certainty until harvest actually occurs, and that has been shown to influence navel orangeworm infestation (Curtis et al. 1984), supporting the recommendation for early crop harvest. When we added harvest date (expressed as a deviation from the mean harvest date for a particular ranch, during a particular year) to the GEE model reported in Table 3, however, it failed to make a significant contribution to explaining variation in nut damage [coefficient = 0.012 ± 0.028 (SE), Wald = 0.17, $P = 0.68$]. This may largely have been a consequence of all blocks being harvested within a very narrow time window (ca. 6 d) within almost all ranch-year combinations.

For our data set, the unit sample used to estimate early hull-split infestation was 30 nuts, replicated at 1–4 locations per block. But to estimate damage relative to a threshold of just 2% infestation, sampling a larger number of nuts may often be necessary. Some private consultants do take larger samples, despite the labor intensiveness of such estimates. An advantage of sampling either adult females or early-split nuts is that these methods can be used either under conventional management or under mating disruption, which should play an expanding role in navel orangeworm management in California (Burks et al. 2016). Furthermore, new monitoring techniques under development, including the use of kairomone lures that are more attractive than almond meal (Beck et al. 2014, Burks et al. 2016), may also enhance the utility of female trapping methods, contributing to our ability to anticipate crop damage more precisely. Our results suggest that damage prediction is not an unrealistic goal, and that focused research on improving our sampling of female moths and infestation of early-split nuts holds promise for enhancing our management practices for navel orangeworm.

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References Cited

Almond Board of California. 2015. Almond Almanac 2015, p. 41. Almond Board of California, Modesto, CA.

Andrews, K. L., M. M. Barnes, and S. A. Josserand. 1980. Dispersal and oviposition by navel orangeworm moths. Environ. Entomol. 9:525–529.

Andrews, K. L., and M. M. Barnes. 1982. Invasion of pistachio orchards by navel orangeworm moths from almond orchards. Environ. Entomol. 11:278–279.

Beck, J. J., D. M. Light, and W. S. Gee. 2014. Electrophysiological responses of male and female *Amyelois transitella* antennae to pistachio and almond host plant volatiles. Entomol. Exp. Appl. 153:217–230.

Burks, C. S., and B. S. Higbee. 2015. Impact of trap design and density on effectiveness of a commercial pheromone lure for monitoring navel orangeworm (Lepidoptera: Pyralidae). J. Econ. Entomol. 108: 600–610.

Burks, C. S., B. S. Higbee, D. G. Brandl, and B. E. Mackey. 2008. Sampling and pheromone trapping for comparison of abundance of *Amyelois transitella* in almonds and pistachios. Entomologia Experimentalis et Applicata 129:66–76.

Burks, C. S., B. S. Higbee, J. P. Siegel, and D. G. Brandl. 2011. Comparison of trapping for eggs, females, and males of the navel orangeworm (Lepidoptera: Pyralidae) in almonds. Environ. Entomol. 40:706–713.

Burks, C. S., L. P. S. 'Bas' Kuenen, and K. M. Daane. 2016. Phenyl propionate and sex pheromone for monitoring navel orangeworm (Lepidoptera: Pyralidae) in the presence of mating disruption. J. Econ. Entomol. 109:958–961.

Coffelt, J. A., K. W. Vick, P. E. Sonnet, and R. E. Doolittle. 1979. Isolation, identification, and synthesis of a female sex pheromone of the navel orangeworm, *Amyelois transitella* (Lepidoptera: Pyralidae). J. Chem. Ecol. 5:955–966.

Curtis, C. E., and J. D. Clark. 1984. Pheromone application and monitoring equipment used in field studies of the navel orangeworm (Lepidoptera: Pyralidae). J. Econ. Entomol. 77: 1057–1061.

Curtis, C. E., R. K. Curtis, and K. L. Andrews. 1984. Progression of navel orangeworm (Lepidoptera: Pyralidae) infestation and damage of almonds on the ground and on the tree during harvest. Environ. Entomol. 13:146–149.

Demkovich, M., J. P. Siegel, B. S. Higbee, and M. R. Berenbaum. 2015. Mechanism of resistance acquisition and potential associated fitness costs in *Amyelois transitella* (Lepidoptera: Pyralidae) exposed to pyrethroids insecticides. Environ. Entomol. 44:855–863.

Hamby, K. A., J. A. Alifano, and F. G. Zalom. 2013. Total effects of contact and residual exposure of bifenthrin and λ-cyhalothrin on the predatory mite *Galendromus occidentalis* (Acari: Phytoseiidae). Exp. Appl. Acarol. 61:183–193.

Hamby, K. A., N. L. Nicola, F. J. A. Niederholzer, and F. G. Zalom. 2015. Timing spring insecticide applications to target both *Amyelois transitella* (Lepidoptera: Pyralidae) and *Anarsia lineatella* (Lepidoptera: Gelechiidae) in almond orchards. J. Econ. Entomol. 108:683–693.

Hardin, J. W., and J. M. Hilbe. 2013. Generalized estimating equations, 2nd ed. CRC Press, Boca Raton, FL.

Higbee, B. S., and C. S. Burks. 2008. Effects of mating disruption treatments on navel orangeworm (Lepidoptera: Pyralidae) sexual communication and damage in almonds and pistachios. J. Econ. Entomol. 101: 1633–1642.

Higbee, B. S., and C. S. Burks. 2011. Effect of bait formulation and number of traps on detection of navel orangeworm (Lepidoptera: Pyralidae) oviposition using egg traps. J. Econ. Entomol. 104: 211–219.

Higbee, B. S., and J. P. Siegel. 2009. New navel orangeworm sanitation standards could reduce almond damage. Calif. Agric. 63:24–28.

Higbee, B. S., C. S. Burks, and T. E. Larsen. 2014. Demonstration and characterization of a persistent pheromone lure for the navel orangeworm, *Amyelois transitella* (Lepidoptera: Pyralidae). Insects 5:596–608.

Højsgaard, S. 2016. Package 'geepack', vers. 1.2-1. https://cran.r-project.org/ last accessed 6 September 2017.

Kuenen, L. P. S., and J. P. Siegel. 2016. Sticky traps saturate with navel orangeworm in a nonlinear fashion. Calif. Agric. 70:32–38.

Kuenen, L. P. S., D. Brandl, and R. E. Rice. 2005. Modification of assembly of Pherocon IC traps speeds trap liner changes and reduces in-field preparation time. Can. Entomol. 137:117–119.

Kuenen, L. P. S., J. S. McElfresh, and J. G. Millar. 2010. Identification of critical secondary components of the sex pheromone of the navel orangeworm (Lepidoptera: Pyralidae). J. Econ. Entomol. 103:314–330.

Leal, W. S., A.-L. Parra-Pedrazzoli, K.-E. Kaissling, T. I. Morgan, F. G. Zalom, D. J. Pesak, E. A. Dundulis, C. S. Burks, and B. S. Higbee. 2005. Unusual pheromone chemistry in the navel orangeworm: novel sex attractants and a behavioral antagonist. Naturwissenschaften 92:139–146.

Naimi, B. 2015. Package 'usdm', vers. 1.1–15. https://cran.r-project.org/, last accessed 6 September 2017.

O'Brien, R. M. 2007. A caution regarding rules of thumb for variance inflation factors. Qual. Quant. 41:673–690.

Palumbo, J. D., N. E. Mahoney, D. M. Light, J. Siegel, R. D. Puckett, and T. J. Michailides. 2014. Spread of *Aspergillus flavus* by navel orangeworm (*Amyelois transitella*) on almond. Plant Dis. 98:1194–1199.

Pedigo, L. P., and M. E. Rice. 2009. Entomology and pest management, 6th ed. Waveland Press, Long Grove, IL.

Rice, R. E. 1976. A comparison of monitoring techniques for the navel orangeworm. J. Econ. Entomol. 69:25–28.

Rosenheim, J. A., and C. Gratton. 2017. Ecoinformatics (big data) for agricultural entomology: pitfalls, progress, and promise. Annu. Rev. Entomol. 62: 399–417.

Rosenheim, J. A., B. S. Higbee, J. D. Ackerman, and M. H. Meisner. 2017. Ecoinformatics can infer causal effects of crop variety on insect attack by capitalizing on 'pseudoexperiments' created when different crop varieties are interspersed: a case study in almonds. J. Econ. Entomol. in press.

Sappington, T. W., and C. S. Burks. 2014. Patterns of flight behavior and capacity of unmated navel orangeworm (Lepidoptera: Pyralidae) adults related to age, gender, and wing size. Environ. Entomol. 43: 696–705.

Schatzki, T. F., and M. S. Ong. 2001. Dependence of aflatoxin in almonds on the type and amount of insect damage. J. Agric. Food Chem. 49: 4513–4519.

University of California. 2002. Integrated Pest Management for Almonds, 2nd ed. University of California, Division of Agriculture and Natural Resources, Oakland, CA.