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

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Abstract

Capturing the complementary strengths of observational and experimental research methods usually requires the researcher to gather separate experimental and observational data sets. In some cases, however, commercial agricultural practices produce the spatial and temporal mixing of 'treatments' independently of other possibly covarving factors that is normally achieved only with formal experimentation. The resulting 'pseudoexperiments' can provide strong evidence for causal relationships. Here, we analyze a large observational data set that creates a series of such pseudoexperiments to assess the effect of different commercial varieties of almond, Prunus dulcis (Mill.) on the impact of two key lepidopteran pests, the navel orangeworm Amyelois transitella (Walker) (Lepidoptera: Pyralidae), and the peach twig borer Anarsia lineatella Zeller (Lepidoptera: Gelechiidae). Almonds are universally planted as polycultures of different varieties to obtain efficient cross-pollination. We find substantial differences across almond varieties in the rates of infestation of almond hulls and nutmeats by the two pests. We find no support for the hypothesis that earlier-maturing varieties sustain higher attack; for A. transitella, later-maturing varieties instead had more frequent infestation. On many almond varieties, A. lineatella reaches high infestation levels by feeding almost exclusively on the hulls, rather than nutmeats. Given the importance of these pests in directly destroying almond nuts and in promoting aflatoxin-producing Aspergillus sp. fungal infections of almonds, further work exploring the impact of these pests is warranted. Because many crops requiring cross-pollination are planted as mixtures of different varieties, commercial agricultural production data hold great potential for studying within-crop variation in susceptibility to insect attack.

Key words: ecoinformatics, pseudoexperiments, variety mixtures, navel orangeworm, peach twig borer

Research in agricultural entomology relies heavily upon experimentation. The gold standard of field research methodology is to conduct experiments, often on research farms, using small replicated plots in which different experimental treatments, created through the experimenter's manipulations, are applied randomly to different plots. Such experiments have, as their central advantage, the ability to create strong evidence for causal relationships between the manipulated variables and the observed response variables. Causal knowledge is crucial to supporting management recommendations that are the end product of much applied research. Purely observational research methods, in contrast, generally produce strictly correlative evidence and thus are often insufficient as a stand-alone research methodology (Rosenheim et al. 2011).

Experimentation is, however, associated with its own set of weaknesses as a stand-alone research methodology. Experiments may be impractical when researchers wish to examine processes that operate at spatial or temporal scales that are too large to be manipulated readily. Experiments are also labor intensive and thus costly, imposing strict limits on the size of data sets that can be generated. Experiments also lack the realism of data gathered from the commercial farming setting. Observational data sets, and, in particular, analysis of pre-existing data from commercial farming (an example

© The Author(s) 2017. Published by Oxford University Press on behalf of Entomological Society of America. All rights reserved. For permissions, please e-mail: journals.permissions@oup.com. of ecoinformatics) can complement these weaknesses of a sole reliance on experimentation (e.g., Jiménez et al. 2009). The usual recommendation for uniting the unique, complementary advantages of experimental and observational research methods thus has been to gather separate experimental and observational data sets within a single broader research program (Rosenheim and Gratton 2017).

In some special cases, however, purely observational data sets derived from commercial farming can capture nearly all of the power of inference that we normally ascribe solely to experimentation and thus combine the advantages of experimentation and ecoinformatics within a single data set. This occurs when what we recognize as traditional experimental designs, in which different treatments are interspersed in space and time and applied randomly to experimental subjects, are produced, essentially inadvertently, by normal farming practices, producing what we will call here a 'pseudoexperiment'. For example, pseudoexperiments are created when farmers purposefully plant a spatial mosaic, or polyculture, of different crop varieties. Different crop varieties are often needed for cross-pollination, as is the case for many tree crops (e.g., almond, apple, cherry, pear, plum, walnut) and also in dioecious crop plants, where male and female plants must be grown together (e.g., fig, kiwifruit, persimmon, pistachio, and sunflower; University of California 2017). Different genotypes, varieties, or genders of crop plants often vary in their susceptibility to pest attack, and agricultural entomologists often wish to evaluate these differences. To avoid confounding spatial heterogeneity with crop variety differences, experimentalists often use a common garden design, in which different crop varieties are spatially interspersed at a single experimental site. Here, we introduce another approach.

We use a case study to demonstrate the utility of analyzing pseudoexperiments created by crop polycultures in commercial agriculture. Almond orchards in California are planted as alternating rows of different varieties to support cross-pollination. Almonds are attacked by two dominant lepidopteran pests, the navel orangeworm Amyelois transitella (Walker) (Lepidoptera: Pyralidae) and the peach twig borer Anarsia lineatella Zeller (Lepidoptera: Gelechiidae) (University of California 2002). These pests may be critical both as direct destroyers of nuts and also as vectors of Aspergillus spp. fungi, which may produce aflatoxins, a potent class of carcinogens (Palumbo et al. 2014, Picot et al. 2017). Caterpillars of both species may develop either on the almond hulls or directly on the nutmeat; for almond variety Nonpareil, Higbee and Siegel (2012) showed that most infested nuts also had damage to the nutmeat. Aflatoxin contamination of nutmeats is a major concern, as too-high levels trigger rejection of almond lots. Aflatoxin contamination of almond hulls has received less attention, but may still be of concern, as hulls are incorporated into feed for dairy cows, and aflatoxins can move into milk products (Flores-Flores et al. 2015). Differences across almond varieties in susceptibility to attack by navel orangeworm have been reported (Crane and Summers 1971, Soderstrom 1977, Higbee and Burks 2008, Higbee and Siegel 2012), but it is unknown if varieties differ in the relative likelihood of navel orangeworm feeding in the hulls versus the nutmeats. Hamby et al. (2011) analyzed a common garden experiment and documented that earlier-maturing varieties (measured as the timing of hull-split) were associated with heavier navel orangeworm infestation. In contrast, little is known about how almond varieties differ in susceptibility to the peach twig borer.

Here, we ask how the impacts of navel orangeworm and peach twig borer on almonds grown commercially vary across almond varieties. Our key response variables are (i) infestation levels observed in the entire almond fruit (hull + nutmeat); (ii) feeding damage to the nutmeat itself; and (iii) the proportion of infested almond fruits in which the caterpillar damaged the nutmeat, as opposed to feeding solely in the hull. We also take a second look at the hypothesis that earlier-maturing varieties sustain heavier fruit infestation.

Materials and Methods

Data Source

Pest damage data came from two large almond ranches (Wonderful Orchards, Bakersfield, CA) located in western Kern County in the southern San Joaquin Valley. The Santa Fe Ranch (36.642059-119.975457), 2500 acres of almonds organized into 21 blocks, was studied from 2009 through 2012, and the Lost Hills Ranch (35.551184-119.651321), 2800 acres of almonds organized into 30 blocks, was studied from 2009 through 2015. Each block was planted as a mixture of two or three almond varieties, as is universal in commercial almond production. Nine almond varieties were studied: Butte, Carmel, Fritz, Monterey, Nonpareil, Padre, Price, Sonora, and Wood Colony, which collectively represent >90% of all California almond hectarage (Almond Board of California 2016). Blocks planted to three varieties were arranged as follows: two rows of variety Nonpareil, two rows of the first pollenizer variety, two rows of Nonpareil, and two rows of the second pollenizer variety, repeated many times across the block. Blocks planted to two varieties simply alternated pairs of rows of the two varieties. The block was the basic management unit for all agricultural operations, including pest control measures, and individual block-years are taken here as the sampling unit for our analyses. Detailed data on almond infestation by navel orangeworm and peach twig borer were gathered at these locations as part of a federally funded area-wide trial of mating disruption-based control for navel orangeworm (2007-2012). Thus, in any particular block-year combination, navel orangeworm populations were managed using mating disruption alone, conventional insecticides alone (usually methoxyfenozide or bifenthrin, rarely other materials), or a combination of the two, along with rigorous sanitation practices (Higbee and Siegel 2009). Peach twig borer was managed using conventional insecticides. Insecticide applications were never made to just certain varieties within a block; rather, the entire block was treated when an application was made.

Within each block, one to four samples of approximately 500 nuts each (mean \pm SD = 529 \pm 118 nuts) 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 either the navel orangeworm or the peach twig borer generated: i) infestation of the almond fruit (hull or nutmeat; defined as the presence of live larvae or pupae or clear evidence of species-specific damage, including characteristic exuvia, frass, webbing, and feeding damage) or ii) damage to the nutmeat itself. From these measurements, we calculated the proportion of infested fruit in which the nutmeat was damaged as a measure of the propensity of a caterpillar, once present in the fruit, to directly attack the nutmeat. All nut samples taken within a block were combined to create a single combined sample for statistical analysis. Across all blocks and years, just over one million nuts (1,005,067) were scored.

Some ecoinformatics studies work with data sets that pool information from many different farms, allowing researchers to study a broad range of farming operations and draw broadly applicable conclusions (Rosenheim et al. 2011). In this study, however, all data came from just a single large commercial farming operation. Therefore, we must exercise caution and recognize that conditions on other farms may be different and thus that results from the current study may not be broadly generalizable. This is a weakness shared with traditional experimental methods (Diamond 1983).

Forming Pseudoexperiments

Different blocks within each ranch were planted with different combinations of almond varieties. We grouped together those blocks, within and across ranches, that had the same combinations of varieties. This produced 10 pseudoexperiments, with between 2 and 10 blocks per pseudoexperiment, observed across the multi-year duration of data collection. By working with the 10 pseudoexperiments, and not combining all observations to create a single, larger dataset, we lost some statistical power. But, we avoided the possibility that some almond varieties might accidentally be located in regions where pest pressure is higher (e.g., where almonds abut pistachio orchards, a known source of navel orangeworm; Higbee and Siegel 2009), or the possibility that farmers might choose to plant particular varieties in locations that have specific characteristics (e.g., farmers might plant more resistant varieties in locations where they expect elevated pressure from the focal pest). In either of these scenarios, spurious associations might be created between almond variety and pest damage level. Statistical measures exist that can ameliorate some of these concerns (e.g., modeling spatial autocorrelation or inclusion of covariates for pest pressure), but it is hard to be confident that these methods produce a complete solution to hidden, but potentially important, confounding variables.

Statistical Analysis

We used generalized estimating equations (GEEs), implemented in R program geepack, version 1.2-1 (Højsgaard 2016), to accommodate the repeated observations made on the same experimental units (Hardin and Hilbe 2013). Because infestation was a binary response variable, we used a binomial variance model and a logit link function. We modeled residual variance using the autoregressive-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. To compare levels of infestation across almond varieties within a pseudoexperiment, we adopted a conservative approach of considering two varieties to be significantly different if their 95% confidence intervals, calculated with the robust variance estimate, did not overlap. To maintain the α-error rate at 0.05 for pseudoexperiments that had three almond varieties (and thus three pairwise contrasts per response variable), we used the Bonferroni correction (i.e., we required nonoverlap of the 98.3% confidence intervals). We report results from a simplest-case model, with fixed effects for almond varieties and a random effect for block. Analyses that included data only for blocks that did not receive pesticide applications at hull split produced very similar results (data not shown). We used a simple model and did not try to include additional covariates, because within a pseudoexperiment each block had the same varieties planted and any block-to-block variation in pest management practices or any other source of variation in insect densities would be expected to affect all varieties equally. Elsewhere we present a more complex model that examines predictors of almond infestation by navel orangeworm (Rosenheim et al. 2017).

To test the hypothesis that earlier-maturing almond varieties suffer higher infestation by navel orangeworm, we needed to devise a means of comparing infestation of varieties across pseudoexperiments. To do this, we used infestation of variety Nonpareil as a reference point. Nonpareil is the dominant almond variety planted in California, making up 37% of all California almond hectares in 2016 (Almond Board of California 2016). Nonpareil was planted in 8 of the 10 pseudoexperiments, and thus, we restricted our analysis to those pseudoexperiments. Within each of these pseudoexperiments, we expressed each variety's main effect for infestation, as calculated by the GEE, relative to the infestation main effect for Nonpareil. So, for example, in Pseudoexperiment 1, the main effect for almond fruit infestation by navel orangeworm was 7.60% for variety Fritz, whereas the corresponding estimate for Nonpareil was 2.95%. Thus, we calculated a relative infestation metric for Fritz of 2.57 (=7.60/2.95) and rescaled Nonpareil's infestation to 1.00 (=2.95/2.95). We then pooled these relative infestation estimates across all pseudoexperiments, averaging values in cases where multiple pseudoexperiments generated estimates for the same variety, to produce values that could be compared across the full data set.

Results

Almond varieties differed substantially in their frequency of attack by navel orangeworm and peach twig borer (Figs 1-4; Supp Figs S1-S6 [online only]). Almond variety also had a very strong effect on whether navel orangeworm and peach twig borers attacked the hulls of the fruit or fed on the nutmeats (Figs 1-4; Supp Figs S1-S6 [online online]). Only 4.0% of navel orangeworm caterpillars that infested an almond fruit moved to damage the nutmeat on variety Padre (the lowest value observed; Supp Fig. S6C [online only]), whereas an average of 69.4% did so when feeding on variety Fritz (the highest value; Figs 1C; Supp Figs S2C, S3C, S5C [online only]). Peach twig borer caterpillars were found to feed primarily in the hulls of almost all almond variety fruits: for all varieties examined other than Nonpareil, the proportion of infested fruits in which peach twig borer damaged the nutmeat ranged from just 0.0-9.81%. Only on variety Nonpareil did peach twig borer commonly attack the nutmeat, with $38.6 \pm 14.0\%$ (mean \pm SD) of infested fruits having damaged nutmeats.

In commercial almond production, each variety within a block is harvested separately when the nuts are sufficiently mature, and mean harvest dates varied widely across the almond varieties in our data set (Fig. 5). We found no support for the hypothesis that earlier-maturing varieties sustain higher damage by navel orangeworm; our data instead suggested the reverse, with later-maturing varieties receiving higher infestation (Spearman's rank correlation, $\rho_s = 0.76$, N = 8, P = 0.028; Fig. 5). Infestation of nutmeats by navel orangeworm and infestation of fruits and nutmeats by peach twig borers all showed the same trend for higher infestation of later-maturing varieties, but the relationships were not significant (navel orangeworm, nutmeat damage vs harvest date: $\rho_s = 0.62$, P = 0.10; peach twig borer, fruit infestation harvest date: $\rho_s = 0.62$, P = 0.10; peach twig borer, nutmeat damage vs harvest date: $\rho_s = 0.43$, P = 0.29). All correlations remained positive, but nonsignificant ($\rho_{e} = 0.36-0.62$, P = 0.10-0.39), when only blocks that received no pesticide applications at hull split were included in the analysis.

Discussion

We have presented a case study of how a pre-existing, observational dataset derived from commercial farming can be analyzed to produce strong evidence for causal influences of almond varieties on attack by two insect pests. To support cross-fertilization, almonds are grown commercially as polycultures of different varieties. The resulting spatial mixture of varieties is similar to what one would achieve with an experimental design, with the advantages of a realworld, commercial setting. Our analyses document significant variation in infestation by navel orangeworm and peach twig borer, and major differences across varieties in the propensity of caterpillars to feed in the hull versus in the nutmeat. We find no support for

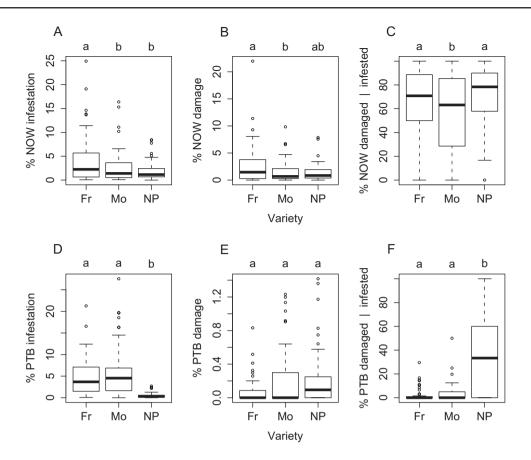


Fig. 1. Boxplot summarizing percent infestation of different almond varieties by navel orangeworm (NOW, panels A–C) and peach twig borer (PTB, panels D–F) for Pseudoexperiment 1, which encompassed 10 blocks and 70 total block-years of data. Shown are infestation of almond fruits (A, D), feeding damage to nutmeats (B, E), and the percentage of infested fruits that also had feeding damage to the nutmeats (C, F). Letters above the bars indicate varieties that are significantly different, as judged by a GEE analysis, and nonoverlapping 95% confidence intervals. Boxplots show the median value (bold black band), the bottom and top of the boxes are the 25th and 75th percentiles of the observations, the whiskers extend to 1.5 times the inter-quartile range, and all remaining values are shown individually. Fr, Fritz; Mo, Monterey; NP, Nonpareil.

the hypothesis that earlier-maturing varieties are subject to heavier attack by these pests, instead finding support for the reverse.

Pseudoexperiment versus Experiment

We have referred to blocks planted to the same mixtures of varieties as pseudoexperiments. What are the differences between data gathered in this setting and data gathered from a purely experimental setting, and how might they influence the strength of the causal inferences that we can draw? Although we think these differences are subtle, it is nonetheless useful to define them.

First, as is often the case with crop variety mixtures in commercial settings, the different almond varieties are often not planted in equal proportions within blocks. Nonpareil almonds provide growers with high financial returns, and thus, the standard planting arrangement is repeated units of two rows of Nonpareil, two rows of the first pollenizer, two rows of Nonpareil, and two rows of the second pollenizer. Thus, for most blocks, there are twice as many Nonpareil trees than either of the pollenizer varieties. Consequently, if there is any frequency-dependent influence of variety on herbivore attack (i.e., if moths prefer to lay on a more [or less] common variety; see Verschut et al. 2016), this could create a pattern of differential attack. We know of no studies exploring this possibility for navel orangeworm or peach twig borer. If such effects were operating, it would, however, be a realistic aspect of variety performance in the commercial almond setting. Second, as just described, the traditional commercial almond polyculture means that varieties differ in their patterns of adjacency; in particular, the pollenizer varieties are always found adjacent to Nonpareil, and never to each other. Thus, if physical proximity of different varieties was important in some way, this could contribute to what we measure here as cultivar effects. Once again, however, any such putative effect would be a realistic component of variety choice for commercial almond production.

Third, insecticides were applied at hull-split to some of the blocks in this study. If applications are timed to coincide with the onset of hull-split of Nonpareil, which is the dominant variety in most blocks and also the earliest-maturing variety examined in this study, then it is possible that this could produce a pattern of better pesticide-based protection of Nonpareil, and thus lower infestation on Nonpareil relative to other, later-maturing varieties. Studies have demonstrated, however, that insecticide residues are fairly persistent on almond hulls (e.g., residues of methoxyfenozide remained at ca. 90% of initial levels 30 days after application; B. S. Higbee, unpublished data). This should reduce the effect of application timing on the protection of different almond varieties. Nevertheless, it was for this reason that we repeated our analyses using only replicates that received no hull split pesticide applications, and which, therefore, were free of any possible confounding influence of insecticide application timing, to see if variety effects changed appreciably. These analyses produced results that were little different from analyses of the full data set, suggesting that this scenario is not important

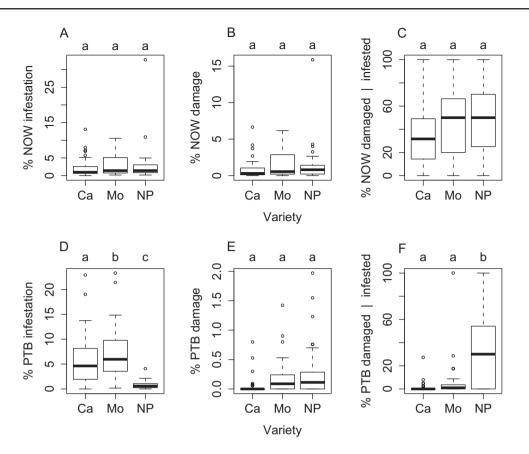


Fig. 2. Boxplot summarizing percent infestation of different almond varieties by navel orangeworm (panels A–C) and peach twig borer (panels D–F) for Pseudoexperiment 2, which encompassed 7 blocks and 37 total block-years of data. Shown are infestation of almond fruits (A, D), feeding damage to nutmeats (B, E), and the percentage of infested fruits that also had feeding damage to the nutmeats (C, F). Letters above the bars indicate varieties that are significantly different, as judged by a generalized estimating equation analysis, and nonoverlapping 95% confidence intervals. Ca, Carmel; Mo, Monterey; NP, Nonpareil.

within this data set. Again, however, we note that if such an effect were occurring, it would capture a real aspect of commercial almond production.

Fourth, commercial almond blocks provide opportunities for direct comparison of only a modest number of almond varieties (in the current study, two or three). We used Nonpareil as a benchmark variety to broaden the comparisons to more varieties, but a common garden experiment, in which many varieties are planted together, provides a more direct means of considering simultaneously a larger number of varieties.

On the other hand, our pseudoexperiments do have some advantages over a traditional common garden experiment, primarily due to enhanced realism. To the extent that varietal differences are mediated by behavioral choices made by ovipositing female moths, the pseudoexperiments that we analyze here reflect the real commercial setting, whereas common garden experiments are unrealistic, providing too many choices on too fine-grained a spatial scale. Decisions made by individual caterpillars (e.g., to attack the hull vs the nutmeat) or differential performance on different cultivars (e.g., ability to penetrate a hard shell to reach the nutmeat) seem much less likely to be influenced by the spatial arrangements of different varieties in the orchard, as the relevant spatial scale for individual caterpillars is almost certainly much smaller than an individual tree.

On the whole, we see substantial benefits from capitalizing on pseudoexperiments that are created by agricultural practices that mix different varieties. Yan et al. (2002) advocated a similar approach as a powerful means of comparing the productivity of different wheat cultivars by aggregating information from farmer strip trials of different cultivars, which are generally unreplicated at the level of an individual farm but can become highly replicated when combining information across farms. The rapid spread of crop and pest management software applications (Fountas et al. 2015) means that the data needed to conduct these analyses will expand rapidly in the near future.

Almond Varieties and Pest Impact

For the almond farmer, navel orangeworm and peach twig borer generate their primary economic damage by feeding on nutmeats. This is true both because of the loss of marketable product and because larval and adult navel orangeworm have been shown to vector *Aspergillus* spp. fungi that produce aflatoxins. Aflatoxins are potent carcinogens, and strict guidelines for aflatoxin contamination of almonds can cause rejections of commercial almond lots intended for domestic, U.S. consumption, and especially export to the EU. Our analyses reveal substantial differences across varieties in susceptibility to caterpillar damage.

We failed to confirm the previously documented pattern that earlier-maturing varieties are subject to higher navel orangeworm damage (Hamby et al. 2011). The two studies used substantially different sets of almond varieties, which might explain the discrepancy. Occasionally, almond shells may fail to seal normally, increasing vulnerability of some almond varieties to attack by navel orangeworm, but no shell seal problems were observed during this work (B. S. Higbee, personal communication). In general, earlier-maturing varieties are also earlier-harvested varieties, and there is no reason to expect that crop phenology should change the

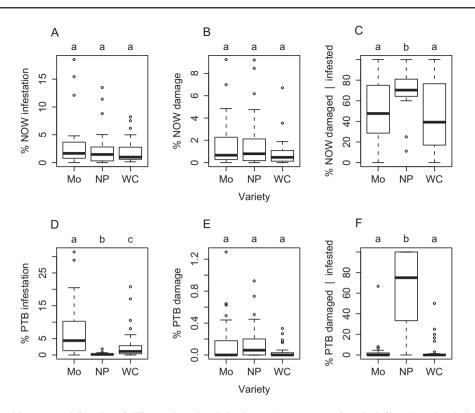


Fig. 3. Boxplot summarizing percent infestation of different almond varieties by navel orangeworm (panels A–C) and peach twig borer (panels D–F) for Pseudoexperiment 3, which encompassed 4 blocks and 28 total block-years of data. Shown are infestation of almond fruits (A, D), feeding damage to nutmeats (B, E), and the percentage of infested fruits that also had feeding damage to the nutmeats (C, F). Letters above the bars indicate varieties that are significantly different, as judged by a generalized estimating equation analysis, and nonoverlapping 95% confidence intervals. Mo, Monterey; NP, Nonpareil; WC, Wood Colony.

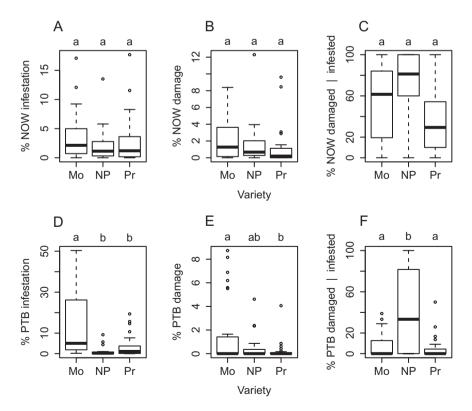


Fig. 4. Boxplot summarizing percent infestation of different almond varieties by navel orangeworm (panels A–C) and peach twig borer (panels D-F) for Pseudoexperiment 4, which encompassed 4 blocks and 28 total block-years of data. Shown are infestation of almond fruits (A, D), feeding damage to nutmeats (B, E), and the percentage of infested fruits that also had feeding damage to the nutmeats (C, F). Letters above the bars indicate varieties that are significantly different, as judged by a generalized estimating equation analysis, and nonoverlapping 95% confidence intervals. Mo, Monterey; NP, Nonpareil; Pr, Price.

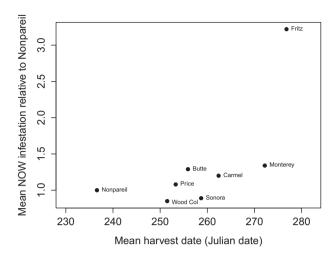


Fig. 5. Positive relationship between mean harvest date (Julian date) and mean infestation of almond fruit by navel orangeworm (NOW; Spearman's rank correlation, $\rho_s = 0.76$, N = 8, P = 0.028). Infestation expressed relative to infestation seen on variety Nonpareil within the same pseudoexperiment. Wood Col = Wood Colony.

total duration of the exposure period to navel orangeworm attack. Leaving mature nuts in the almond orchard for an extended period will lead to a steady accumulation of damage (Curtis and Barnes 1977, Curtis et al. 1984); thus, prompt harvest of mature nuts is a key plank of navel orangeworm cultural management, and this is true for both early- and late-maturing varieties. Because navel orangeworm populations tend to surge toward the end of the harvest season (Rice 1976, Curtis and Barnes 1977, Burks et al. 2008, Higbee and Burks 2008, Burks and Higbee 2015, Rosenheim et al. 2017), later-maturing varieties may, all other things being equal, be subject to more intense attack by this pest, and this may explain the modest trends that we found toward higher navel orangeworm attack on later-maturing varieties. Additional work will be required to determine the mechanistic bases for observed differences across varieties in attack by navel orangeworm and peach twig borer, including preferences of ovipositing female moths and feeding caterpillars, host plant resistance traits (chemical defenses and physical defenses, including shell hardness and shell seal), and the interplay of tree and moth phenology.

We found that peach twig borer, in particular, can heavily infest the hulls of many almond varieties. The economic importance of these infestations is uncertain. Whereas the navel orangeworm has a strong, mutualistic association with aflatoxin-producing Aspergillus fungi (Ampt et al. 2016), we are unaware of any studies examining the potential role of peach twig borer as a vector of these fungi. Navel orangeworms overwinter in mummy nuts and continue to develop in these mummies until the new crop of nuts is nearly mature; mummies frequently harbor Aspergillus fungi, and adult navel orangeworm moths thus can vector fungal spores to new-season nuts at hull split (Palumbo et al. 2014, Picot et al. 2017). Peach twig borers, in contrast, overwinter in small hibernacula, which they create by boring into the almond tree bark at the junctions of branches and the main trunk, and the spring generation feeds by boring into new tender shoots (University of California 2002). Peach twig borers may, therefore, be less likely to vector Aspergillus spores. Nevertheless, peach twig borers do deposit frass in attacked hulls. Almond hulls in California are primarily used as feed for dairy cows (Almond Board of California 2016). Aflatoxins in feed, including in almond hulls, are a perennial concern for the dairy industry, as aflatoxins can be

passed into milk products (Flores-Flores et al. 2015). We suggest that future work should examine if peach twig borer attack of almond hulls is associated with any change in risk of *Aspergillus* infection of hulls or nutmeats.

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Supplementary Data

Supplementary data is available at Journal of Economic Entomology online.

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