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Hull Split and Damaged Almond Volatiles Attract Male and Female Navel Orangeworm Moths

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Almond Volatiles Attract Navel Orangeworm

ABSTRACT

A blend of volatiles derived from the emissions of almonds at hull split and mechanically damaged almonds was compared to almond meal, the current monitoring standard for the insect pest navel orangeworm (NOW). Field trapping studies were performed to determine the blend's ability to attract adult NOW. The blend comprised racemic 1-octen-3-ol, ethyl benzoate, methyl salicylate, acetophenone, and racemic (*E*)-conophthorin. Ethyl acetate was used as a solvent with a blend component concentration of 100 mg/mL. The blend attracted both sexes of NOW when tested in five two-week intervals spanning the first three flights of NOW in commercial almond orchards in the southern Central Valley of California. The blend demonstrated consistently higher capture rates for female NOW throughout the evaluation period, but unlike almond meal significantly attracted males. Reported is a survey of the major and minor volatiles emitted from almonds at hull split, the key period of vulnerability to NOW infestation. Also reported is the attractancy of a formulated test blend based on the host plant volatile emissions, electroantennographic screening experiments, and field trapping studies. The results of this test blend highlight progress toward a host plant-based attractant for NOW, a major insect pest of California tree nuts and presently lack an adequate monitoring tool.

KEYWORDS: almond; *Amyelois transitella*; field trapping; navel orangeworm; *Prunus dulcis*; semiochemical; volatile

INTRODUCTION

The navel orangeworm (NOW), *Amyelois transitella* Walker (Lepidoptera: Pyralidae) is the key insect pest of tree nuts in California. Feeding by NOW larvae is responsible for damage to the kernel as well as the introduction of mycotoxigenic fungi; both resulting in considerable economic loss to growers.[1,2] Current methods to monitor NOW populations include traps baited with virgin female NOW moths or egg traps baited with almond meal.[3] The use of virgin female NOW moths is primarily for research purposes and not practical for commercial use. The use of almond meal in egg traps is the current standard for monitoring female NOW.[4]

The female sex pheromone of NOW is known[5,6] and recent work with the components is promising as an attractant for male NOW.[7,8] The main pheromone component (Z,Z)-11,13-hexdecadienal is unstable under orchard conditions; nevertheless formulations in aerosol puffer delivery devices have been successfully used in mating disruption control of NOW in almond[9,10] and walnut orchards. The pheromone components in lure formulations are hindered by stability and degradation issues in the field.[4]

The identification and use of semiochemicals to attract and monitor NOW moths in almond orchards remains elusive. In 2009 Burks et al.[11] investigated use of the single non-host compound phenyl propionate in almond and pistachio orchards to capture adult NOW. Although attractive to NOW moths, the authors concluded phenyl propionate was unlikely to be practical for economic and perceived safety reasons (personal communication C. Burks).[11]

Based on field observations (B.S.H.) that female NOW are attracted to the odor of damaged almonds[12] attention was focused on mechanically damaged almonds[13] and almonds undergoing hull split. Collection of volatiles from these sources provided the identification of both ubiquitous and distinctive volatiles of interest. Electroantennographic (EAG) analysis was used

as a method to screen host plant volatiles for potential semiochemical associated behavioral responses. Compounds eliciting the greatest chemoreception responses were formulated into various blends and screened over several growing seasons for attractancy in preliminary field trapping experiments.

The objectives of this study were to: 1) perform a survey of the volatiles emitted from almonds undergoing hull split; 2) screen the individual hull split and select damaged[13] almond volatiles via EAG and field trapping studies; and, 3) determine the attractiveness of a blend, primarily comprised of hull split volatiles, to NOW in almond orchards and compare its efficacy to the current standard monitoring lure, almond meal. The host plant volatile blend, designated as the Blend, was evaluated over five two-week intervals spanning the first three flights of NOW in the southern Central Valley of California.

MATERIALS AND METHODS

Chemical Sources. Chemicals were purchased from commercial sources and used without further purification: (+/-)-1-octen-3-ol (98%), ethyl benzoate (99%), methyl salicylate (98%), acetophenone (99%), and ethyl acetate (99.5%) (VWR International, Wayne, PA); (+/-)-(*E*)-conophthorin (Contech, Victoria, BC); and, (*Z*,*Z*)-11,13-hexadecadienal (Suterra, Bend, OR).

Collection of volatiles. An intact branch of Nonpareil or Monterey variety almonds, ca. 15 almonds, at the hull split stage were bagged with a large custom Teflon bag (40 × 25 cm) similar to the collection bag used in previous *in situ* studies[14] but with 0.64 cm ports for connecting Teflon tubing and utilizing a closed push/pull collection system (**Figure 1**) similar to the device used in 2011 by Beck et al. for ambient volatiles.[15] Briefly, a glass cartridge containing 25 g of

Tenax-TA (40-60 mesh, Alltech, Deerfield, IL) was connected to a Venturi vacuum adapter using compressed air to generate the desired flow rate (35-70 mL/min). Fresh compressed air and Tenax-cleansed air were directed to the collection bag via the closed system to maintain neutral pressure inside the collection bag. Collected volatiles were chemically desorbed, concentrated and analyzed by GC-MS as previously described.[15] Briefly, volatiles were desorbed with diethyl ether (100 mL), concentrated to ca. 1 mL via water bath (ca. 40 ^{o}C) and Vigreux condenser, and 1 μL aliquots injected onto J&W Scientific (Folsom, CA) DB-Wax and DB-1 (60 m × 0.32 mm i.d. × 0.25 μm) columns. The average of two separate collection periods during the 2008 growing season (July 21 to August 5, and August 5-13, for Nonpareil variety; and August 12-18, and August 18-25 for Monterey variety) were each analyzed on the two different columns, and reported as the percentage of minor and major volatiles in the odor profiles. All volatile identities, with the exception of β-bourbonene, were verified by comparison of retention times and fragmentation patterns to authentic standards.

Blend Composition. Host plant blend of 1-octen-3-ol : ethyl benzoate : methyl salicylate : acetophenone : conophthorin (12:4:4:1:1 ratio), calculated for 2.5 g in 25 mL; 1-octen-3-ol (1.53 mL, 9.9 mmol); ethyl benzoate (0.48 mL, 3.3 mmol); methyl salicylate (0.43 mL, 3.3 mmol); acetophenone (0.10 mL, 0.8 mmol); and, conophthorin (0.13 mL, 0.8 mmol) were diluted in ethyl acetate (22.3 mL). Rectangles (ca. 1 × 4 cm) of cotton plugs (U.S. Cotton, Gastonia, NC) were inserted into 8 mL Nalgene bottles (VWR International, Wayne, PA), 2 mL of blend (200 mg/2 mL) added, and bottles capped.[16] In the field, caps were replaced with emission caps having a 1.5 mm hole.

For evaluation of emission release rates, loaded and tared Nalgene bottles identical to field samples were placed in a one-liter Mason jar, capped with a lid fitted with an inlet for purified air (150 mL /min) and vented via tubing to a fume hood. The emission experiment was run in triplicate. The Mason jars containing the bottles with the Blend were then placed in an oven set at 30 ^{o}C. At approximate 24 h intervals bottles were removed, weighed, and 1.0 μL injected onto a GC-FID (Shimadzu GC-2010 Plus, Pleasanton, CA) with a J&W Scientific (Folsom, CA) DB-Wax column for quantitation. The GC-FID conditions were: initial temperature, 40 ^{o}C; initial time 0.0 min; ramp 1, 4 ^{o}C/min to 160 ^{o}C; hold 0.0 min; ramp 2, 25 ^{o}C/min to 210 ^{o}C, hold 3.0 min; inlet temperature, 200 ^{o}C, split 10:1; total flow 22.7 mL/min; column flow, 1.79 mL/min; and linear velocity, 25.2 cm/sec.

Electroantennography. The experimental and normalization procedure previously described[14] were used for this study using the same subtraction of the female antennal response to the negative control (pentane) and the female sex pheromone component (*Z*,*Z*)-11,13-hexadecadienal. For females the crude assumption was made that female antennae should not have a full response to the female sex pheromone and their response was therefore subtracted along with the antennal response to the negative control, pentane. Moreover, this assumption was made since at the time no compound was available as positive control for female antennal responses unlike the sex pheromone component used for male antennal responses. Current EAG bioassays used in our laboratory compare and correct antennal responses to the host plant volatile acetophenone for both male and female NOW.[17] Male antennal responses to host plant volatiles were normalized by correcting the deflection amplitude elicited by the major component of the

sex pheromone to 500 μV. Responses to volatiles were duplicated for each sex and reported as the average.

The EAG responses for the Blend concentration dose-response experiment were run using updated methods recently reported.[17] Briefly, 50 μg of the Blend in concentrations of 10 mg of the blend in increasing dilution amounts of ethyl acetate (neat, 25, 50, 75, and 100 μL) were loaded onto assay discs, allowed to dry briefly for 30 seconds and administered across the excised antennae in two second puffs. Reported responses were corrected by subtraction of antennal response to the negative control.

Field Trials. For the 2011 season-long evaluation of the attractiveness of the host plant blend a randomized complete block design with five replicates per treatment was configured in five almond orchards (64-256 ha) located in Kern County, CA. Traps used were standard orange plastic delta traps with glue liners (Suterra, Bend, OR). The treatments were traps baited with: 1) Nalgene bottles containing 200 mg of the Blend solution; 2) bottles with only cotton serving as a negative control; 3) traps baited with almond meal (ca. 18.6 g) in standard egg traps (Trécé, Adair, OK) as a female attractant; and, 4) wire mesh cages containing three virgin females as a male attractant (30 m downwind of other treatments). Trap catches were collected weekly, NOW adult moths were counted, sexed, females dissected to determine their mating status, and fresh blend bottles placed in the traps. Each moth capture experiment comprised two one-week trapping intervals in 2011: April, April 30 to May 5; May, May 13-27; June, June 17 to July 1; July, July 2-17; and, August, August 5-19. Data from periods of no NOW pressure – blend and almond meal treatments having no captures and no or significantly low male capture in virgin female baited traps – were not included in the analysis. Trap data were analyzed with one-way,

repeated measures ANOVA and followed by pairwise multiple comparisons with the Fisher LSD method.

Preliminary field trapping screening of attractiveness of individual volatiles and simple component blends were performed in Kern County, CA over the 2008-2010 growing seasons. For testing of individual components rubber white septa (Sigma-Aldrich, St. Louis, MO) impregnated with pentane solutions containing 5 mg of each test component were hung in orange delta traps. Moth captures of individual components were compared to traps baited with almond meal. For simple blends septa with 5 mg of total components were placed in delta traps, hung in 5 replicate blocks, and again compared to traps baited with almond meal.

RESULTS

Twenty-one compounds were observed and identified in minor to major amounts from Nonpareil and Monterey varieties of almonds during the hull split period (**Table 1**). Emissions between the cultivars were relatively similar in composition with generally minor differences in relative amounts. Some notable differences between the two cultivars were a greater proportion of monoterpenes, and the sesquiterpenes β-caryophyllene and α-humulene in the Nonpareil odor profile. The amounts of benzaldehyde and conophthorin were greater in the Monterey odor profile. Twelve of the 21volatiles in **Table 1** were also detected from damaged almonds[13] and are indicated with a superscript. The compound 1-octen-3-ol, not detected in the hull split experiment but detected from damaged almond emissions, elicited a relatively large female antennal response.

Evaluation of the EAG data in **Table 1** for elicitation of responses from primarily females provided the main basis for the almond odor-derived blend reported; of these methyl salicylate

and (+/-)-conophthorin, ranked eighth and tenth most stimulating, were the only components included in the final blend that elicited female antennal responses of less than 200 μV. A more complex, eight-component volatile blend that included α-humulene, 2-phenylethanol, and nonanal was field tested and provided no improvement in attraction. The more complex blend was statistically equivalent, yet numerically lower in responses than the five-component blend reported here (1.25 ± 0.29 vs. 1.50 ± 0.24, overall NOW captured respectively), thus demonstrating that a simpler blend was similarly effective in trapping NOW. The compound nonanal was detected in the damaged almond study;[13] however, in the hull split volatile collection experiment nonanal was only detected on one GC-MS column thus listed as a transient and not included in **Table 1**.

Traps baited with almond meal captured 19 female and one male NOW over the entire seasonal test period with capture rates in the April interval significantly greater than blank traps, which caught a total of one female and one male NOW (**Table 2**).

For captures of both male and female NOW combined (row labeled NOW in **Table 2**) the Blend significantly exceeded almond meal captures in all test intervals. For female moth captures the Blend significantly exceeded almond meal captures in the April (early 1st flight) and June (early- to mid 2nd flight). The Blend attracted a large percentage of mated females (93%) versus virgin females with the only virgin females captured during the April trapping interval. For male moth captures the Blend significantly exceeded almond meal captures in April, June, July, and August intervals.

Male, female, and combined-gender captures for the Blend statistically exceeded almond meal for the overall test period, which averaged the pooled April through August intervals (Overall block in **Table 2**). Male captures significantly exceeded female captures for the Blend

during the April interval and the overall averages, while female captures significantly exceeded male captures for almond meal during the April interval and the overall averages.

Blend emission release rates over the one-week evaluation period provided the following data (average emission rates in mg/h, average blend composition ratio based on emission rates, and comparison to original composition blend ratio as determined by GC-FID): ethyl acetate (8.23, 74.0 cf: 100.4); conophthorin (0.11, 1.0 cf: 1.0); 1-octen-3-ol (1.46, 13.1 cf: 12.5); acetophenone (0.12, 1.1 cf: 1.0); ethyl benzoate (0.50, 4.5 cf: 4.3); and methyl salicylate (0.40, 3.6 cf: 3.4). Graphs of emission rates as a function of time are provided in **Figure 2**. The linear regression trendline for each component is provided in the graphs in **Figure 2**. However, when an exponential regression analysis was performed for each component the r^2 values were nearly identical, albeit slightly numerically lower (excluding ethyl acetate) than the linear trendline. Graphing of the average vial weights over time during the gravimetric analysis gave an r^2 = 0.9998 when a linear trendline was applied.

Four point calibration curves for each component gave the following r^2 values at the specified concentration range: 1-octen-3-ol, 0.9964, 3.13 to 50.00 mM; ethyl benzoate, 0.9978, 1.56 to 12.50 mM; methyl salicylate, 0.9917, 1.56 to 12.50 mM; acetophenone, 0.9988, 0.39 to 3.13 mM; and, conophthorin, 0.9967, 0.39 to 3.13 mM.

The dose-response experiment analyzing female NOW antennal response via EAG as a function of concentration of the Blend in ethyl acetate (10 mg/x μl of solvent) gave the data presented as a graph in **Figure 3**. The neat blend (no solvent) was statistically greater than the blends at high dilution rates with ethyl acetate as solvent. The four concentrations (100, 75, 50 and 25 μL ethyl acetate) were statistically equivalent. The field rate corresponds to 10 mg of blend components in 100 μL of ethyl acetate.

DISCUSSION

The volatile survey of Nonpareil and Monterey almond cultivars at hull split provided a relatively similar composition with a total of 21 compounds, of which 18 were shared between the two cultivars. A number of the trace and transient peaks not included in **Table 1** have been identified in other almond host plant volatile studies and have been analyzed via EAG for potential activity.[14] For instance, the compound nonanal was detected in the 2009 *in situ* study by Beck and co-workers[14] and was later reported by Leal and co-workers to bind with NOW olfactory proteins.[18] The volatiles identified in **Table 1** were intended as a survey and as a starting point for the testing of compounds and blends for attracting NOW. The emissions between the two cultivars had relatively minor differences in reported amounts except for benzaldehyde, β-caryophyllene, α-humulene, and conophthorin. This disparity in relative amounts may be explained by the cultivar hull differences; a more thorough comparison study of the hull volatile emissions would be necessary to provide a more definitive volatile output difference between the two cultivars.

To begin the EAG investigation of hull split volatiles, an initial blend that represented the overall bouquet of volatiles was formulated and comprised the relatively high-EAG value components. These included: (*E*)-β-ocimene, (+/-)-conophthorin, acetophenone, ethyl benzoate, α-humulene, (*E*,*E*)-α-farnesene (impure), methyl salicylate, and 2-phenylethanol in the ratio of 3:10:6:2:10:8:1:3. The ratio was based on the average GC-MS responses of Nonpareil and Monterey emissions noted in **Table 1**. However, during early EAG and field-trapping studies many of the other components were eliminated and 1-octen-3-ol, a volatile from damaged almonds[13] and eliciting a high EAG response, was eventually inserted to provide the basic

framework of successful blend components. During the blend component screening process, over 50 field-trapping iterations using the components from this initial mixture were performed and a basic blend comprising 1-octen-3-ol : ethyl benzoate : methyl salicylate in a 3:1:1 ratio was identified as being the minimum basic components necessary for adult moth attraction. However, the number of moths captured with this basic blend never exceeded the number of moths caught in the almond meal during the limited screening studies. During these trials, the addition of acetophenone into the blend helped to increase the number of observed NOW captures. Subsequent addition of (+/-)- conophthorin into the blend of (+/-)-1-octen-3-ol, ethyl benzoate, methyl salicylate, and acetophenone provided higher and more consistent capture numbers during these field trapping studies. This was an interesting result since (+/-)-conophthorin by itself performed relatively poorly in our limited field trial studies, as did all other volatiles when tested individually. However, the unique structure of conophthorin and its appearance in damaged[13] and hull split almonds prompted further field evaluation when combined with other candidate host plant volatiles. Conversely, the compound (*E*)-β-ocimene demonstrated a good antennal response (ranked third for males and sixth for females) but did not perform well when evaluated either alone or in various simple blends during the screening field trapping studies. Concurrent EAG studies of all of the iterations were not performed and thus no correlations between EAG and capture results can be made at this time.

The components of the Blend are common plant volatiles with demonstrated semiochemical-elicited behavior in insects.[19] 1-Octen-3-ol is a ubiquitous volatile from both fungal and plant hosts,[13] as well as a breakdown product of linoleic acid.[20] The unassigned stereoisomer has been classified as an attractant for numerous insects.[19] The *R*- and *S*-enantiomers of 1-octen-3-ol were evaluated during the field trapping screening studies but their inclusion in blends did not

demonstrate differential chiral capture activity greater than similar blends using the less expensive racemic 1-octen-3-ol. The spiroacetal conophthorin, most commonly known as a semiochemical for bark beetles[21,22] has origins from plants and insects,[23] but was detected from mechanically damaged almonds.[13]

Interestingly, the attraction of males to the Blend has positive implications for the potential use of the Blend, or an optimized version, for monitoring NOW populations in orchards undergoing mating disruption treatments, wherein males are not readily captured in pheromone-baited monitoring traps in orchard environments inundated with synthetic pheromone.

The initial assumption that the formulated blend would only attract female NOW was incorrect and in fact the results in **Table 2** demonstrate that the Blend attracted more male adult moths than female adult moths. One possible explanation for this result may be explained by a report[24] that observed male *Heliconius* butterflies displaying mate-searching behavior around the host plant. In their report they suggest that the host plant volatiles may be alerting the males to a location to find potential partners.

Another interesting result from the current study was the mating status of the captured females. The traps in the test intervals May through August captured only mated females (with one spermatophore each) and in the April trap test interval three of the 44 females captured were virgins. However, it is not unusual to capture the occasional unmated female NOW in oviposition attractant traps (personal observation, B.S.H.). Based on the initial assumption that the primary behavior of gravid females is searching for an optimal ovipositional site it makes sense for the mated females to be attracted to these particular host plant volatiles.[25]

Ethyl acetate was used as a solvent to help stabilize the blend components. Traps baited with ethyl acetate were not attractive to NOW moths in preliminary 2010 experiments. Initial field

trapping screening indicated ethyl acetate did not have an adverse effect on the Blends' ability to attract NOW moths, which concurs with a study of the strawberry sap beetle that included ethyl acetate as a blend component in high ratio.[26] Conversely, ethyl acetate as a host plant volatile has been shown to induce pheromone production from the African palm weevil and is a necessary component during field attractancy studies.[27] Hypothetically, the possible hydrolysis of ethyl acetate would generate acetic acid, which has been shown to increase codling moth captures when combined with other plant volatiles.[28] This possibility and in depth emission rates of the blend components in ethyl acetate will be considered in future studies. It should be noted that ethyl acetate in addition to ethanol and acetic acid have been detected in other *in vitro* almond emission studies and all three compounds are ubiquitous volatiles emitted in large amounts from most ripe or ripening fruits. The use of ethanol vs. ethyl acetate as a solvent for the Blend was compared concurrently in field trapping studies. The lower number of observed moths captured in the Blend with ethanol as the solvent prompted the choice of ethyl acetate; however, this comparison was for internal purposes only and should not be considered definitive.

Overall, the demonstrated attractancy of the Blend shows promise for the development of a host plant-based attractant; however, there are several aspects of the Blend formulation that require further optimization, with the method of release an obvious first step. Initially, septa charged with the host plant volatiles were used during the screening process but it was decided to switch to the vials with cotton and a hole in the cap for the season-long trapping study to ensure a relatively high and consistent release rate. Additionally, the use of a solvent appeared to have stabilized the emission of the ratio of the host plant volatiles and lengthened the duration of emission. An isothermal (30 ^{o}C) study of the evaporative emissions of the Blend components in ethyl acetate indicated a steady emission at the desired ratios for more than seven days with the

relative percentage of ethyl acetate dropping from ca. 80% to 70% over the evaluation period (**Figure 2**). It should be noted that the total amount of each component lost over time (**Figure 2**) appears to be linear; however, when both the linear and exponential trendline is applied the r^2 values are essentially equivalent. This similarity of trendline fits among regression analyses has been observed[29] with other kairomone release rates in other systems and thus no conclusions are drawn at this juncture. If the use of solvent as a host plant emission mediator is continued then a study that varies both the concentration and solvent type may be beneficial. Some preliminary studies using ethanol in place of ethyl acetate indicated ethyl acetate was more effective in terms of comparative trap numbers when used as a co-solvent; however these two solvents may play varying roles at different concentrations and different cap hole sizes.

To address any ambiguity regarding the role of ethyl acetate as a solvent, the use of the Blend in a membrane dispenser will be explored in subsequent studies. An EAG dose-response experiment that measured the female antennal response to varying concentrations of the Blend in ethyl acetate was conducted (**Figure 3**) and the EAG responses indicated that the Blend without the solvent would be more effective than the 100 mg/mL concentration that was used in this study. However, the initial field trapping studies that compared neat Blend to the Blend in ethyl acetate showed slight adult moth preference for the Blend in ethyl acetate.

The host plant-based Blend from almonds was more attractive to adult NOW than the standard almond meal bait. The higher capture numbers and increased consistency present an opportunity for an improved host plant-based monitoring approach for NOW in orchards managed either conventionally or by mating disruption. While these results do not infer commercial use, they do highlight an important incremental step in host plant-based volatile blends for monitoring NOW, which has proven elusive. A comprehensive, high-replicate

electroantennographic screening of all available almond emission volatiles is currently underway and will assist in further optimization of host plant volatiles as a potential monitoring tool.

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FIGURE CAPTIONS

Figure 1. Closed volatile collection system used to collect *in situ* hull split volatiles from an enclosed almond branch.

Figure 2. Emission release rates for each Blend component and solvent at 30 ^{o}C with amount lost (g of material) versus time. Insets show concentration of emitted component (mg/h) versus time. Shown are linear regression analyses for modeling emission release rates.

Figure 3. Female NOW EAG response to concentrations of 10 mg of Blend mixed with varying dilution rate amounts of ethyl acetate. EAG conditions were 50 μg total of mixtures and N = 7 for each concentration. Responses with different letters are significantly different ($P < 0.05$), one-way ANOVA followed by pairwise comparison by Student's t-test.

Figure 1

Figure 2.

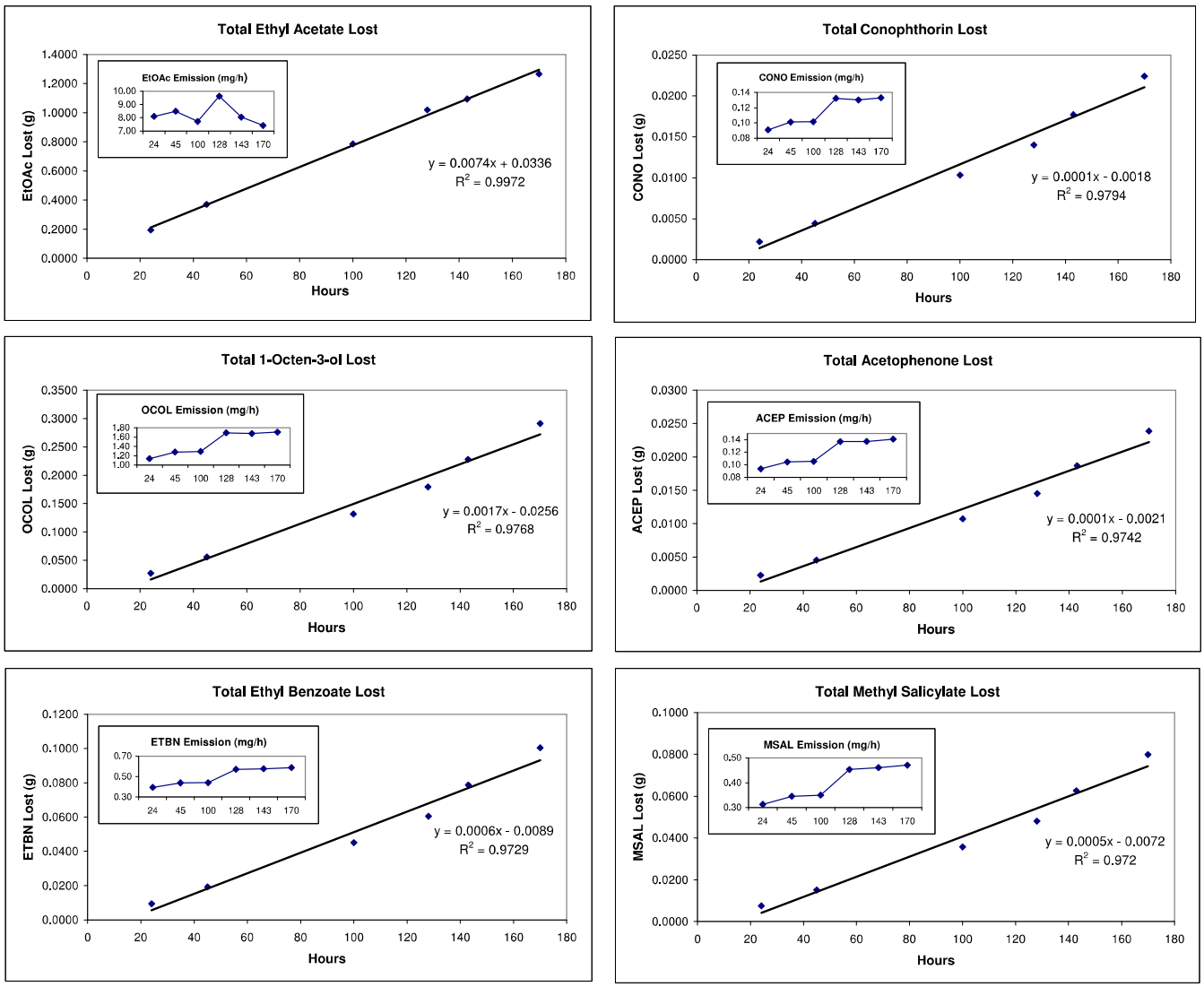
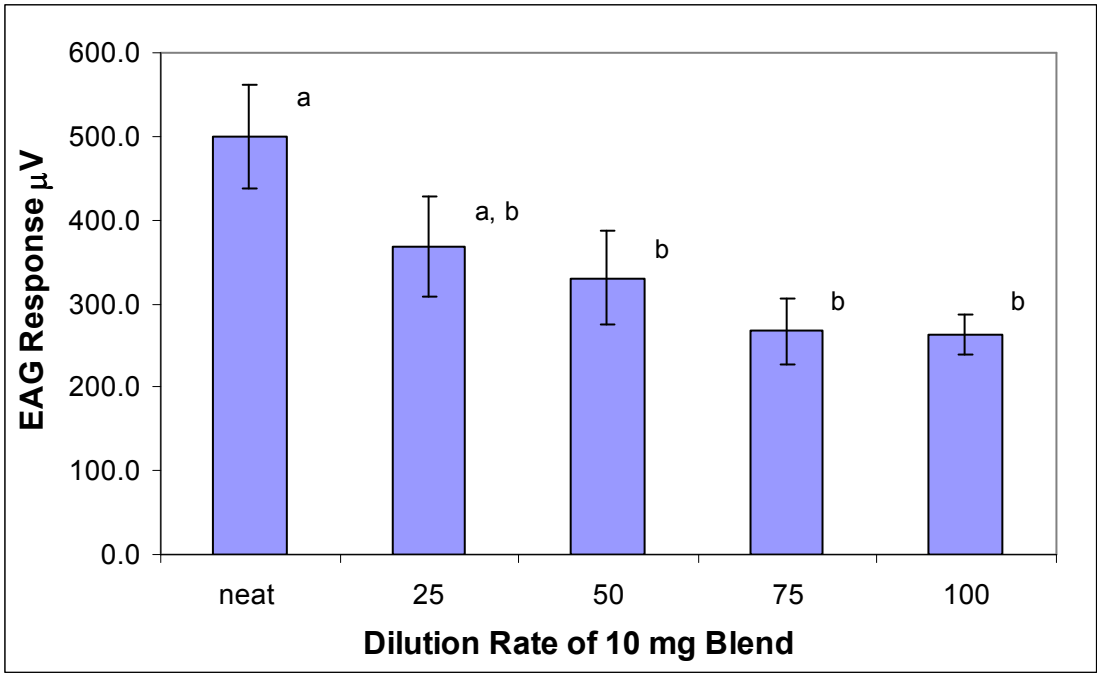

Total Ethyl Acetate Lost
EtOAc Lost (g)
Hours
EtOAc Emission (mg/h)
y = 0.0074x + 0.0336
R2 = 0.9972
Total Conophthorin Lost
CONO Lost (g)
CONO Emission (mg/h)
y = 0.0001x - 0.0018
R2 = 0.9794
Total 1-Octen-3-ol Lost
OCOL Lost (g)
OCOL Emission (mg/h)
y = 0.0017x - 0.0256
R2 = 0.9768
Total Acetophenone Lost
ACEP Lost (g)
ACEP Emission (mg/h)
y = 0.0001x - 0.0021
R2 = 0.9742
Total Ethyl Benzoate Lost
ETBN Lost (g)
ETBN Emission (mg/h)
y = 0.0006x - 0.0089
R2 = 0.9729
Total Methyl Salicylate Lost
MSAL Lost (g)
MSAL Emission (mg/h)
y = 0.0005x - 0.0072
R2 = 0.972

Figure 3.


600.0
500.0
400.0
300.0
200.0
100.0
0.0
EAG Response µV
a
a, b
b
b
b
neat
25
50
75
100
Dilution Rate of 10 mg Blend

1

Table 1. Major (≥10%) and minor (< 10% and > 1%) volatiles collected *in situ* from hull split Nonpareil (NP) and Monterey (MO) almonds, Kern County, CA 2008

Identity	Source[b]	Average %		EAG Response (μV)[a]	
		NP	MO	♂	♀
α-pinene[c]	Alph	3.31	0.87	0	0
camphene[c]	Iso	1.84	1.50	0	10
β-pinene[c]	Iso	3.49	0.00	0	0
myrcene[c]	Iso	1.58	0.48	25	0
limonene[c]	Alph	15.00	6.78	0	0
(*E*)-β-ocimene	Iso	2.13	1.33	183	240
acetoin[c]	Ald	1.55	3.05	0	95
conophthorin[c]	Con	1.84	7.98	55	130
(*E*)-4,8-dimethyl-1,3,7-nonatriene[c]	Iso	1.90	1.65	0	0
(*Z*)-hex-3-enyl acetate	Bed	2.06	3.91	0	0
benzaldehyde[c]	Ald	22.46	49.95	0	0
β-bourbonene[c, d]	Tent	3.27	0.00	-	-
β-caryophyllene	Iso	20.35	2.72	0	190
methyl benzoate	Ald	1.48	2.91	10	5
acetophenone	Alph	2.23	4.02	0	260
ethyl benzoate[c]	Alph	0.00	1.69	0	525
α-humulene	Ald	7.89	1.64	187	250
(*E*,*E*)-α-farnesene	Iso	4.79	3.07	160	155
methyl salicylate	Alph	0.01	0.76	245	175
2-phenylethanol[c]	Ald	1.61	3.95	54	320
phenol	Ald	0.62	1.02	0	0
1-octen-3-ol[e]	Alph	-	-	0	475

[a] Corrected to (*Z*,*Z*)-13,16-hexadecadienal and pentane; [b] Source of compound for authentication and EAG studies: Ald, Sigma Aldrich; Alph, Alpha Aesar; Bed, Bedoukian; Con, Contech; Iso, previously isolated by a researcher in this laboratory (Buttery, Flath, or Binder); Tent, tentative assignment; [c] Compounds also seen in mechanically damaged almond study[ref 13]; [d] Compound not available for EAG or authentication; [e] From damaged almond study

Table 2. Mean captures of male and mated female NOW per trap per week, Kern County, CA 2011

Test Interval[b]	Flt	N=	Moths	Treatment[a] Host Plant Blend[c]	Almond Meal	Blank	One-way ANOVA	Males in Virgin Female-Baited Traps (range)
April[d]	1	10	NOW	11.30 ± 1.97 a	1.60 ± 0.34 b	0.10 ± 0.10 c	F=27.87, df:2,27; *P*<0.001	
			Female	4.40 ± 0.97 a *	1.60 ± 0.34 b *	0 c	F=13.64, df:2,27; *P*<0.001	
			Male	6.90 ± 1.10 a *	0 b *	0.10 ± 0.10 b	F=38.86, df:2,27; *P*<0.001	86.57 ± 18.74 (27-183)
May	1	5	NOW	1.60 ± 0.60 a	0.20 ± 0.20 b	0 b	F=6.91, df:2,12; *P*=0.018	
			Female	0.60 ± 0.25	0.20 ± 0.20	0	F=3.50, df:2,12; *P*=0.081	
			Male	1.00 ± 0.63	0	0	F=2.50, df:2,12; *P*=0.143	20.60 ± 13.34 (0-105)
June	2	7	NOW	1.14 ± 0.26 a	0 b	0 b	F=19.20, df:2,18; *P*<0.001	
			Female	0.43 ± 0.20 a	0 b	0 b	F=4.50, df:2,18; *P*=0.035	
			Male	0.76 ± 0.29 a	0 b	0 b	F=6.25, df:2,18; *P*=0.014	26.80 ± 10.00 (0-102)
July	2	9	NOW	2.00 ± 0.58 a	0.22 ± 0.22 b	0.11 ± 0.11 b	F=11.74, df:2,24; *P*<0.001	
			Female	0.44 ± 0.24	0.11 ± 0.11	0.11 ± 0.11	F=1.14, df:2,24; *P*=0.344	
			Male	1.56 ± 0.63 a	0.11 ± 0.11 b	0 b	F=6.34, df:2,24; *P*=0.009	17.80 ± 6.54 (0-52)
August	3	7	NOW	1.14 ± 0.40 a	0.14 ± 0.14 b	0 b	F=6.22, df:2,18; *P*=0.014	
			Female	0.71 ± 0.42	0.14 ± 0.14	0	F=2.25, df:2,18; *P*=0.148	
			Male	0.43 ± 0.20 a	0 b	0 b	F=4.50, df:2,18; *P*=0.035	30.00 ± 10.28 (2-85)
Overall		38	NOW	4.08 ± 0.89 a	0.53 ± 0.15 b	0.05 ± 0.04 b	F=20.19, df:2,111; *P*<0.001	
			Female	1.55 ± 0.39 a *	0.50 ± 0.15 b *	0.03 ± 0.03 b	F=12.72, df:2,111; *P*<0.001	
			Male	2.53 ± 0.54 a *	0.03 ± 0.03 b *	0.03 ± 0.03 b	F=21.46, df:2,111; *P*<0.001	
Total[e]			NOW	155	20	2		
			Female	59	19	1		
			Male	96	1	1		

[a] Capture values are means ± S.E. Data in rows followed by different letters are significantly different ($P < 0.05$), one-way, repeated measures ANOVA followed by all pairwise multiple comparisons by Fisher LSD method. [b] April, 4/30 - 5/5 May, 5/13 - 5/27; June, 6/17 - 7/1; July, 7/2 - 7/17; August, 8/5 - 8/19**;** [c] Females captured in May-August traps baited with the Blend were all mated and with one spermatophore; [d] Five-day period; [e] Total, combined captures over all test periods; * Significant difference between male and female captures, paired t-test, $P < 0.005$.

TOC GRAPHIC

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Hull Split and Damaged Almond Volatiles Attract Male and Female Navel Orangeworm Moths