

Comparison of the volatile emission profiles of ground almond and pistachio mummies: Part 1 – Addressing a gap in knowledge of current attractants for navel orangeworm[☆]

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ABSTRACT

Over the years various tissues of almond and pistachio have been evaluated for their ability to attract the navel orangeworm moth, a major insect pest in California tree nut orchards. Almond meal, which typically consists of ground almond kernels, is a monitoring tool for navel orangeworm populations in almond and pistachio orchards. Recently, ground pistachio and almond mummies have been re-visited in field trapping studies for their potential to attract navel orangeworm moths. Surprisingly, the volatile profiles of these two systems have not been reported. The objective of this study was to survey and then compare and contrast the volatile profiles of both ground almond and pistachio mummies over the course of a week. Principal component analysis (PCA) of the headspace volatiles showed a distinct difference between ground almond and pistachio mummies. The volatile emission profile of the almond mummies remained consistent over the one-week period, albeit in low content and composition; whereas the profile of the pistachio mummies changed over time as shown by PCA. Seven compounds were identified as being common volatiles to both matrices. These data may help explain results from recent navel orangeworm field trapping studies using almond and pistachio mummies, as well as with the formulation of future synthetic blends.

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1. Introduction

The navel orangeworm (*Amyelois transitella*) has been an insect pest of California tree nuts since the 1950s and 1960s (Ebeling, 1959; Michelbacher and Davis, 1961; Wade, 1961; Beck and Higbee, 2013) and continues to inflict significant economic damage to the tree nut industry (Higbee and Siegel, 2009; Niederholzer, 2012). Despite numerous efforts over the years, effective control of the navel orangeworm has relied primarily on insecticide sprays, proper orchard sanitation (Higbee and Siegel, 2009), and some use of mating disruption (Higbee and Burks, 2008; UC IPM, 2013).

The utilization of the egg trap, a plastic cylinder with mesh-covered openings to allow bait volatiles to escape and a ridged surface for female oviposition, to monitor navel orangeworm populations began circa mid-1970s (Rice, 1976), with the original bait being a modification of the medium used to rear navel orangeworm and consisting of wheat bran, glycerine, honey, and water (Rice, 1976; Rice et al., 1976; Rice and Sadler, 1977). The wheat bran ovipositional attractant gave way to the use of almond press cake with and without the addition of crude almond oil (Rice et al., 1984; Van Steenwyk and Barnet, 1985). Since that time, almond meal-based products have been a standard egg trap bait for monitoring of navel orangeworm populations in almond orchards (Kuenen et al., 2008; Burks et al., 2011; Higbee and Burks, 2011). Other tree nut-based attractants have included fatty acid components (Phelan et al., 1991), extracts of navel orangeworm frass mixed with kernels, hulls and shells of almonds (Curtis and Clark, 1979), and more recently pistachio kernels (Kuenen et al., 2008; Higbee and Burks, 2011).

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Through the years several experiments have evaluated almond mummies in the field to determine navel orangeworm infestation levels. A 1982 report (Andrews and Barnes, 1982) evaluated the ovipositional attractiveness of navel orangeworm infested- and non-infested almond mummies in almond orchards, and in 1987 Phelan and Baker used a wind-tunnel bioassay followed by field trapping studies to compare the attractancy of navel orangeworm to press cake and almond mummies (Phelan and Baker, 1987). More recently, almond mummies and pistachio mummies were evaluated for the ability to attract navel orangeworm moths (Nay et al., 2012) in almond and pistachio orchards in field trapping studies.

Despite the span of years and modifications to the egg trap bait formulation very little is known about their volatile emissions. Our laboratories have evaluated the volatile headspace of numerous other almond and pistachio-based matrices (Beck et al., 2008, 2009, 2011a, 2011b, 2012a; Roitman et al., 2011; Mahoney et al., 2014), yet no publications have provided volatile profiles of specific tree nut mummy matrices. With the apparent lack of information on bait volatiles we undertook the present project to address two distinctive objectives: to survey the volatile profiles of ground almond and pistachio mummies over the course of a week; and to compare and contrast the volatile emission profiles of these two matrices to discuss probable semiochemicals with respect to known field trapping studies.

2. Results and discussion

The water activity and water content were determined to establish the overall level in each matrix. For the almond mummies the water activity was 0.512 (± 0.001 s.e.m.) and the water content was 10.10% (± 0.35 s.e.m.). The water activity of the pistachio mummies was 0.514 (± 0.003 s.e.m.) and the water content was 8.81% (± 0.09 s.e.m.). The water activity values for both matrices were well below the value of 0.85, which is the value necessary for most non-xerophilic fungi to develop (Hocking, 2001). For the almond mummies, the water activity level of 0.512 is close to the value of 0.454 noted for the almond hulls during hull split in September (Mahoney et al., 2014). The water activity of the pistachio mummies, however, decreased from 0.840 as pistachio hulls in October (Mahoney et al., 2014) to the present value of 0.514. Comparison of these water activity levels between previous season hull and mummies may not directly correlate since the mummies of both matrices include the hull, shell, and kernel, and are being compared to just the hull tissues from the previous season. However, they do provide a basis for understanding the mummy matrices and the possible interactions with the present fungi.

Tables 1 and 2 list the volatiles detected on day seven from the headspace volatile analyses of the pistachio and almond mummy matrices, respectively. A total of 64 volatiles were detected from pistachios (Table 1) seven of which were common to both matrices (Fig. 1). The vast majority of the volatiles detected were terpenoids with the major monoterpenes showing similar composition and content to *ex situ* analyses performed previously on the fruits of the Kerman cultivar (Roitman et al., 2011). The unusual diversity of other classes of compounds emitted by the ground pistachio mummies are likely due to the various molds typically present in most tree nut orchards (Bayman et al., 2002). None of the compounds listed in Table 1 are presently known semiochemicals of navel orangeworm. However, some monoterpenes (i.e., limonene, myrcene, and linalool) have been reported to be attractive to other Pyralidae moths (Meagher and Landolt, 2008); and, a pyran-type of compound, similar to compound 2009, has been reported as a sex pheromone component of a Pyralidae moth (Sasaerila et al., 2003).

The volatiles from the pistachio mummies were consistent in their relative abundances emitted. Of the 64 compounds detected, 55 showed little variation (less than 7%) in the four days of

Table 1

Volatiles produced by ground pistachio mummies. Volatile relative amounts are the average relative abundances (Rel. Abund.) $d = 7$, \pm standard error means (\pm s.e.m.). Volatiles are listed by their calculated retention indices (RI) in ascending order.

Pistachio mummy volatiles	RI	Rel. abund.	(\pm s.e.m)
Tricyclene ^a	1007	12,917,206	(761,313)
α -Pinene ^{a,b}	1020	168,777,888	(8,446,904)
α -Thujene ^{a,b}	1024	212,887,434	(10,923,240)
Camphene ^a	1060	37,786,598	(1,585,935)
β -Pinene ^a	1106	32,341,250	(1,835,460)
Sabinene ^a	1118	9,146,822	(616,519)
Δ -4-Carene ^c	1129	4,555,157	(286,799)
1-Methylpyrrole ^a	1134	2,770,517	(187,803)
Δ -3-Carene ^a	1146	38,813,125	(1,393,492)
β -Myrcene ^a	1161	18,875,988	(938,775)
α -Terpinene ^a	1177	3,288,522	(153,027)
Limonene ^{a,b}	1197	628,743,171	(13,468,474)
β -Phellandrene ^a	1206	8,026,775	(469,266)
2-Pentylfuran ^{a,b}	1230	1,340,179	(131,033)
<i>cis</i> - β -Ocimene ^a	1234	1,109,069	(58,880)
γ -Terpinene ^a	1243	1,361,774	(75,870)
<i>trans</i> - β -Ocimene ^a	1250	1,244,972	(59,311)
Styrene ^a	1253	809,697	(30,331)
<i>p</i> -Cymene ^a	1267	4,403,231	(162,992)
α -Terpinolene ^{a,b}	1281	90,486,747	(3,348,011)
2,6-Dimethylpyrazine ^a	1327	405,913	(56,388)
Mentha-1,4,8-triene ^c	1391	555,586	(106,506)
Thujol ^c	1420	1,002,667	(194,131)
<i>p</i> -Mentha-1,5,8-triene ^c	1422	459,323	(82,780)
<i>p</i> -Cymenyl ^c	1433	1,410,075	(31,047)
β -Thujone ^c	1437	868,323	(58,801)
<i>cis</i> -Limonene oxide ^c	1443	657,154	(180,432)
Acetic acid ^{a,b}	1451	4,962,956	(389,359)
<i>trans</i> -Limonene oxide ^c	1456	947,250	(47,893)
α -Longipinene ^a	1465	30,426,284	(171,434)
α -Ylangene ^a	1481	593,376	(33,575)
Longicyclene ^a	1495	636,644	(44,519)
Camphor ^a	1511	453,416	(210,223)
Linalool ^a	1549	807,493	(149,438)
Pinocarvone ^a	1564	1,078,632	(175,718)
Junipene ^c	1565	1,491,608	(201,392)
Unk Sesq A (161, 93, 69, 108, 133) ^d	1571	2,382,589	(103,204)
Bornyl acetate ^a	1579	23,877,080	(714,223)
Unk Sesq B (41, 69, 79, 91, 161) ^d	1593	1,478,579	(192,991)
Unk Sesq C (121, 56, 136, 41) ^d	1600	3,904,707	(249,957)
Widdrene (Thujopsene) ^a	1618	4,161,532	(159,469)
Myrtanal ^a	1623	793,197	(108,251)
<i>trans</i> - <i>p</i> -Mentha-2,8-dien-1-ol ^c	1629	2,415,015	(51,976)
Unk A (93, 96, 108, 81, 55) ^d	1642	3,162,828	(155,731)
<i>trans</i> - β -Farnesene ^a	1665	799,884	(105,326)
<i>p</i> -Mentha-2,8-dien-1-ol ^c	1671	1,828,901	(205,424)
Unk Sesq D (119, 121, 41, 105, 93) ^d	1675	1,424,742	(167,476)
<i>trans</i> -Verbenol ^a	1680	833,349	(132,243)
1,8-Menthadien-4-ol ^c	1688	3,562,233	(149,232)
Eucarvone ^c	1695	690,462	(67,035)
4,7-Dimethylbenzofuran ^c	1698	949,531	(34,316)
Verbenone ^a	1701	4,348,136	(167,333)
Germacrene D ^a	1705	1,324,406	(72,324)
Unk B (121, 107, 79, 136, 41) ^d	1726	1,335,427	(62,643)
Carvone ^a	1730	1,386,404	(278,567)
β -Himachalene ^c	1731	1,559,723	(97,311)
Unk C (84, 83, 41, 91, 69) ^d	1753	3,648,000	(203,573)
<i>trans</i> - α -Bisabolene ^a	1772	608,356	(113,474)
Unk Sesq E (119, 91, 132, 105, 41) ^d	1777	2,127,143	(79,796)
Cuparene ^a	1818	2,172,229	(98,533)
Isopiperitenone ^c	1834	1,228,084	(81,893)
<i>p</i> -Cymen-8-ol ^a	1847	2,483,498	(127,604)
Piperitenone ^c	1915	621,218	(95,822)
5,6-Dihydro-4-methyl-2-H-pyran-2-one ^{b,c}	2009	3,236,706	(135,830)

^a Compounds verified by comparison of retention times and fragmentation patterns to authentic standards.

^b Compounds were also detected in the headspace of almond mummies.

^c Tentative assignment.

^d Five highest fragmentation peaks (*m/z*) provided for unknown sesquiterpenes (Unk Sesq) or unknown class of compounds (Unk).

Table 2

Volatiles produced by ground almond mummies. Volatile relative amounts are the average relative abundances (Rel. Abund.) $d=7$, \pm standard error means (\pm s.e.m.). Volatiles are listed by their calculated retention indices (RI) in ascending order.

Almond mummy volatiles	RI	Rel. abund.	(\pm s.e.m)
α -Pinene ^{a,b}	1020	402,718	(20,762)
α -Thujene ^{a,b}	1024	420,830	(77,755)
2-Ethyl-3-methylbutanal ^c	1064	298,017	(61,952)
Limonene ^{a,b}	1197	5,096,758	(411,157)
2-Pentylfuran ^{a,b}	1230	73,333	(13,333)
3-Methyl-3-buten-1-ol ^c	1248	346,725	(19,445)
α -Terpinolene ^{a,b}	1281	532,614	(40,197)
Nonanal ^b	1391	394,901	(88,977)
Acetic acid ^{a,b}	1451	6,805,702	(410,756)
2-Furfural ^c	1458	300,000	(28,868)
Benzaldehyde ^b	1516	201,714	(18,440)
2,3-Butanediol A ^c	1541	734,735	(22,252)
2,3-Butanediol B (meso) ^c	1578	1,369,446	(76,581)
γ -Pentalactone ^b	1601	615,699	(17,491)
γ -Butyrolactone ^b	1618	685,547	(69,169)
Unk D (41, 43, 59, 69, 113) ^d	1660	304,583	(27,459)
Unk E (45, 43, 69, 126, 85) ^{d,e}	1666	311,385	(74,367)
γ -Hexalactone ^b	1694	279,500	(18,378)
5,6-Dihydro-4-methyl-2H-pyran-2-one ^a	2009	391,927	(114,915)
Unk F (126, 43, 98, 140, 53) ^d	2092	1,067,337	(83,178)

^a Compounds were also detected in the headspace of pistachio mummies.

^b Compounds verified by comparison of retention times and fragmentation patterns to authentic standards.

^c Tentative assignment.

^d Five highest fragmentation peaks (m/z) provided for unknown class of compounds (Unk).

^e Relative abundance listed is for $d=1$, the only day this volatile was emitted.

sampling during the seven-day period. Principal component analysis (PCA) of the volatile emission profiles (Fig. 2) showed differences between all days other than days 3 and 7, which appeared to be similar. These day-to-day differences are due to the compounds with the RI values of 1481, 1495, 1593, 1665, 1675, 1695, 1705, 1772, and 1915, and are primarily sesquiterpenes with the exception of two oxygenated monoterpenes (1695 and 1915). Only two other volatiles, 1230 and 2009, showed changes in their relative abundances over the four sampling days (see Supplemental Data).

Evaluation of the almond mummy data (Table 2) showed a marked decrease in both the volatile composition and content relative to pistachio mummies. Ground almond mummies produced a total of 20 volatiles over the seven-day period covering a broad range of compound classes, which included monoterpenes, benzenoids or other aromatics, alcohols, and several lactones. It was interesting to note that six of the 20 almond mummy volatiles 1197, 1230, 1391, 1451, 1618, and 1694 were also detected in a study of the volatile profiles of aspergilli-infected almonds, and that most of these have been associated as fungal or fatty acid breakdown volatiles (Beck et al., 2011b).

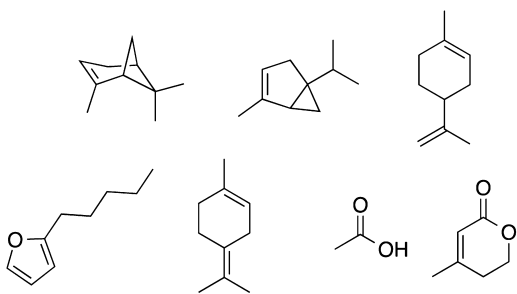


Fig. 1. Volatiles common to both pistachio and almond mummies. Detected by headspace analysis over a 7 d period at 30 °C. From left to right and top to bottom: α -pinene, α -thujene, limonene, 2-pentylfuran, α -terpinolene, acetic acid, and 5,6-dihydro-4-methyl-2H-pyran-2-one.

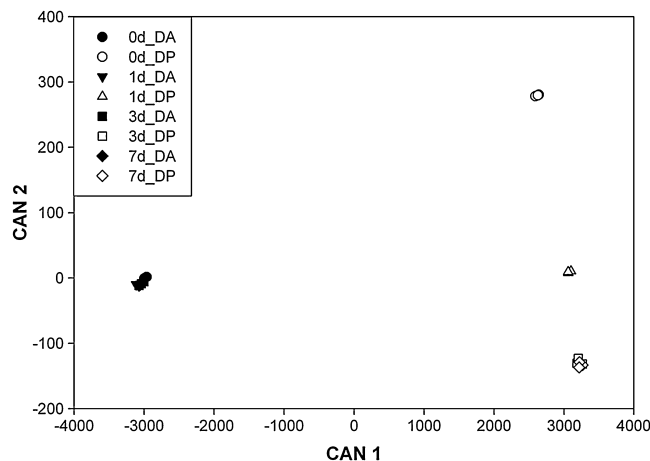


Fig. 2. Principal component analysis of the first two canonical variables of the volatiles emitted by almond mummies and pistachio mummies. DA = almond mummies, DP = pistachio mummies.

One of the most distinctive differences between almond and pistachio mummy volatile emissions was the overall amount of the detected volatiles. For instance, the sum of total volatile relative abundances was ca. 8×10^7 (4×10^6 average per volatile) for almond and 1×10^9 (2×10^7 average per volatile) for pistachio, though the same matrix amounts and the same collection parameters were used. Volatile composition aside, this difference in volatile output amount may play an important role in the ability of almond mummies to attract navel orangeworm moths (Nay et al., 2012) relative to the pistachio mummy matrix.

The PCA results shown in Fig. 2 illustrate that there were no major differences of almond mummy emissions over the four sampling periods. This is due to the fact that PCA is performed on quantitative values and thus the low relative abundance of the almond mummy volatiles over time did not contribute to observable differences via PCA (Fig. 2). However, of the 20 compounds emitted from almond mummies (Table 2), four compounds (1064, 1516, 1666, and 2009) did show intermittent volatile emissions (see Supplemental Data), albeit in low relative abundances. It should be noted that none of the volatiles from almond mummies have documented semiochemical activity for navel orangeworm, except for nonanal which has been reported to bind with olfactory proteins from navel orangeworm (Liu et al., 2010).

Principal component analysis of the volatile profiles showed a clear separation of the two matrices on the x -axis where 92.5% of the experimental variation was explained. The large separation between almond and pistachio mummy volatile profiles, as demonstrated on the x -axis of Fig. 2, was due to host-specific volatiles and/or common volatiles that accumulated differentially in the two matrices. This is further illustrated in the Venn diagram (Fig. 3), which illustrates the differences in volatile emissions between these two matrices, and also highlights the surprising number of volatiles common to both (Fig. 1). There were 57 volatiles that were host-specific to pistachio mummies, 13 volatiles host-specific to almond mummies, and 7 volatiles, which represented 35% of the almond volatile profile, common to both matrices. These volatiles are denoted in Tables 1 and 2 with superscripts and include α -pinene (1020), α -thujene (1024), limonene (1197), 2-pentylfuran (1230), α -terpinolene (1281), acetic acid (1451), and 5,6-dihydro-4-methyl-2H-pyran-2-one (2009). All of these compounds were detected at significantly greater amounts ($P < 0.01$) in pistachio mummies and ranged from ca. 8 to 500 times more than the amounts emitted by almond mummies. The only exception was acetic acid, which accumulated

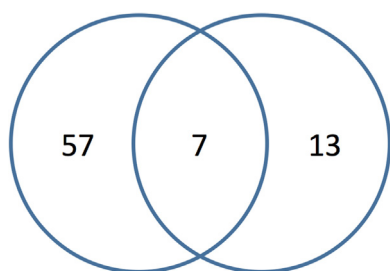


Fig. 3. Venn diagram illustrating the differences between the volatile profiles of pistachio and almond mummies. The circle on the left represents the 57 volatiles that are host-specific to pistachio mummies, the circle on the right the 13 volatiles host-specific to almond mummies, and the 7 volatiles in the overlapping area represent the volatiles common to both matrices.

to similar amounts in the two matrices. Moreover, all of these compounds except for 5,6-dihydro-4-methyl-2H-pyran-2-one (2009) in almond mummies had consistent emission over the four sampling periods. Acetic acid (1451) has been documented to enhance the trap efficacy of a host plant volatile (Knight, 2010).

One important aspect of this research was that it provided the volatile profiles of tree nut matrices that have been evaluated for their ability to attract navel orangeworm. A drawback of these matrices has been their inconsistent physical make-up (Rice, 1976; Andrews and Barnes, 1982), which is likely to influence their chemical make-up as well (i.e. infested versus non-infested, frass, molds, cultivar type, seasonal variations). As a result, these matrices may provide inconsistent results from year to year (Higbee and Burks, 2011). The results of this study will help researchers delineate important semiochemicals that may act as attractants for the navel orangeworm, and subsequently progress toward a synthetic blend of volatiles for monitoring this insect pest (Beck et al., 2012a). Also highlighted in this study was the apparent important role the mummy fungi play in the emission of volatiles that may attract navel orangeworm (Mahoney et al., 2014; Beck et al., 2012b). There exists a need to delineate the fungi present on almond and pistachio mummies and to more accurately explore their individual volatile profiles from these matrices. Many of these volatiles are being explored for any potential semiochemical behavior to navel orangeworm.

3. Experimental

3.1. Almond and pistachio mummy collection

Almond mummies from an orchard containing Nonpareil and Monterey cultivars, and pistachio mummies from an orchard of the cultivar Kerman were collected from the ground and/or trees in the winter months of 2012 and 2013. All mummies were from commercial orchards located in the southern Central Valley of California (Kern County). The mummies were transported to the USDA-ARS laboratory in Albany, CA in a burlap sack for almonds or brown paper bag for pistachios and kept at room temperature.

3.2. Almond and pistachio mummy water and volatile analyses

For each matrix, 2.7 kg of the mummy tissue (hull, shell, kernel, and any present navel orangeworm larvae and frass) was ground to a powder in a blender (Waring, Torrington, CT). The almond and pistachio powdered materials were used with no additional treatment. Unused powdered materials were stored in separate plastic bags at room temperature and in the dark. The water content of the mummy powders was measured via a moisture analyzer (Mettler HB43-S, Columbus, OH) in triplicate 5 g samples, and the water activity was measured via a water activity meter

(AquaLab 4TE, Pullman, WA) in triplicate 2 g samples. For collection of volatile headspace 12 g of either almond or pistachio mummy powder was transferred to a 4-ounce wide-mouth Mason jar (Ace Hardware, El Cerrito, CA) with a modified lid containing a port for volatile sampling. Triplicates of mummy samples were incubated in a closed system for seven days at 30 °C. The headspace volatiles of the ground almonds or pistachio mummies were adsorbed onto 100 μm solid-phase microextraction (SPME), polydimethylsiloxane fibers (Supelco, Bellefonte, PA). Headspace volatiles of the triplicates were monitored on days 0, 1, 3, and 7 in a closed system, and each at exactly the same intervals of 0, 24, 72, and 168 h. The exposure time of the fiber to the headspace volatiles for each collection was 30 min at 30 °C, except for the 0 h sample which was 30 min at room temperature.

3.3. Volatile analysis

All adsorbed volatiles were desorbed onto a DB-Wax column (60 m × 0.32 mm i.d. × 0.25 μm) (J&W Scientific, Folsom, CA) installed on a 6890 gas chromatograph (GC) coupled to HP-5973 mass selective detectors (MS; Palo Alto, CA). Desorbed volatiles were analyzed with identical parameters as noted in a previous publication (Mahoney et al., 2014). Volatiles were included in the final tables if detected in all three replicates of each sampling period. Volatile identities were verified by comparison of retention time and fragmentation patterns to authentic standards. Volatile identities not verified were listed as tentative assignments and are based on NIST or Wiley databases.

3.4. Statistical analysis

Principal component analysis of each matrix ($n = 2$) and time ($n = 4$) was performed using BioNumerics 4.6 (Applied Maths, Inc.). PCA was performed using the log-transformed data of each volatile representing a specific matrix and time point.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.phytol.2014.04.010>.

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