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Comparison of the volatile emission profiles of ground almond and pistachio mummies: Part 2 – Critical changes in emission profiles as a result of increasing the water activity

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ABSTRACT

Pistachio and almond mummies have been reported to attract adult navel orangeworm moths in field trapping studies. The volatile profiles of these matrices were recently described. Concurrently, recent investigations have demonstrated that the water activity of almond hulls plays an important role in the production of semiochemicals known to attract the navel orangeworm in almond orchards. In the present study, the water activity of pistachio and almond mummies was increased and the resultant headspace volatiles monitored over the course of a week. The volatile profile of wet pistachio mummies contained 86 volatiles, of which 22 were unique to the wet matrix. The volatile profile of the wet pistachio matrix increased in chemical diversity to include small chain alcohols, benzenoids, and fatty acid breakdown products relative to the dry matrix, which primarily emitted terpenoids. The wet almond mummies emitted a total of 57 volatiles, 37 more than dry almond mummies. Among the volatiles detected in the wet almond mummies were three of the five compounds that are found in the synthetic blend of host plant volatiles known to attract navel orangeworm moths. The volatile bouquets from the wet and dry pistachio mummies, and wet almond mummies were evaluated by electroantennographic (EAG) analysis.

1. Introduction

The navel orangeworm (*Amyelois transitella*) has been a major insect pest in California tree nut orchards for the past five decades (Michelbacher and Davis, 1961; Wade, 1961). Egg traps – a plastic cylinder with mesh-covered openings and ridged surfaces that females oviposit upon – have been used to monitor mated female navel orangeworm moth populations in tree nut orchards for ca. four decades (Rice, 1976) and are still considered a standard commercial monitoring tool for females (Burks et al., 2011).

The original bait for egg traps was a modification of the medium used to rear navel orangeworm and included wheat bran, glycerine, honey, and water (Rice, 1976; Rice et al., 1976; Rice and Sadler, 1977). Rice and Sadler (1977) found that the wheat bran mix had to be kept moist by the addition of water every 3- to 4-days to maintain trap activity. This observation has noteworthy relevance to navel orangeworm attraction in terms of the volatile emission profiles produced by the bait. Recent studies in our laboratories have investigated the complex relationship of fungi and developing fungal spores on almond and pistachio hosts, and the resultant volatiles that appear to be dependent upon the water activity of the host matrix (Beck, 2013; Beck et al., 2011b, 2012a,b, 2014; Mahoney et al., 2014). For example, during the investigation of ground almond hull emissions (Mahoney et al., 2014) the water activity of a dry sample (0.454) was increased to a higher level. Several days after the addition of water the headspace of the sample was again monitored and the volatile profile of the sample had changed substantially in both content and composition.

Nay et al. (2012) recently performed field trapping studies using pistachio and almond mummies – nuts remaining after harvest activity and aged in the field over the autumn and winter – as bait to attract navel orangeworm moths in pistachio and almond

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orchards. Their results demonstrated navel orangeworm preference for pistachio mummies over almond mummies. More importantly, the results of their study highlighted the need for further exploration of these matrices for potential semiochemicals that could be used in synthetic blends to attract the navel orangeworm. Because of the possible compositional variability of baits such as tree nut mummies (Rice, 1976; Andrews and Barnes, 1982), the volatile composition may be inconsistent and the ensuing field trapping results therefore uncertain (Higbee and Burks, 2011). The compositional variability can include cultivar or phenology differences, larvae infested or non-infested, and frass or fungal bouquets present.

Despite being ubiquitous and generally known, the exact identities and amounts of fungi present on tree nut mummies are not currently documented. The high amount of fungi present on mummies (Fig. 1) and the associated oxidative decay products (Beck et al., 2011b) offer several opportunities for semiochemicals to be emitted and attract navel orangeworm. The volatile emission profiles of the dry matrices of pistachio and almond mummies were reported in Part 1 of this series (Beck et al., 2014), and provided the identities of several potential semiochemicals involved in the chemical ecology of the navel orangeworm. The objectives of this investigation were: (1) to increase the water activity of each of the matrices and determine the corresponding volatile profiles over 1 week; and, (2) to compare the volatile profiles and identify compounds that were shared among the matrices, unique to the wet matrix, or increased in relative amounts as a result of the change in water activity. In addition to analysis of the static headspace, the dynamic headspace volatiles were collected over the course of several days, and the resultant volatile bouquets analyzed by electroantennography (EAG) using antennae from both male and female navel orangeworm.

2. Results and discussion

The overarching premise that an increase in water activity of the pistachio and almond mummies would increase the volatile emissions was successfully demonstrated in both matrices. The volatile profiles in each system increased in both composition and content as illustrated in Fig. 2, which shows the wet pistachio mummy volatile emissions increased from 64 total volatiles in the dry matrix to 86 total volatiles in the wet matrix – an increase of 134%. This change in profile resulted in 22 volatiles that were unique to the wet pistachio matrix. The almond mummy volatile emissions increased by 285%, from 20 volatiles in the dry matrix to 57 total volatiles in the wet matrix, and thus 37 volatiles that were unique to the wet matrix. Also, where there were seven volatiles shared between the dry matrices (Part 1, this issue), the wet matrices had 19 volatiles in common including the same seven volatiles detected in the dry matrices, making these seven volatiles

Fig. 1. A typical pistachio mummy (left) and almond mummy (right), both showing fungal growth and decay.

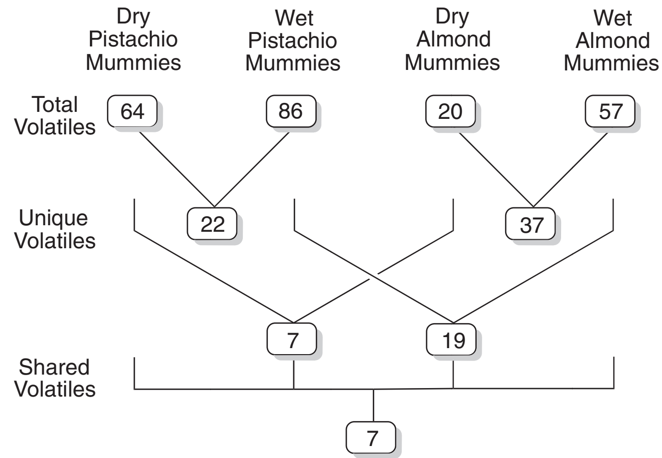

Fig. 2. Illustration depicting the total number of volatiles detected from the four matrices. The row labeled "unique volatiles" shows the number of volatiles detected that were unique to the almond and pistachio matrices when the water activity of each was increased and compared to the respective dry matrices. The row labeled "shared volatiles" shows the volatiles that were common for the dry matrices of almond and pistachio, and those common for the wet matrices of almond and pistachio. The final number at the bottom was the number of volatiles that were common across all four matrices.

(α-pinene, α-thujene, limonene, 2-pentylfuran, α-terpinolene, acetic acid, 5,6-dihydro-4-methyl-2*H*-pyran-2-one) common to all matrices – wet, dry, pistachio, almond (Fig. 2).

The water activity and water content values for the matrices were determined for comparison to the dry mummy matrices. The water activity of the wet pistachio mummies was 0.937 (±002 s.e.m.) and the water content 28.28 (±0.11 s.e.m.). The water activity of the wet almond mummies was 0.898 (±0.004 s.e.m.) and the water content 30.41 (±0.39 s.e.m.). The determined water activities of both the wet pistachio and wet almond mummy matrices were well above the needed value of 0.85 for most fungal growth (Hocking, 2001).

The volatiles unique to the wet pistachio mummies, as well as their detected relative abundances over the four sampling periods are provided in Table 1 (see Part 1 for the 64 volatiles from the dry matrix). There were several interesting features of the over-time volatile analyses for wet pistachio mummies. Firstly, volatiles consistently detected throughout all four sampling days (RI = 1090, 1209, 1252, 1387, 1452, 1908) were also in the wet almond matrix, albeit not emitted as consistently in the almond matrix. Secondly, the volatiles with an ethyl ester moiety (RI = 962, 1049, 1065, 1233, 1537, 1639) were all produced on day 3 or 7 of the analysis possibly implying fungal growth, time, or certain concentrations were required to oxidize the corresponding alcohols to acids necessary for esterification. Lastly, presence of methyl ketones and γ-nonalactone (RI = 1077, 1180, 1387, 2021) were similar to the volatiles emitted by almonds during the enzymatic or oxidative breakdown of fatty acids commonly found in both pistachio and almonds (Beck et al., 2011b). Principal component analysis (PCA) of the volatiles from the dry and wet pistachio mummies (Fig. 3) illustrated the differences in emissions over time between the two matrices where significant changes were observed in volatile emission due to the change in moisture. This large separation could be due in part to the volatile emissions from developing fungal spores in the wet matrix and not in the dry matrix (Table 1 and Supplemental Data).

While the number of unique volatiles from the wet almond matrix was greater than the number of unique volatiles from wet pistachio (37 vs. 22, respectively), the sum of all unique wet almond volatile relative abundance (1.15×10^8) was essentially equal to the sum of unique wet pistachio volatile relative abundance (1.25×10^8). This suggests that on average, the wet

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