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Differentiation of Volatile Profiles from Stockpiled Almonds at Varying Relative Humidity Levels Using Benchtop and Portable GC-MS

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Supporting Information

ABSTRACT: Contamination by aflatoxin, a toxic metabolite produced by *Aspergillus* fungi ubiquitous in California almond and pistachio orchards, results in millions of dollars of lost product annually. Current detection of aflatoxin relies on destructive, expensive, and time-intensive laboratory-based methods. To explore an alternative method for the detection of general fungal growth, volatile emission profiles of almonds at varying humidities were sampled using both static SPME and dynamic needle-trap SPE followed by benchtop and portable GC-MS analysis. Despite the portable SPE/GC-MS system detecting fewer volatiles than the benchtop system, both systems resolved humidity treatments and identified potential fungal biomarkers at extremely low water activity levels. This ability to resolve humidity levels suggests that volatile profiles from germinating fungal spores could be used to create an early warning, nondestructive, portable detection system of fungal growth.

KEYWORDS: *aflatoxin, fungal detection, signaling volatiles, spore, water activity*

INTRODUCTION

In the 2013/2014 crop year California almond orchards produced 2.11 billion pounds (957 million kg) of saleable product, of which 1.30 billion pounds (590 million kg) were exported.[1] Aflatoxins are hepatotoxic and carcinogenic metabolites produced by the fungi *Aspergillus flavus* and *A. parasiticus*, two microbes that are ubiquitous in California tree nut orchards.[2] Aflatoxin contamination of almonds and pistachios results in the loss of millions of dollars annually. Per the Voluntary Aflatoxin Sampling Plan (VASP) program,[3] the current method of detection and analysis involves removal and destruction of 20 kg of product, utilizes laboratory-based methods (e.g., HPLC), is expensive, and can take weeks for results. Because aflatoxin contamination can be limited to one or two highly contaminated nuts, current sampling methods may not guarantee detection of the infected nuts, thus posing serious health and safety concerns for the public. Despite an increase in self-regulation and aflatoxin testing protocols by the almond industry, the costly rejection of exported product continues. A reliable, early warning aspergilli detector has been voiced as a critical need of the industry, and work toward alternative detection methods has strong support of the almond industry, as well as state and federal agencies.

Volatiles could be used in such alternative detection methods and have been described as likely indicators of plant diseases.[4,5] In recent years there has been an increase in the use of portable mass spectroscopy (MS)-based technology for detection of damaged or diseased plant volatiles in both agricultural and field applications.[6−8] Additionally, our laboratory recently reported the use of a portable GC-MS system to distinguish between undamaged and damaged flower heads of yellow starthistle using specific plant-emitted volatile biomarkers.[8] These biomarkers can represent signaling odors for possible plant−insect or plant−plant communication.[9,10]

Extending these ideas to almonds, we sought to determine if the previously described portable GC-MS platform[8] could distinguish between whole almonds exposed to varying humidity levels under controlled laboratory experiments and from commercial almond stockpiles (Figure 1). Harvested almonds, with their hull and shells intact, are stored in these large stockpiles under tarps while awaiting processing, and thus present the possibility of increased humidity levels. With increased humidity, there is increased risk of general fungal growth as well as aflatoxin production from *Aspergillus* fungi.[11,12] Fungal spore growth is activated at certain water activity levels for various types of fungi.[13,14] This activation and transition from the resting to germinating phase is associated with release of specific volatiles.[15,16] In addition to volatile release following moisture activation of fungal growth, almond tissue enzymes may also influence release of certain volatiles at different humidities.[17,18]

Due to the risks associated with aflatoxin production by fungi in almonds at higher humidities, the Almond Board of California (ABC) has recommended that almonds not be stockpiled if hull or kernel moistures exceed 13% and 6%,

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Figure 1. Commercial almond stockpiles in the Central Valley of California prior to tarp coverage (top) and after tarps are in place (bottom). Pictures courtesy of B. Higbee.

respectively.[11,12] These values correspond to a relative humidity of 80% in ambient conditions.[11] Accordingly, the aims of this study were to collect and analyze the volatile profiles of almonds maintained at varying relative humidity levels using both benchtop and portable GC-MS platforms. Using these results, we sought to ascertain whether the portable GC-MS platform was sensitive enough to warrant further investigation as an in-field detection system for fungal biomarker volatiles.

■ MATERIALS AND METHODS

Chemicals. Compounds used as standards for peak authentication were purchased from commercial chemical companies or as noted in tables. Specific biomarker volatiles include 2-methylpropanol and nonanal (Alfa Aesar, Tewksbury, MA), pentanal, heptanal, and 5-methylfurfural (Sigma-Aldrich, St. Louis, MO), nonane (Poly Science, Niles, IL), and acetic acid (Fisher, Fair Lawn, NJ).

Almonds. Several bags of almonds, Nonpareil variety, were picked from a commercial stockpile (SE corner of R-382) located in Lost Hills, CA, during the month of August 2014 and shipped overnight in paper bags contained within cardboard boxes. Almonds were randomly chosen from near the edges of the stockpiles.

Water Activity and Percent Moisture Analyses. Intact and in-hull, stockpile almonds were placed into groups of seven weighing approximately 24 g and placed in 237 mL (8 ounce) Mason jars (Figure 2). A total of 39 groups of seven almonds were assembled, comprising triplicates for each treatment (63%, 75%, and 84%) at each time point (50 min and 4, 8, and 12 d) and one triplicate for the time point 0 min. Relative humidity environments were achieved using 17 mL vials placed within the Mason jars and each containing 6 mL of saturated salt solutions of 63% ($NaNO_2$), 75% (NaCl), and 84% (KCl) at 30 °C (Figure 2). For the time zero (d = 0) treatment, no salt solutions were added, almonds were removed from the bag and separated into hull, shell, and kernel, and moisture levels taken immediately. All humidity treatments for moisture analyses were analyzed at times identical to the corresponding headspace analyses. After the incubation period at each relative humidity, the hull, shell, and kernel tissues were again separated by hand and weighed. Each tissue was ground in a small blender cup and measured for water activity using an AquaLab 4TE and percent moisture using a Mettler HB43-S moisture balance.

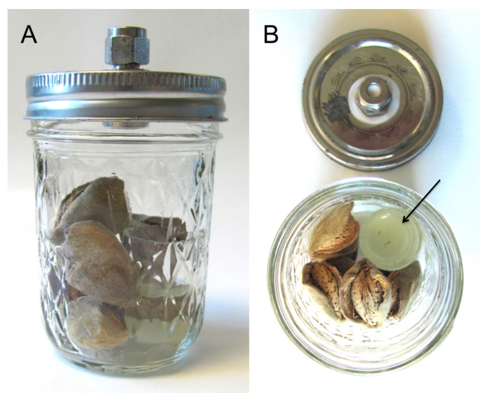

Figure 2. Containment and volatile collection devise used to provide varying relative humidity levels for intact almonds obtained from commercial stockpiles. Arrow denotes the vial filled with salt and water used for specific relative humidity levels.

Collection of Volatiles—Static Headspace for Benchtop GC-MS. In collection chambers identical to those used in the moisture analyses, seven intact and in-hull stockpile almonds weighing approximately 24 g were placed into 237 mL (8 ounce) Mason jars with a modified lid for insertion of SPME fibers (Figure 2). Each humidity level (ambient, 63%, 75%, and 84%) was performed in triplicate. Vials containing salt solutions for humidity levels, described above, were placed into the Mason jars next to the almonds. Empty vials were placed in the collection chambers for the no humidity added (ambient) treatments. For the day zero analyses the lids were sealed and volatiles allowed to permeate the headspace for 30 min, and then PDMS-DVB SPME fibers (Supelco, Bellefonte, PA) were inserted and exposed to the headspace volatiles for 20 min, thus providing the day zero, 50 min collection samples. The PDMS-DVB fibers were chosen after preliminary analyses of almond tissues at increased relative humidity and determining the best volatile profile adsorption among PDMS, PDMS/DVB, and PDMS/DVB/Carboxen. All volatile collections were performed at 30 °C. After volatile collections, the lids were removed, and the headspace was vented for 1 min and then resealed for continued permeation. Headspace volatiles were again adsorbed at exactly four-day (96 h) intervals (d = 4, 8, and 12), with venting performed for 1 min after each volatile collection.

Collection of Volatiles—Dynamic Needle Trap for Portable GC-MS. The collection conditions and protocols employed for dynamic headspace volatile collections were nearly identical to those used for the static collection with the exception of the following: two ports were used on the modified lid of the collection chamber—one for attaching the needle trap and pump, and one for the introduction of charcoal-purified air; the use of a needle trap and pump assembly connected to the collection jar via Teflon tubing inserted through a port on the modified lid; and, actual headspace volatile collection times. Each humidity level (ambient, 63%, and 75%) was performed in triplicate. Volatiles were collected using parameters identical to those previously published for this needle trap/pump assembly.[8] The needle trap interface (PerkinElmer, American Fork, UT) connected via

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Teflon tubing was inserted into the collection device. The needle trap contained the polymer Tenax TA (1 mg), Carboxen 1016 (1.6 mg), and Carboxen 1003 (1.5 mg) as the adsorbent (Supelco, Bellefonte, PA). Volatiles were drawn through the needle trap media via a 12 VDC eccentric diaphragm pump (Schwarzer Precision, Germany) set at a flow rate of 20 mL/min for 15 min. The total volume collected was per the manufacturer's recommendation and identical to previous studies.[8] Collections of volatiles from a blank system were performed to determine background and contaminant volatiles. The corresponding peaks of these volatiles were subtracted during the data analyses of adsorbed volatiles from the almond tissues.

Benchtop GC-MS Analysis of Volatiles. Volatiles adsorbed onto SPME fibers were thermally desorbed onto an Agilent 7890B GC coupled to a 5977A MSD (Palo Alto, CA) outfitted with an Agilent DB-1 column (60 m × 0.320 mm × 0.25 μm). Volatiles were analyzed via the following method parameters: injection and inlet temperatures 200 °C, splitless mode, constant flow of 2.0 mL/min, oven initial temperature 40 °C, hold time 0 min, ramp 4 °C/min, final temperature 190 °C, hold 0 min, and post run 270 °C for 3 min, for a total run time of 40.5 min. For further confirmation, identification, and peak resolution, additional injections were occasionally performed on an Agilent 7890B GC coupled to a 5977A MSD and outfitted with an Agilent DB-Wax column (60 m × 0.320 mm × 0.25 μm) and analyzed via the following method parameters: injection and inlet temperatures 200 °C, splitless mode, constant flow of 2.1 mL/min, oven initial temperature 40 °C, hold time 0 min, ramp 4 °C/min, final temperature 190 °C, hold 0 min, and post run 230 °C of 3 min, for an inject to chromatogram time of 40.5 min. Retention indices (RIs) were calculated using a homologous series of *n*-alkanes on both the DB-1 and DB-Wax columns. RI values from both columns were used to assist with initial identification, and identities were further confirmed by comparison to retention times and fragmentation patterns of standards. Compound identities not verified on both instruments with a commercial or other available standard were marked as tentatively identified. Total time per sample, from headspace collection to visualization of the chromatogram for the benchtop system, was approximately 91 min.

Portable GC-MS Analysis of Volatiles. Adsorbed volatiles on the needle trap were thermally desorbed directly onto the injection port of the portable GC-MS, set at 270 °C. The injector assembly was modified to accommodate the needle trap and allow for proper flow of helium through the needle trap during desorption. Other instrument parameters were identical to previously published experimental conditions.[8] Briefly, injector and transfer line temperatures 270 °C, split off mode, initial temperature 40 °C, hold time 10 s, ramp 2 °C/s, final temperature 270 °C, hold 10 s, and desorption time 20 s, for an inject to chromatogram time of 155 s. Fragmentation patterns from the toroidal mass spectrometer (TMS) were compared with those of the benchtop GC-MS, and available standards were used to confirm retention times and TMS fragmentation patterns. Compound identities not verified by a commercial or available standard were marked as tentatively identified. Total time per sample, from headspace collection to visualization of the chromatogram for the portable system, was approximately 18 min.

Statistical Analysis. Peak areas for volatiles (n = 48 benchtop, n = 36 portable) were used for all statistical comparisons. For benchtop GC-MS work, linear discriminant analysis was used to evaluate differences in volatile profiles among humidity treatments. From those linear discriminants, multivariate analysis of variance (MANOVA) was performed after assuring adherence to assumptions of normality and homoscedasticity through visual inspection of residual diagnostic plots (including quantile-quantile) plots and interrogation with Shapiro-Wilk's test and Levene's test. Posthoc comparisons were accomplished with Tukey's HSD test. Potential biomarkers were identified using variable importance measures from random forests models fit with 5 repeated 10-fold cross validation. For portable GC-MS analysis, random forests models fit as above were used to determine potential biomarkers and the suitability for prediction and were able to handle the more nonlinear, non-normal data. To estimate overall out-of-sample accuracy while accommodating the limited data, training and testing of the model was done using a 75:25 training:testing split. MANOVA analysis and canonical discriminant analysis was then applied to the top three volatiles identified from variable importance measures from the random forests models after verifying adherence to assumptions as described above.

For these analyses, data were collated in Microsoft Excel then analyzed using R version 3.3.1 using the RStudio version 0.99.902 development environment. The following packages facilitated analysis: *dplyr* and *tidyr* for data formatting,[19,20] *ggplot2* and *ellipse* for graphics,[21,22] *MASS* and *candisc* for discriminant analysis,[23,24] *readxl* for the R–Excel interface,[25] *car* and *lsmeans* for MANOVA and posthoc analysis,[26] and *randomForest* and *caret* for machine learning algorithms.[27,28]

■ RESULTS AND DISCUSSION

As anticipated and previously noted, increases in relative humidity led to increased water activity and moisture levels in almond hulls, kernels, and shells. Hull moisture data (Figure 3) were particularly relevant given earlier findings that hulls provide the vast majority of volatile emissions.[18] Additionally, the almond hulls consistently started at and maintained a higher water activity value and responded the quickest to the relative humidity environments. The hulls did not reach the 13% moisture level after 12 days at 63% relative humidity (Figure 3), corroborating research by others.[11] Conversely, the hull percent moisture values were greater than 13% after 8 days and 4 days for the 75% and 84% relative humidity levels, respectively (Figure 3).

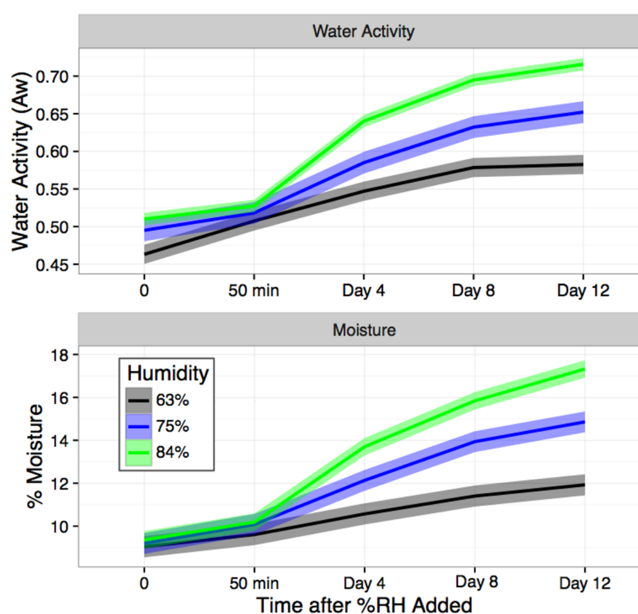

Figure 3. Water activity and percent moisture levels from almond hulls over time at 63%, 75%, and 84% relative humidity. Lines and shaded areas denote mean (n = 3) and bootstrapped 95% confidence intervals, respectively.

Particularly relevant for investigations of fungal growth, none of the almond tissues achieved a water activity level of greater than 0.73 (Figure 3). Our earlier work with almond tissues, water activities, and volatiles emitted focused on the role of spores as they transitioned from resting to germinating.[15−18] The results obtained from these studies were based on the premise that spore development requires relatively high water activity levels of their host, 0.85 or greater for most fungi,[14] and

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as low as 0.77 for some xerophilic fungi such as *Aspergillus niger*.[28]

Despite these low water activity levels, volatile profiles from analysis on benchtop GC-MS allowed separation across both time and humidity levels. Linear discriminant analysis separated volatile profiles at time zero and those profiles at later time points with different levels of humidity (Figure 4). MANOVA

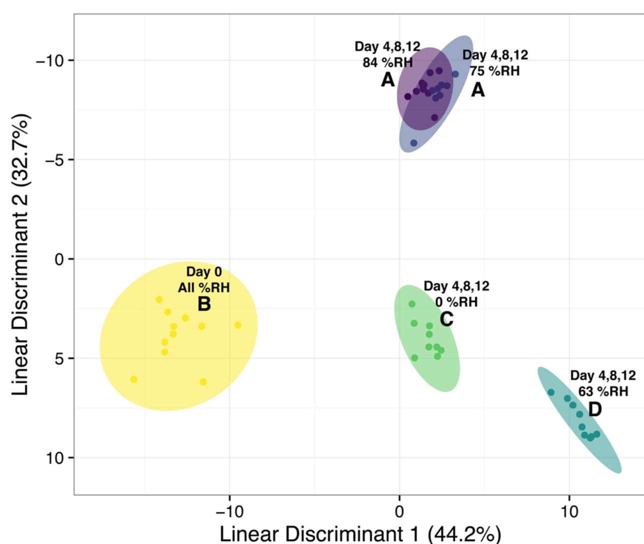

Figure 4. Linear discriminant analysis of volatile profiles from static headspace SPME collection of stockpiled almonds at ambient (denoted 0%, no moisture added), 63%, 75%, and 84% relative humidity levels and analyzed on a benchtop GC-MS. Shaded ellipses denote 95% confidence intervals around means of groups. Individual letters not shared by the groups indicate significant differences ($P < 0.05$) by Tukey's HSD test.

analysis using the first two linear discriminants showed a significant effect of humidity class ($F = 628.06$; $df = 8{,}84$; $P < 0.0001$) and significantly ($P < 0.05$) resolved humidity classes (with the exception of 75% and 84% humidity) by Tukey's HSD test (Figure 4). The overlap seen in the 75% and 84% humidity treatments on the benchtop GC-MS (Figure 4) suggests that higher humidity levels may be functionally similar in terms of volatile emissions, and thus the 84% humidity treatment was not performed for the portable GC-MS instrument experiments.

Interestingly, such analysis of volatile profiles clearly resolved day = 0 for all relative humidities and days = 4, 8, and 12 for the ambient relative humidity treatment (Figure 4). This suggests an activation of the almond fungi and/or tissue to the resident moisture (water activity average 0.4894 ± 0.0070; percent moisture average 9.1978 ± 0.0694) of the dry almond hulls over time and while stored in a closed system. This topic warrants in-depth investigation regarding possible spore activation at water activity levels clearly below that typically considered for xerophilic fungi[29] but is beyond the scope of the current investigation and report.

This degree of treatment separation from benchtop GC-MS analysis suggests that volatile profiles could potentially be used to discriminate humidity levels and identify fungal growth in the field. Indeed, variable importance measures from random forests machine learning algorithms identified three potential biomarkers for monitoring ubiquitous fungal growth. The compounds isobutanol, **1**, pentanal, **2**, and 5-methyl-3-isoxazolamine, **3**, as a tentatively assigned molecule (Table 1 and Figure 5), were found to be the highest ranking for providing

Table 1. Benchtop Biomarker Volatiles with Highest Importance Ranking by Random Forest Testing,[a] out of 156 Detected Peaks by Benchtop GC-MS

compound identity	RT DB-1	RT DB-Wax	calculated RI[b] DB-1	calculated RI[b] DB-Wax
isobutanol[c]	3.94	7.27	612	1099
pentanal[d]	4.63	5.02	671	980
5-methyl-3-isoxazolamine[e]	7.01	16.45	797	1413
heptanal[d]	9.36	9.59	879	1187
nonane[f]	10.08	3.97	900	900
5-methylfurfural[d]	11.02	21.40	930	1577

[a]Delineates which compounds are responsible for the difference between treatments. [b]Retention indices relative to *n*-alkanes on DB-1 and DB-Wax columns for compound identification including comparison to retention times and fragmentation to authentic samples. [c]Sources were Alfa Aesar. [d]Sources were Sigma-Aldrich. [e]Sources were tentatively identified due to no standard available. [f]Sources were Poly Science.

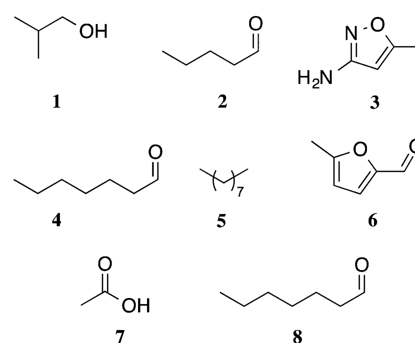

Figure 5. Biomarker volatiles identified by benchtop GC-MS analysis: isobutanol, **1**; pentanal, **2**; 5-methyl-3-isoxazolamine, **3** (tentatively identified); heptanal, **4**; nonane, **5**, and 5-methylfurfural, **6**. Biomarker volatiles identified by the portable GC-MS system: acetic acid, **7**, and nonanal, **8**.

differences between treatments. Three other compounds were identified as biomarkers, but with a lower ranking for distinguishing between treatments: heptanal, **4**, nonane, **5**, and 5-methylfurfural, **6** (Table 1 and Figure 5). Interestingly, none of these biomarker were detected when ubiquitous almond orchard fungal spores were placed on fatty acids,[15] yet the biomarker isobutanol, **1**, was consistently detected when moisture was present on almond tissues.[17,18] In general, many of the other compounds detected on wet almond hulls[17,18] and fungal spores on fatty acids[15] were also detected in the present study, but not as biomarkers. These include the fairly common fungal volatiles alkanals, 2-alkanones, alkyl lactones, spiroketals, and furan analogues. To further investigate the ability of volatile profiles to predict moisture levels in the field, we compared the results from benchtop GC-MS analysis with results from the portable GC-MS.

Direct comparison of the linear discriminant analyses of the benchtop volatile data (Figure 4) to that of the portable volatile data (Figure 6) illustrates the similarities of both systems and also significantly ($P < 0.05$) resolves humidity treatment. Using volatile profiles from the portable GC-MS, cross validated

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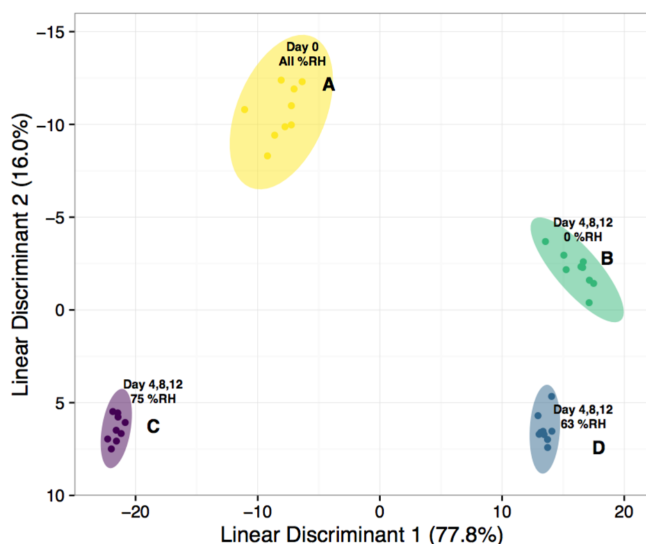

Figure 6. Linear discriminant analysis of volatile profiles from dynamic headspace needle trap collection of stockpiled almonds at ambient (denoted 0%, no moisture added), 63%, and 75% relative humidity levels and analyzed on the portable GC-MS. Shaded ellipses denote 95% confidence intervals around the means of groups. Individual letters not shared by the groups indicate significant differences ($P < 0.05$) by Tukey's HSD test.

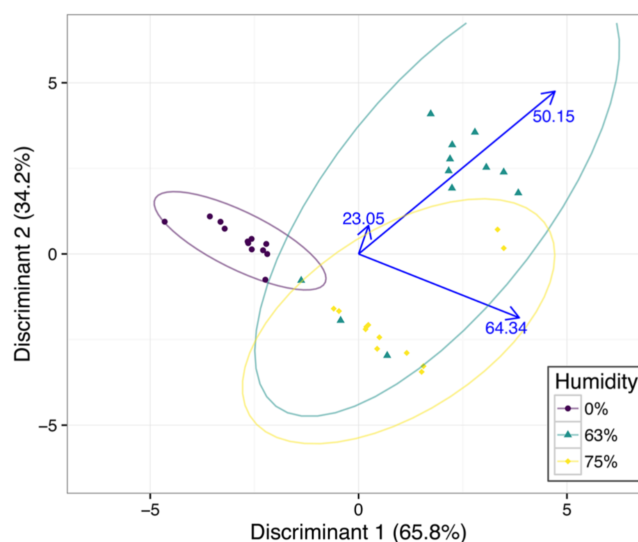

Figure 7. Canonical discriminant analysis of three volatiles obtained from portable GC-MS analysis of dynamic headspace needle trap collection of stockpiled almonds at ambient (denoted 0%, no moisture added), 63%, and 75% relative humidity levels. Magnitude and direction of arrows denote relative contribution and axis of discrimination for each volatile, respectively. Ellipses denote 95% confidence intervals around mean of groups.

random forests models were able to predict humidity levels with 94.4% [95% CI: 81.3%, 99.3%] out of sample overall accuracy (Table 2). This high level of overall accuracy suggests

Table 2. Confusion Matrix from Random Forest Model Fit to Portable GC-MS Volatile Profiles

	reference[a]		
prediction	0%	63%	75%
0%	12	0	0
63%	1	10	1
75%	0	0	12

[a]Accuracy, 0.9444; 95% confidence interval, 0.8134, 0.9932.

that this type of analysis could potentially be applied in the field to provide a reasonably accurate means of detection for growers. From the portable GC-MS volatile profiles, variable importance measures identified three primary volatile biomarkers, in order of importance (Table 3 and Figure 7), responsible for discrimination between treatments: unknown compound at 50.15 s, nonanal, **8**, at 64.34 s, and acetic acid, **7**, at 23.05 s. Applying a MANOVA analysis with these three volatiles showed significant effects of humidity ($F = 3.29$; $df = 6{,}46$; $P = 0.008$), day ($F = 2.15$; $df = 9{,}72$; $P = 0.04$), and their interaction ($F = 1.94$; $df = 18{,}72$; $P = 0.03$). While posthoc analysis with Tukey's HSD was able to significantly ($P < 0.05$) resolve differences between humidities, canonical discriminant analysis showed separations that were not nearly as clean as those obtained from the benchtop GC-MS. Specifically, while the three biomarker volatiles from the portable GC-MS do show resolution between humidities, the overlap of confidence intervals among the mean of the treatments in Figure 7 were due to the outliers from day = 0 in the 63% treatment (green triangles in lower portion of 63% ellipse) and from day = 12 in the 75% (yellow diamonds in upper right of 75% ellipse). Thus, Figure 7 shows the inability of the portable instrument to distinguish between the day groupings. The intent of the report was to demonstrate the ability of the two GC-MS platforms to distinguish among the relative humidity treatments using the overall profiles and distinct biomarkers but not necessarily the identity of the biomarkers. Biomarkers for both instruments included unknown or tentatively identified compounds. Yet, posthoc analyses utilized these unidentified detected signals to achieve distinction among the treatments.

Table 3. Identified Biomarker Volatiles[a] with Highest Contribution to Delineating Treatments, out of 42 Detected Peaks by the Portable GC-MS System

compound identity	RT (s)
acetic acid[b]	23.05
unknown	50.15
nonanal[c]	64.34

[a]Identified using random forest, which delineated compounds responsible for differences between treatments. [b]Compounds verified using commercial standards from Fisher. [c]Compounds verified using commercial standards from Alfa Aesar.

Despite the less resolved results from the portable GC-MS, the primary objective of monitoring and delineating the headspace volatiles from stockpiled almonds with a typical benchtop GC-MS and a portable GC-MS system was successfully achieved. Overall, the results from both GC-MS systems demonstrated similarities in terms of their ability to distinguish the relative humidity levels, with the benchtop system providing greater resolution and detail of specific biomarkers. In addition to the ability to distinguish humidities in the lab, and potentially the field, other characteristics from volatile profiles shed light on the role of fungal growth at different humidities. In the discriminant analyses, the overlap of the 75% and 84% relative humidity levels suggests that the volatile profiles of almonds at these humidities are very similar. This result corroborates the observation by other researchers

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that mold growth occurs more quickly at higher relative humidity and water activity levels.[11,29] In this instance, the change in volatile profiles from the intact almond tissue in the environment at less than 70% to the profiles greater than 70% was significantly different (t = 36.5, df = 42, $P < 0.0001$). This result was likely due to a change in fungal status, dormant or resting to germination or growth, as a function of water activity of the corresponding host tissue.[13,18] In their 2009 report, Lampinen and co-workers[11] showed an increase in aflatoxin detected in stockpiled almonds when the relative humidity levels were greater than 70%. Our findings suggest that there may be a distinct point that certain volatiles were emitted when there was a change in fungal activity as a result of available moisture and thus a possible point of detection using either benchtop or portable GC-MS technology.

When compared to the benchtop system, some limitations of the portable GC-MS platform were revealed. These include decreased sensitivity and instrument variability (as seen in the overlap in Figure 7). More important was the finding that the portable system did not clearly demonstrate an ability to reliably discriminate among the treatments. This was evident by the lower number of biomarkers relative to the benchtop, and more importantly the identities of the biomarkers, which were questionable as actual unique fungal volatiles since acetic acid, **7**, and nonanal, **8**, have both been detected on dry ground almond mummy hulls.[30] However, the portable GC-MS system did provide a means of detection of fungal volatiles at varying relative humidity levels for this particular plant−microbe system. The disadvantages may be acceptable for some systems, given sufficient analyte amounts. Additionally, the advantages of portability and rapid run time (18 min vs 91 min) may be an attractive feature for other applications.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.6b04220.

> Benchtop peaks detected with retentions times (min) from DB-Wax and DB-1 columns, corresponding RI values, and authentication sources (Table S1); portable GC-MS peaks detected, retention times in seconds, fragmentation patterns, and authentication sources (Table S2); water activity and moisture levels from separated almond tissues (hulls, kernels, shell) over time at 63%, 75%, and 84% ambient relative humidity (Figure S1); and moisture data for all days, at all humidity levels, and for individual almond tissues (Figure S2) (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) ABC, Almond Board of California. Almond Almanac. http://www.almonds.com/sites/default/files/content/attachments/2014_almanac_final.pdf (accessed Sep. 20, 2016).

(2) Molyneux, R. J.; Mahoney, N.; Kim, J. H.; Campbell, B. C. Mycotoxins in edible tree nuts. *Int. J. Food Microbiol.* **2007**, *119*, 72−78.

(3) ABC, Almond Board of California, Voluntary Aflatoxin Sampling Plan (VASP) Program. http://www.almonds.com/sites/default/files/content/attachments/vasp-manual.pdf (accessed Nov. 3, 2016).

(4) Sankaran, S.; Mishra, A.; Ehsani, R.; Davis, C. A review of advanced techniques for detecting plant diseases. *Comp. Electron. Agric.* **2010**, *72*, 1−13.

(5) Jansen, R. M. C.; Wildt, J.; Kappers, I. F.; Bouwmeester, H. J.; Hofstee, J. W.; van Henten, E. J. Detection of diseased plants by analysis of volatile organic compound emission. *Annu. Rev. Phytopathol.* **2011**, *49*, 157−174.

(6) Schott, M.; Wehrenfennig, C.; Gasch, T.; Düring, R. A.; Vilcinskas, A. A portable gas chromatograph with simultaneous detection by mass spectrometry and electroantennography for the highly sensitive in situ measurement of volatiles. *Anal. Bioanal. Chem.* **2013**, *405*, 7457−7467.

(7) Aksenov, A. A.; Pasamontes, A.; Peirano, D. J.; Zhao, W.; Dandekar, A. M.; Fiehn, O.; Ehsani, R.; Davis, C. E. Detection of Huanglongbing disease using differential mobility spectrometry. *Anal. Chem.* **2014**, *86*, 2481−2488.

(8) Beck, J. J.; Porter, N.; Cook, D.; Gee, W. S.; Griffith, C. M.; Rands, A. D.; Truong, T. C.; Smith, L.; San Román, I. In-field volatile analysis employing a hand-held portable GC-MS: emission profiles differentiate damaged and undamaged yellow starthistle flower heads. *Phytochem. Anal.* **2015**, *26*, 395−403.

(9) Unsicker, S. B.; Kunert, G.; Gershenzon, J. Protective perfumes: the role of vegetative volatiles in plant defences against herbivores. *Curr. Opin. Plant Biol.* **2009**, *12*, 479−485.

(10) Heil, M.; Karban, R. Explaining evolution of plant communication by airborne signals. *Trends Ecol. Evol.* **2010**, *25*, 137−144.

(11) Lampinen, B.; Michalaides, T. *Almond stockpile monitoring for aflatoxin potential*; Final research report 08-AFLA2-Lampinen; Almond Board of California: Modesto, CA, U.S.A., 2008−2009.

(12) ABC, Almond Board of California. Field quality: stockpile management + concealed damage. http://www.almonds.com/sites/default/files/content/attachments/12_field_quality_stockpile_management_and_concealed_damage_2013.pdf (accessed Sep. 20, 2016).

(13) Ayerst, G. The effects of moisture and temperature on growth and spore germination in some fungi. *J. Stored Prod. Res.* **1969**, *5*, 127−141.

(14) Hocking, A. D. *Fungal xerophiles (osmophiles). eLS.*; John Wiley & Sons Ltd.: Chichester, U.K., 2001.

(15) Beck, J. J.; Mahoney, N. W.; Cook, D.; Gee, W. S. Generation of the volatile spiroketals conophthorin and chalcogran by fungal spores on polyunsaturated fatty acids common to almonds and pistachios. *J. Agric. Food Chem.* **2012**, *60*, 11869−11876.

(16) Beck, J. J.; Baig, N.; Cook, D.; Mahoney, N. E.; Marsico, T. D. Semiochemicals from ex situ abiotically stressed cactus tissue: a contributing role of fungal spores? *J. Agric. Food Chem.* **2014**, *62*, 12273−12276.

(17) Beck, J. J.; Mahoney, N. E.; Cook, D.; Higbee, B. S.; Light, D. M.; Gee, W. S.; Baig, N. 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. *Phytochem. Lett.* **2014**, *8*, 220−225.

(18) Mahoney, N. E.; Gee, W. S.; Higbee, B. S.; Beck, J. J. Ex situ volatile survey of ground almond and pistachio hulls for emission of spiroketals: analysis of hull fatty acid composition, water content, and water activity. *Phytochem. Lett.* **2014**, *7*, 225−230.

(19) Wickham, H.; Francois, R. dplyr: A grammar of data manipulation. R package version 0.5.0. https://CRAN.R-project.org/package=dplyr (accessed Sep. 20, 2016).

(20) Wickham, H. tidyr: Easily tidy data with 'spread()' and 'gather()' functions. R package version 0.6.0. https://CRAN.R-project.org/package=tidyr (accessed Sep. 20, 2016).

(21) Wickham, H. *ggplot2: Elegant graphics for data analysis*; Springer-Verlag: New York, NY, 2009.

(22) Murdoch, D.; Chow, E. D. ellipse: Functions for drawing ellipses and ellipse-like confidence regions. R package version 0.3-8. https://CRAN.R-project.org/package=ellipse (accessed Sep. 20, 2016).

(23) Venables, W. N.; Ripley, B. D. *Modern applied statistics with S*, 4 ed.; Springer: New York, 2002.

(24) Friendly, M.; Fox, J. Candisc: visualizing generalized canonical discriminant and canonical correlation analysis. R package version 0.7−0. https://CRAN.R-project.org/package=candisc (accessed Sep. 19, 2016).

(25) Wickham, H. readxl: Read Excel riles. R package version 0.1.1. https://CRAN.R-project.org/package=readxl (accessed Sep. 20, 2016).

(26) Fox, J.; Weisberg, S. *An {R} companion to applied regression*, 2nd ed.; Sage: Thousand Oaks, CA, 2010.

(27) Liaw, A.; Wiener, M. Classification and regression by randomForest. *R News* **2002**, *2*, 18−22.

(28) Kuhn, M. Caret: classification and regression training. R package version 6.0-71. https://CRAN.R-project.org/package=caret (accessed Sep. 20, 2016).

(29) Bayman, P.; Baker, J. L.; Mahoney, N. E. *Aspergillus* on tree nuts: incidence and associations. *Mycopathologia* **2002**, *155*, 161−169.

(30) Beck, J. J.; Mahoney, N. E.; Cook, D.; Gee, W. S.; Baig, N.; Higbee, B. S. 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. *Phytochem. Lett.* **2014**, *9*, 102−106.

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