## Comparison of volatile emissions from undamaged and mechanically damaged almonds<sup>†</sup>



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#### Abstract

BACKGROUND: The navel orangeworm (NOW) *Amyelois transitella* (Walker) is a major insect pest of almonds causing considerable monetary setbacks for both growers and processors, and thus control of NOW is one of the top priorities for the almond industry. Field observations purport that NOW is attracted to previously injured almonds. Accordingly, in this study the volatile output of damaged almonds was investigated in an effort to identify potential attractants for further studies into the control and/or monitoring of NOW. Mature almonds from the Monterey variety were evaluated for their volatile composition after mechanical damage and compared with the volatile composition of undamaged almonds.

**RESULTS:** Volatile organic compounds (VOCs) were collected on Tenax, desorbed and identified via gas chromatography/mass spectrometry analysis. VOCs unique to the damaged tree nuts included trace amounts of 3-pentanol and isomers of the spiroketal chalcogran. VOCs that increased in relative amounts after damage include the spiroketal conophthorin and numerous four-carbon ester and ketone as well as alcohol derivatives, in addition to two eight-carbon chain compounds.

CONCLUSION: Several VOCs, both unique and in increased amounts, were identified from damaged almonds. Their presence in damaged almonds warrants further investigation into their role in NOW response to damaged almonds, which may lead to insights into the control and/or monitoring of NOW. Published in 2008 by John Wiley & Sons, Ltd.

Keywords: almond; damaged; spiroketal; Tenax; volatile

#### INTRODUCTION

The navel orangeworm (NOW) Amyelois transitella (Walker) is a major insect pest of almonds grown in California and causes considerable monetary setbacks for both growers and processors. Control of NOW has been stated as one of the top priorities for the almond industry, with another priority being the development of new pest management tools.<sup>1</sup> There is twofold interest in controlling NOW, namely its direct damage to tree nuts and the associated contamination of toxin-producing fungi (mycotoxins) resulting from NOW feeding damage, which provides avenues for infection by mycotoxigenic fungi. The point of damage into the tree nut from the pest insect exposes the protective layers (hull, shell, seed coat) surrounding the kernel. This point of entry allows for ambient spores of aspergilli to enter and thus contaminate the nut.<sup>2</sup> Contamination of tree nuts by mycotoxins is a chief concern for both human food and animal feed safety, with both areas experiencing major export issues as a result of the contamination.<sup>3</sup> The aflatoxin-producing (aflatoxigenic) fungi most relevant to agriculture include Aspergillus flavus and Aspergillus parasiticus. Aflatoxin is presently a significant food safety problem owing to its carcinogenic and teratogenic attributes. The current total aflatoxin action threshold for international export of tree nuts is set at 4 ppb compared with the domestic level of 20 ppb set by the Food and Drug Administration (FDA).<sup>2,3</sup> California is the top producer of almonds, supplying 75% of the world's needs.<sup>1</sup> Approximately 5% of California's cropland is dedicated to almond production.<sup>4</sup> The

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California almond industry generates approximately \$2 billion annually, with the total California tree nut industry reporting over \$3.5 billion. About 50–70% of California tree nuts are exported overseas annually, with 80% of almond production alone being exported.<sup>1</sup> The strict export action levels for aflatoxin have resulted in mycotoxin management issues for producers as well as state and federal governments. Actual costs of crop loss due to aflatoxin contamination in California were estimated to have been \$23–47 million over the period 1995–2001.<sup>3</sup> Moreover, the economic and health impacts of mycotoxins have been stated to be severe for developing nations.<sup>5</sup>

In a recent investigation, researchers reported the observation that female NOW moths were attracted to injured almonds.<sup>6</sup> Current attractants used in the field and/or lab for NOW include the female sex pheromone of NOW, (Z,Z)-11,13-hexadecadienal,<sup>7</sup> a pheromone blend of (Z,Z,Z,Z,Z)-3,6,9,12,15-tricosapentaene, (*Z*,*Z*,*Z*,*Z*,*Z*)-3,6,9,12,15-pentacosapentaene, ethvl palmitate and ethyl-(Z,Z)-11,13-hexadecadien-1-yl acetate<sup>8</sup> and the almond oil fatty acids myristic, palmitic, stearic, oleic and linoleic.9 Investigations on VOCs from almonds report the detection of 2hexyl-3-methylmaleic anhydride<sup>10</sup> and various alkane, alkene, alkanol, aromatic and furan VOCs.11 However, a search of the literature does not provide examples of VOC emission as a result of injury to the almond. As part of our ongoing efforts to address the concerns regarding NOW, our labs investigated the VOC output of mechanically damaged (DMG) almonds from the Monterey variety and compared the VOC fingerprint with that of undamaged (CTRL) almonds to ascertain what VOCs, if any, were unique to DMG almonds. The major VOCs from the CTRL and DMG experiments were compared and contrasted.

## MATERIALS AND METHODS Plant material

Fruits of Prunis dulcis (P. Mill.) D.A. Webb, variety Monterey, common name sweet almond, were collected in two batches during mid to late June 2006 from the groves of Paramount Farming Company, Bakersfield, CA, USA. Each batch was replicated in triplicate over different days. Batch 1 consisted of almonds that had been injured while intact on the tree, allowed to remain on the tree for approximately 14 days, then removed and placed in glass jars with a Teflon paper seal between the cap and jar. The injury/damage consisted of hull penetration with an 8 penny nail (3 mm diameter). Batch 2 consisted of control almonds that were not injured, removed from the tree and placed in glass jars with a Teflon paper seal between the cap and jar. Batches 1 and 2 were collected during concurrent time frames. Batches were sent via overnight delivery to the USDA-ARS facility in Albany, CA, USA for volatile evaluation.

## Collection of VOCs<sup>6</sup>

Almonds (*ca* 500 per experiment) were transferred to a 12 L round-bottomed flask fitted with an inlet for purified airflow at 1 L min<sup>-1</sup> and a Tenax (25 g) collection system. VOCs were collected for 18 h and desorbed with freshly distilled diethyl ether (100 mL), then the ether was concentrated to a volume of *ca* 1 mL with a warm water bath and a Vigreux distillation column.

# Gas chromatography/mass spectrometry (GC/MS) analysis

Separation of the collected VOC mixture was achieved with a DB-Wax column ( $60 \text{ m} \times 0.32 \text{ mm i.d.} \times$ 0.25 µm; J&W Scientific, Folsom, CA, USA) installed on an HP 6890 gas chromatograph (GC) coupled to an HP 5973 mass selective detector (MSD) (Hewlett Packard, Palo Alto, CA, USA). Extracts were analysed with the following method:  $1 \mu L$  injections; injector temperature, 150°C; splitless mode; inlet temperature, 150 °C; inlet pressure, 7.7 psi; total flow, 11.9 mL min<sup>-1</sup>; He carrier gas at 7.7 psi; flow, 1.5 mL min<sup>-1</sup>; velocity, 31 cm min<sup>-1</sup>; constant flow; oven settings: initial temperature, 30°C; hold time, 4 min; ramp, 2 °C min<sup>-1</sup>; final temperature, 200 °C; hold time, 30 min. The MSD parameters were as follows: source temperature, 230 °C; MS quadrupole temperature, 150 °C; electron impact (EI) mode, 70 eV; solvent delay, 1 min; scan group 1, 40-300 amu; scan group 2 at 20 min, 40-450 amu. National Institute of Standards and Technology (NIST), Wiley and internally generated databases were used for fragmentation pattern identification. Retention indices (RIs) were calculated using a homologous series of *n*alkanes on a DB-Wax column. Compounds that did not match the RIs of known VOCs from our database and/or did not provide sufficient mass fragmentation pattern matches were assigned as unknown in Table 1.

## Statistical analysis

GC/MS analysis was performed on each of the three separate samples for both the CTRL and DMG batches of almonds. The relative areas for each of the compounds from the GC/MS runs were normalised to the internal standard cyclodecanone  $(15 \,\mu g)$  and the means, standard deviations and confidence limits (95%) in Table 1 and Fig. 3 were calculated with Microsoft Excel software (Redmond, WA, USA).

## **RESULTS AND DISCUSSION**

Analysis of the major VOCs emitted by both the CTRL and DMG almonds provides a wide range of compounds, which corroborated other reports and added to the volatile fingerprint of almonds in the literature. Table 1 provides a list of the major VOCs detected from both experiments. Examination of Table 1 showed a number of monoterpenes common to citrus and other plants,<sup>12</sup> namely  $\alpha$ -pinene, camphene,  $\beta$ -pinene,  $\beta$ -myrcene, limonene



Figure 1. Total ion chromatogram (relative abundance *versus* time) illustrating a typical elution pattern of DMG almond VOCs. Unique, increased and/or notable compounds are labelled with numbers corresponding to compounds listed in Table 1.

and cymene. The compounds  $\alpha$ -pinene and camphene were noted to be in relatively large amounts in the CTRL almonds and underwent a small decrease in volatile output for the DMG almonds (Fig. 1 illustrates a typical GC elution pattern for DMG VOCs). Camphene and  $\alpha$ -pinene are both common, non-specific plant VOCs that have a wide range of semiochemical activity,<sup>13–15</sup> but neither has been reported for activity against NOW. The remaining monoterpenes are ubiquitous as plant VOCs, and several have been noted as semiochemicals.<sup>14</sup>

The spiroketal conophthorin (7-methyl-1,6-dioxaspiro[4.5]decane), in unknown configuration, was also observed to undergo a small increase in relative amounts in several of the DMG almond volatile analyses. Conophthorin is present in several insects and plants and in varying concentrations of isomers (Fig. 2).<sup>16</sup>

The sesquiterpenes bourbonene (as a mixture with benzaldehyde),  $\beta$ -copaene and aromadendrene also increased in relative amounts in the DMG almonds. These particular sesquiterpenes have been noted to occur together in potato leaf VOCs.<sup>17</sup> Bourbonene and  $\beta$ -copaene are pheromones for the European birch aphid,<sup>18</sup> and aromadendrene has been reported to be an attractant for the Brazilian eucalyptus brown looper.<sup>15</sup> However, none of the noted sesquiterpenes has been implicated as possessing activity against NOW.

The only compounds to demonstrate corroboration of previous reports of almond VOCs were 2pentylfuran, nonanal, 1-octen-3-ol, benzaldehyde



Figure 2. Stereoisomers of conophthorin (7-methyl-1,6-dioxaspiro [4.5]decane).

*J Sci Food Agric* **88**:1363–1368 (2008) DOI: 10.1002/jsfa and 2-phenylethyl alcohol.<sup>11,19</sup> Notable differences between the work performed by Buttery et al.,<sup>11</sup> which evaluated VOCs from almond hulls, and the VOCs collected in the present study were the detection here of numerous four-carbon ester and ketone as well as alcohol derivatives. Specific examples were the compounds that also showed a general increase in amounts between the CTRL and DMG almond VOCs, namely 2-butanol, ethyl 2methylbutyrate, ethyl isovalerate, ethyl 2-butenoate, ethyl 3-methylbut-2-enoate, ethyl tiglate and 3hydroxy-2-butanone. Several of these VOCs have been attributed to fruity, wine aroma and smoky odours<sup>20,21</sup> and are known semiochemicals,<sup>22-24</sup> yet are not associated with NOW semiochemicals. The compounds that demonstrated statistically valid increases were ethyl 2-methylbutyrate, 2-methyland 3-methyl-1-butanol, ethyl tiglate and  $\beta$ -copaene (Fig. 3), in addition to one unknown compound.

Several compounds in Table 1 were noted to be indicative of fungal growth. Of particular interest were 2-methyl- and 3-methyl-1-butanol and 2-pentyfuran owing to their relatively large amounts. The butanol



Figure 3. VOCs showing a statistically significant increase (95% confidence limit) in DMG almonds.

#### $\label{eq:table_to_constraint} \textbf{Table 1.} \ \text{Major volatile components of Monterey (MO) damaged (DMG) and control (CTRL) almonds^a$

No.         Compound         Rl <sup>6</sup> Rl (PMR) <sup>d</sup> Rl (it.)         MO CTRL         MO C           1 <i>α</i> -Pinene         1016         1020         1014         2.21 (1.42)         1.78 (0)           2         2-Butanol         1032         1027         1019, 1025         0.24 (0.12)         0.35 (0.37)         0.59 (0.37)           3         Ethyl butyrate         1053         1046         0.32 (0.09)         0.62 (0.37)         0.69 (0.37)           4         Unknown         1044         ND         0.16 (0.37)         0.69 (0.37)         0.63 (0.32 (0.09)         0.62 (0.9)         0.62 (0.9)         0.62 (0.9)         0.62 (0.9)         0.62 (0.9)         0.62 (0.9)         0.62 (0.9)         0.62 (0.9)         0.62 (0.9)         0.62 (0.9)         0.62 (0.9)         0.62 (0.14)         0.33 (0.14)         0.33 (0.14)         0.33 (0.14)         0.33 (0.14)         0.33 (0.14)         0.33 (0.14)         0.33 (0.14)         0.32 (0.9)         0.56 (0.14)         0.36 (0.14)         0.36 (0.14)         0.36 (0.14)         0.36 (0.14)         0.36 (0.14)         0.36 (0.14)         0.36 (0.14)         0.36 (0.14)         0.36 (0.14)         0.36 (0.14)         0.36 (0.36)         0.36 (0.36)         0.36 (0.36)         0.36 (0.36)         0.36 (0.36)         0.36 (0.	No.	Compound	RI <sup>c</sup>	RI (PMR) <sup>d</sup>	RI (lit.)	Relative amount <sup>b</sup>	
1 $a$ -Pinene         1016         1020         1014         2.21 (1.42)         1.78 (t           2         2-Butanol         1032         1027         1019, 1025         0.24 (0.12)         0.35 (0.37)         0.59 (0.37)           3         Ethyl butyrate         1033         1044         ND         0.10 (0.37)         0.56 (0.37)         0.56 (0.37)         0.56 (0.37)         0.56 (0.37)         0.56 (0.37)         0.56 (0.37)         0.56 (0.37)         0.56 (0.37)         0.56 (0.37)         0.56 (0.37)         0.56 (0.37)         0.56 (0.37)         0.56 (0.37)         0.56 (0.37)         0.56 (0.37)         0.56 (0.37)         0.56 (0.47)         0.56 (0.47)         0.56 (0.47)         0.56 (0.47)         0.56 (0.47)         0.33 (0.14)         0.32 (0.21)         0.31 (0.02)         0.20 (0.14)         0.32 (0.21)         0.31 (0.25)         0.20 (0.16)						MO CTRL	MO DMG
2         2-Butanol         1032         1027         1019, 1025         0.24 (0.12)         0.39           3         Ethyl butyrate         1037         1029         0.51 (0.37)         0.59 (0.59)           5         Ethyl 2-methylbutyrate         1053         1046         0.32 (0.09)         0.62 (0.9)           6         Camphene         1056         1063         1048         2.27 (1.24)         1.98 (0.22)           7         Ethyl isovalerate         1067         1062         2.57 (0.25)         3.34 (0.23)           8         β-Pinene         1092         1106         1088, 1094         0.35 (0.67)         NL           10         3-Pentanol         1111         1103         1108         ND         0.020 (0.22)           11         Ethyl 2-butenoate         1158         1157         1154, 1159         0.77 (0.04)         0.28 (0.22)           12         β-Myrcane         1186         1197         1182, 1195         1.06 (0.36)         0.80 (0.75)           13         Limonene         1281         1222         1280         0.28 (0.75)         NN           14         3-Methyl-and 2-methyl-1-butanol         1207         1220         124         183 (0.76)         2.55 (0.20	1	α-Pinene	1016	1020	1014	2.21 (1.42)	1.78 (0.74)
3         Ethyl butyrate         1037         1029         0.51 (0.37)         0.59 (0.57)           4         Unknown         1044         ND         0.19 (0.52)           5         Ethyl 2-methylbutyrate         1053         1046         0.32 (0.09)         0.62 (0.52)           6         Camphene         1057         1058         1063         1048         2.27 (1.24)         1.98 (0.52)           8         β-Pinene         1092         1106         1086, 1094         0.36 (0.14)         0.32 (0.70)           9         Diethyl carbonate         1110         1111         1103         1108         ND         0.08 (0.71)           11         Ethyl 2-butenoate         1158         1158         0.14 (0.25)         0.20 (0.71)           12         β-Myrcene         1188         1197         1182, 1195         1.06 (0.38)         0.30 (0.20)           13         Limonene         1227         1226         1190         1.66 (0.19)         3.63 (0.77)         NL           14         3-Methyl- and 2-methyl-1-butanol         1227         1226         0.30 (0.02)         0.52 (0.75)         NL           15         Ethyl tigtae, ethyl hexanoate         1231         1232         0.38 (0.07)	2	2-Butanol	1032	1027	1019, 1025	0.24 (0.12)	0.32 (0.20)
4         Unknown         ND         0.19(           5         Ethyl 2-methylbutyrate         1053         1063         1048         0.32 (0.09)         0.62 (           6         Camphene         1058         1063         1048         2.27 (1.24)         1.38 (           7         Ethyl isovalerate         1067         1062         2.57 (0.25)         3.34 (           9         Diethyl carbonate         1100         1101         0.53 (0.67)         NL           10         3-Pentanol         1111         1103         1108         ND         0.08 (           11         Ethyl 2-butenoate         1158         1158         0.14 (0.25)         0.20 (           12         #-Myrcene         1160         1157         1154, 1159         0.77 (0.04)         0.56 (           13         Limonene         1188         1197         1182, 1195         1.06 (0.36)         0.80 (           14         3-Methyl-and 2-methylu-1-butanol         1207         1225         1190         1.66 (0.19)         3.63 (           15         Ethyl 3-methylubu-2-enoate         1217         1226         0.30 (0.02)         0.52 (           16         2-Penhylfuran         1227         1226	3	Ethyl butyrate	1037	1029		0.51 (0.37)	0.59 (0.35)
5         Ethyl 2-methylbutyrate         1053         1046         0.32 (0.09)         0.62 (           6         Camphene         1067         1062         2.27 (1.24)         1.98 (           7         Ethyl isovalerate         1067         1062         2.57 (0.25 )         3.34 (           8         β-Pinene         1092         1106         1088, 1094         0.36 (0.14)         0.32 (           9         Diethyl carbonate         1110         1101         0.53 (0.67)         NI           10         3-Pentanol         1111         1103         1108         ND         0.06 (           11         Ethyl 2-butencate         1158         1157         1154, 1159         0.77 (0.04)         0.56 (           13         Limonene         1188         1197         1182, 1195         1.06 (0.36)         0.80 (           14         3-Methyl-and 2-methyl-1-butanol         1207         1205         1190         1.66 (0.19)         3.63 (           15         Ethyl 3-methylubut2-encoate         1219         0.16 (0.28 (0.7))         NT           16         2-Pentyliuran         1227         1226         120, 1224         1.81 (0.04)         0.33 (           17         Ethyl 3-meth	4	Unknown	1044			ND	0.19 (0.26)
6         Camphene         1068         1068         1048         2.27 (1.24)         1.98 (l           7         Ethyl isovalerate         1067         1062         2.57 (0.25)         3.34 (l           8         β-Pinene         1092         1106         1088, 1094         0.36 (0.14)         0.33 (l           9         Diethyl carbonate         1100         1101         0.53 (0.67)         NR           10         3-Pentanol         1111         1103         1108         ND         0.08 (l           11         Ethyl 2-butenoate         1158         1158         0.14 (0.25)         0.22 (l           12         β-Myrcene         1188         1197         1182, 1195         1.06 (0.36)         0.80 (l           14         3-Methyl-and 2-methyl-1-butanol         1207         125         1190         1.66 (0.19)         3.63 (l           15         Ethyl 3-methylbut-2-enoate         1231         1232         0.38 (0.07)         0.74 (l           16         2-Pentylfuran         1247         1252         0.39 (0.02)         0.52 (l           17         Ethyl iglate, ethyl hexanoate         1247         1252         0.39 (0.02)         0.52 (l           18         1-Dodecen	5	Ethyl 2-methylbutyrate	1053	1046		0.32 (0.09)	0.62 (0.04)
7         Ethyl isovalerate         1067         1062         2.57 (0.25)         3.34 (           8 <i>β</i> -Pinene         1092         1106         1088, 1094         0.36 (0.14)         0.32 (f)           9         Diethyl carbonate         1100         1101         0.53 (0.67)         NU           10         3-Pentanol         1111         1103         1108         ND         0.08 (f)           11         Ethyl 2-butenoate         1158         1158         0.14 (0.25)         0.20 (f)           12 <i>β</i> -Myrcene         1160         1157         1154, 1159         1.06 (0.36)         0.80 (f)           13         Limonene         1188         1197         1182, 1195         1.06 (0.36)         0.83 (f)           14         3-Methyl-and 2-methyl-1-butanol         1207         1205         1190         1.66 (0.19)         3.63 (f)           15         Ethyl 3-methylbut-2-enoate         1219         0.16 (0.28)         0.23 (f)         0.73 (f)           16         2-Pentyltiran         1227         1226         1220, 1224         1.81 (0.04)         0.39 (f)           17         Ethyl isplate, ethyl hexanoate         1231         1232         0.30 (0.02)         0.52 (f) <td>6</td> <td>Camphene</td> <td>1058</td> <td>1063</td> <td>1048</td> <td>2.27 (1.24)</td> <td>1.98 (0.92)</td>	6	Camphene	1058	1063	1048	2.27 (1.24)	1.98 (0.92)
8 $\beta$ -Pinene       1092       1106       1088, 1094       0.36 (0.14)       0.52 (0.97)         9       Diethyl carbonate       1100       1101       0.53 (0.67)       NU         10       3-Pentanol       1111       1103       1108       ND       0.08 (0.71)         11       Ethyl 2-butenoate       1158       1158       0.14 (0.25)       0.20 (0.71)         12 $\beta$ -Myrcene       1160       1157       1154, 1159       0.77 (0.04)       0.56 (0.71)         14       3-Methyl-and 2-methyl-1-butanol       1207       1205       1190       1.66 (0.19)       3.63 (0.71)         15       Ethyl 3-methylbut-2-enoate       1217       1226       1220, 1224       1.81 (0.04)       0.38 (0.77)       0.74 (0.72)         16       2-Penhyffuran       1227       1226       0.30 (0.02)       0.52 (0.20)       0.38 (0.07)       0.74 (0.20)         19       Styrene       1247       1252       0.30 (0.02)       0.52 (0.20)       0.36 (0.76)       NU         20       3-Octanone       1247       1250       0.16 (0.04)       0.11 (0.10)       0.15 (0.04)       0.11 (0.10)       0.15 (0.04)       0.11 (0.10)       0.15 (0.26)       0.79 (0.23)       0.24 (0.26) <t< td=""><td>7</td><td>Ethyl isovalerate</td><td>1067</td><td>1062</td><td></td><td>2.57 (0.25)</td><td>3.34 (2.10)</td></t<>	7	Ethyl isovalerate	1067	1062		2.57 (0.25)	3.34 (2.10)
9         Diethyl carbonate         1100         1101         0.53 (0.67)         NNI           10         3-Pentanol         1111         1103         1108         ND         0.08 (           11         Ethyl 2-butenoate         1158         1158         1157         1154, 1159         0.77 (0.04)         0.56 (           12 $\beta$ -Myrcene         1160         1157         1154, 1159         0.77 (0.04)         0.56 (           13         Limonene         1188         1197         1182, 1195         1.06 (0.36)         0.80 (           14         3-Methyl-and 2-methyl-1-butanol         1207         1205         1190         1.66 (0.28)         0.23 (           15         Ethyl 3-methylbut-2-enoate         1219         0.16 (0.28)         0.23 (           16         2-Pentylfuran         1227         1226         1220, 1224         1.81 (0.04)         0.93 (           18         1-Dodecene         1243         1242         0.69 (0.75)         NE           19         Styrene         1247         1252         0.30 (0.02)         0.52 (           21         Cymene isomer ( <i>bara-</i> 1264)         1261         1270         N16 (0.04)         0.111 (           22	8	$\beta$ -Pinene	1092	1106	1088, 1094	0.36 (0.14)	0.32 (0.12)
10         3-Pentanol         1111         1103         1108         ND         0.08 (I           11         Ethyl 2-butenoate         1158         1158         0.14 (0.25)         0.20 (I           12         β-Myrcene         1160         1157         1154, 1159         0.77 (0.04)         0.56 (I           13         Limonene         1188         1197         1182, 1195         1.06 (0.36)         0.80 (I           14         3-Methyl-and 2-methyl-1-butanol         1207         1205         1190         1.66 (0.28)         0.23 (I           16         2-Pentylfuran         1227         1226         1220, 1224         1.81 (0.04)         0.93 (I           17         Ethyl iglate, ethyl hexanoate         1231         1232         0.38 (0.07)         0.74 (I           18         1-Dodecene         1247         1252         0.30 (0.02)         0.52 (I           20         3-Octanone         1249         1251         Tr         0.12 (I           21         Cymene isomer (para-1264)         1261         1250         0.16 (IO.04)         0.11 (I           22         3-Hydroxy-2-butanone         1274         1278         1.83 (IO.76)         2.56 (I           23         Co	9	Diethyl carbonate	1100	1101		0.53 (0.67)	ND
11       Ethyl 2-butenoate       1158       1158       0.14 (0.25)       0.20 (0)         12 $\beta$ -Myrcene       1160       1157       1154, 1159       0.77 (0.04)       0.56 (0)         13       Limonene       1188       1197       1182, 1195       1.06 (0.36)       0.88 (0)         14       3-Methyl- and 2-methyl-1-butanol       1207       1205       1190       1.66 (0.19)       3.63 ()         15       Ethyl 3-methylbut-2-encate       1219       0.16 (0.28)       0.23 ()       0.38 ()       0.73 ()       0.74 ()         16       2-Pentyffuran       1227       1226       1220, 1224       1.81 ().0.4)       0.93 ()         17       Ethyl Bjate, ethyl hexanoate       1231       1232       0.38 ().0.7)       0.74 ()         18       1-Dockecene       1243       1242       0.39 ().0.2)       0.52 ()         03       O.021       0.52 ()       0.30 ().0.2)       0.52 ()       0.30 ().0.2)       0.52 ()         23       Oxprene isomer (para-1264)       1261       1250       0.16 ().0.4)       0.11 ()       0.12 ()         24       Unknown       1286       ND       Tr       ND       Tr         25 <i>E</i> -4,8-Dimethyl-1,3,7-no	10	3-Pentanol	1111	1103	1108	ND	0.08 (0.08)
12 $\beta$ -Myrcene       1160       1157       1154, 1159       0.77 (0.04)       0.56 (0.13)         13       Limonene       1188       1197       1182, 1195       1.06 (0.36)       0.80 (0.136)         14       3-Methyl-and 2-methyl-1-butanol       1207       1205       1190       1.66 (0.19)       3.63 (0.16)         15       Ethyl 3-methylbut-2-enoate       1219       0.16 (0.28)       0.23 (0.21)       0.74 (0.41)       0.33 (0.07)       0.74 (0.16)         16       2-Pentylfuran       1227       1226       1220, 1224       1.81 (0.04)       0.33 (0.07)       0.74 (0.16)         17       Ethyl tiglate, ethyl hexanoate       1231       1232       0.30 (0.02)       0.52 (0.16)         18       1-Dodecene       1247       1252       0.30 (0.02)       0.52 (0.22)         20       3-Octanone       1274       1276       1.83 (0.76)       2.55 (2.3)         21       Cymene isomer (para-1264)       1261       1250       0.16 (0.04)       0.11 (0.10)         23       Conophthorin       1280       0.34 (0.26)       0.79 (0.79 (0.79 (0.77))       0.71 (0.10)       0.15 (0.22 (0.77))         24       Unknown       1286       ND       Tr       1.60 (0.03)       0.30	11	Ethyl 2-butenoate	1158	1158		0.14 (0.25)	0.20 (0.27)
13       Limonene       1188       1197       1182, 1195       1.06 (0.36)       0.80 (0         14       3-Methyl- and 2-methyl-1-butanol       1207       1205       1190       1.66 (0.19)       3.63 ()         15       Ethyl 3-methylbut-2-enoate       1219       0.16 (0.28)       0.23 ()         16       2-Pentylfuran       1227       1226       1220, 1224       1.81 (0.04)       0.93 ()         17       Ethyl tiglate, ethyl hexanoate       1231       1232       0.38 (0.07)       0.74 ()         18       1-Dodecene       1243       1242       0.69 (0.75)       NE         20       3-Octanone       1247       1251       Tr       0.12 ()         21       Cymene isomer (para-1264)       1261       1250       0.16 (0.04)       0.11 ()         23       Conophthorin       1280       0.34 (0.26)       0.790 ()         24       Unknown       1286       ND       Tr         25 $E-4,8$ -Dimethyl-1,3,7-nonatriene       1301       1302       0.11 (0.10)       0.15 ()         26       Chalcogran isomer #1       1343       ND       Tr       126       124,8       1446       1.02 (1.77)       ND       Tr <t< td=""><td>12</td><td><math>\beta</math>-Myrcene</td><td>1160</td><td>1157</td><td>1154, 1159</td><td>0.77 (0.04)</td><td>0.56 (0.13)</td></t<>	12	$\beta$ -Myrcene	1160	1157	1154, 1159	0.77 (0.04)	0.56 (0.13)
14       3-Methyl- and 2-methyl-1-butanol       1207       1205       1190       1.66 (0.19)       3.63 (         15       Ethyl 3-methylbut-2-enoate       1219       0.16 (0.28)       0.23 (         16       2-Pentylfuran       1227       1226       1220, 1224       1.81 (0.04)       0.93 (         17       Ethyl tiglate, ethyl hexanoate       1231       1232       0.38 (0.07)       0.74 (         18       1-Dodecene       1243       1242       0.69 (0.75)       NI         19       Styrene       1247       1252       0.30 (0.02)       0.52 (         20       3-Octanone       1249       1251       Tr       0.16 (0.04)       0.11 (         21       Cymene isomer ( <i>para</i> - 1264)       1261       1250       0.16 (0.04)       0.11 (         22       3-Hydroxy-2-butanone       1274       1278       1.83 (0.76)       2.55 (         23       Conophthorin       1280       0.34 (0.26)       0.79 (       0.71 (       0.10 (0.10)       0.15 (         24       Unknown       1280       ND       Tr       ND       Tr         25 <i>E</i> -4,8-Dimethyl-1,3,7-nonatriene       1301       1302       0.11 (0.10)       0.35 (       0.36 (0	13	Limonene	1188	1197	1182, 1195	1.06 (0.36)	0.80 (0.18)
15       Ethyl 3-methylbut-2-enoate       1219       0.16 (0.28)       0.23 (t         16       2-Pentylfuran       1227       1226       1220, 1224       1.81 (0.04)       0.93 (t)         17       Ethyl tiglate, ethyl hexanoate       1231       1232       0.38 (0.07)       0.74 (t)         18       1-Dodecene       1243       1242       0.69 (0.75)       NII         19       Styrene       1247       1252       0.30 (0.02)       0.52 (t)         20       3-Octanone       1249       1251       Tr       0.16 (0.04)       0.11 (t)         21       Cymene isomer (para- 1264)       1261       1250       0.16 (0.04)       0.11 (t)         22       3-Hydroxy-2-butanone       1274       1278       1.83 (0.76)       2.55 (t)         23       Conophthorin       1280       0.34 (0.26)       0.79 (t)         24       Unknown       1286       ND       Tr         25 <i>E</i> -4,8-Dimethyl-1,3,7-nonatriene       1301       1302       0.11 (0.10)       0.15 (t)         26       Chalcogran isomer #1       1343       ND       Tr       ND       Tr         26       Chalcogran isomer #2       1348       1446       0.28 (0.06) <td>14</td> <td>3-Methyl- and 2-methyl-1-butanol</td> <td>1207</td> <td>1205</td> <td>1190</td> <td>1.66 (0.19)</td> <td>3.63 (2.95)</td>	14	3-Methyl- and 2-methyl-1-butanol	1207	1205	1190	1.66 (0.19)	3.63 (2.95)
16       2-Pentylfuran       1227       1226       120, 1224       1.81 (0.04)       0.93 (0         17       Ethyl tiglate, ethyl hexanoate       1231       1232       0.38 (0.07)       0.74 (0         18       1-Dodecene       1243       1242       0.69 (0.75)       NI         19       Styrene       1247       1252       0.30 (0.02)       0.52 (0         20       3-Octanone       1249       1251       Tr       0.16 (0.04)       0.11 (0         21       Cymene isomer (para-1264)       1261       1250       0.34 (0.26)       0.79 (0         23       Conophthorin       1280       0.34 (0.26)       0.79 (0       0.44 (0.26)       0.79 (0         24       Unknown       1286       ND       Tr       0.34 (0.26)       0.79 (0         24       Unknown       1286       ND       Tr       0.44 (0.26)       0.79 (0         25 <i>E</i> -4,8-Dimethyl-1,3,7-nonatriene       1301       1302       0.11 (0.10)       0.15 (0         26       Chalcogran isomer #2       1348       ND       Tr       128       ND       Tr         26       Chalcogran isomer #2       1348       1446       0.22 (0.07)       0.47 (0       12	15	Ethyl 3-methylbut-2-enoate	1219			0.16 (0.28)	0.23 (0.30)
17       Ethyl tiglate, ethyl hexanoate       1231       1232 $0.38 (0.07)$ $0.74 (0.18)$ 18       1-Dodecene       1243       1242 $0.69 (0.75)$ NU         19       Styrene       1247       1252 $0.30 (0.02)$ $0.52 (0.16)$ 19       Styrene       1247       1252 $0.30 (0.02)$ $0.52 (0.16)$ 20       3-Octanone       1249       1251 $Tr$ $0.12 (0.26)$ $0.79 (0.26)$ 21       Cymene isomer (para-1264)       1261       1250 $0.16 (0.04)$ $0.11 (0.26)$ $0.79 (0.26)$ 22       3-Hydroxy-2-butanone       1274       1278 $1.83 (0.76)$ $2.55 (0.27)$ 23       Conophthorin       1280 $0.34 (0.26)$ $0.79 (0.76)$ $0.79 (0.76)$ 24       Unknown       1280 $ND$ $Tr$ $7.7$ $7.4.8-Dimethyl-1.3,7-nonatriene       1301       1302 0.11 (0.10) 0.51 (0.26) 0.79 (0.76)         25       C-4.8-Dimethyl-1.3,7-nonatriene       1301       1302 0.11 (0.10) 0.71 (0.76) 7.7 (0.76)         26       Chalcogran isomer #1       1343       1389 1390, 1400$	16	2-Pentylfuran	1227	1226	1220, 1224	1.81 (0.04)	0.93 (0.38)
18       1-Dodecene       1243       1242 $0.69 (0.75)$ NI         19       Styrene       1247       1252 $0.30 (0.02)$ $0.52 (0.2)$ 20       3-Octanone       1249       1251       Tr $0.12 (0.2)$ 21       Cymene isomer ( <i>para</i> -1264)       1261       1250 $0.16 (0.04)$ $0.11 (0.10)$ $0.52 (0.2)$ 23       Conophthorin       1280 $0.34 (0.26)$ $0.79 (0.2)$ 24       Unknown       1280 $0.34 (0.26)$ $0.79 (0.2)$ 24       Unknown       1280 $0.34 (0.26)$ $0.79 (0.2)$ 24       Unknown       1280 $0.34 (0.26)$ $0.79 (0.2)$ 25 <i>E</i> -4,8-Dimethyl-1,3,7-nonatriene       1301       1302 $0.11 (0.10)$ $0.15 (0.2)$ 26       Chalcogran isomer #1       1343       ND       Tr       ND       Tr         27       Chalcogran isomer #2       1348       ND       ND       Tr         28       Tetradec-1-ene       14451       1448       1428, 1446 $0.28 (0.06)$ $0.42 (0.2)$ 31       Bourbonene/benzaldehyde mix       1505       1516 $0.21 (0.07)$	17	Ethyl tiglate, ethyl hexanoate	1231	1232		0.38 (0.07)	0.74 (0.14)
19       Styrene       1247       1252 $0.30 (0.02)$ $0.52 (t)$ 20       3-Octanone       1249       1251       Tr $0.12 (t)$ 21       Cymene isomer (para- 1264)       1261       1250 $0.16 (t)$ $0.11 (t)$ 22       3-Hydroxy-2-butanone       1274       1278 $1.83 (0.76)$ $2.55 (t)$ 23       Conophthorin       1280 $0.34 (0.26)$ $0.79 (t)$ 24       Unknown       1286       ND       Tr         25 <i>E</i> -4,8-Dimethyl-1,3,7-nonatriene       1301       1302 $0.11 (0.10)$ $0.15 (t)$ 26       Chalcogran isomer #1       1343       ND       Tr         27       Chalcogran isomer #2       1348       ND       Tr         28       Nonanal       1387       1389       1390, 1400 $0.30 (0.36)$ NE         29       Tetradec-1-ene       1444       1446 $1.02 (1.77)$ NE         30       1-Octen-3-ol       1451       1448       1428, 1446 $0.28 (0.06)$ $0.42 (t)$ 31       Bourbonene/benzaldehyde mix       1505       1516 $0.21 (0.07)$ $0.47 (t)$	18	1-Dodecene	1243	1242		0.69 (0.75)	ND
20       3-Octanone       1249       1251       Tr       0.12 (         21       Cymene isomer (para-1264)       1261       1250       0.16 (0.04)       0.11 ()         22       3-Hydroxy-2-butanone       1274       1278       1.83 (0.76)       2.55 ()         23       Conophthorin       1280       0.34 (0.26)       0.79 ()         24       Unknown       1286       ND       Tr         25       E-4,8-Dimethyl-1,3,7-nonatriene       1301       1302       0.11 (0.10)       0.15 ()         26       Chalcogran isomer #1       1343       ND       Tr         27       Chalcogran isomer #2       1348       ND       Tr         28       Nonanal       1387       1389       1390, 1400       0.30 (0.36)       NE         29       Tetradec-1-ene       1444       1446       0.28 (0.06)       0.42 ()         30       1-Octen-3-ol       1451       1448       1428, 1446       0.28 (0.06)       0.42 ()         31       Bourbonene/benzaldehyde mix       1505       1516       0.21 (0.07)       0.47 ()         32       trans- $\alpha$ -Bergamotene       1577       1582       0.19 (0.19)       0.13 ()         33	19	Styrene	1247	1252		0.30 (0.02)	0.52 (0.56)
21Cymene isomer (para- 1264)126112500.16 (0.04)0.11 (223-Hydroxy-2-butanone127412781.83 (0.76)2.55 (23Conophthorin12800.34 (0.26)0.79 (24Unknown1286NDT25 $E$ -4,8-Dimethyl-1,3,7-nonatriene130113020.11 (0.10)0.15 (26Chalcogran isomer #11343NDT27Chalcogran isomer #21348NDT28Nonanal138713891390, 14000.30 (0.36)NE29Tetradec-1-ene144414461.02 (1.77)NE301-Octen-3-ol145114481428, 14460.28 (0.06)0.42 (31Bourbonene/benzaldehyde mix150515160.21 (0.07)0.47 (32 $frans-a$ -Bergamotene157715820.19 (0.19)0.13 (33 $\beta$ -Copaene166516470.63 (1.09)NE34Aromadendrene160616050.11 (0.18)0.24 (351-Hexadecene165416470.63 (1.09)NE36Ethyl benzoate165416470.63 (1.09)NE371-Methyl-2-pyrrolidinone isomer16620.60 (0.09)0.55 (38Cyclodecanone <sup>6</sup> 1726174415.00 (0.00)15.00 (39Unknown18700.58 (0.28)0.53 (402-Phenylethyl alcohol1899191018480.16 (0.02)	20	3-Octanone	1249	1251		Tr	0.12 (0.11)
223-Hydroxy-2-butanone127412781.83 (0.76)2.55 (23Conophthorin12800.34 (0.26)0.79 (24Unknown1286NDTr25 $E-4,8$ -Dimethyl-1,3,7-nonatriene130113020.11 (0.10)0.15 (26Chalcogran isomer #11343NDTr27Chalcogran isomer #21348NDTr28Nonanal138713891390, 14000.30 (0.36)NE29Tetradec-1-ene144414461.02 (1.77)NE301-Octen-3-ol145114481428, 14460.28 (0.06)0.42 (31Bourbonene/benzaldehyde mix150515160.21 (0.07)0.47 (32trans- $\alpha$ -Bergamotene157715820.19 (0.19)0.13 (33 $\beta$ -Copaene158215890.64 (0.20)1.04 (34Aromadendrene160616050.11 (0.18)0.24 (351-Hexadecene164516470.63 (1.09)NE36Ethyl benzoate165416610.51 (0.60)0.18 (371-Methyl-2-pyrrolidinone isomer16620.60 (0.09)0.55 (38Cyclodecanone <sup>6</sup> 1726174415.00 (0.00)15.00 (39Unknown18700.58 (0.28)0.53 (411-Dodecanol1899191018480.16 (0.02)0.42 (422-Phenoxyethanol212621420.21 (0.06)0.14	21	Cymene isomer (para- 1264)	1261	1250		0.16 (0.04)	0.11 (0.10)
23       Conophthorin       1280 $0.34 (0.26)$ $0.79 (0.26)$ 24       Unknown       1286       ND       Tr         25 $E-4,8$ -Dimethyl-1,3,7-nonatriene       1301       1302 $0.11 (0.10)$ $0.15 (0.26)$ 26       Chalcogran isomer #1       1343       ND       Tr         27       Chalcogran isomer #2       1348       ND       Tr         28       Nonanal       1387       1389       1390, 1400 $0.30 (0.36)$ NE         29       Tetradec-1-ene       1444       1446 $1.02 (1.77)$ NE         30       1-Octen-3-ol       1451       1448       1428, 1446 $0.28 (0.06)$ $0.42 (0.2)$ 31       Bourbonene/benzaldehyde mix       1505       1516 $0.21 (0.07)$ $0.47 (0.2)$ 32       trans- $\alpha$ -Bergamotene       1577       1582 $0.19 (0.19)$ $0.13 (0.2)$ 33 $\beta$ -Copaene       1682       1589 $0.64 (0.20)$ $1.04 (0.2)$ 34       Aromadendrene       1606       1605 $0.11 (0.18)$ $0.24 (0.2)$ 35       1-Hexadecene       1664       1661 $0.51 (0.60)$ $0.18 (0.2)$	22	3-Hydroxy-2-butanone	1274	1278		1.83 (0.76)	2.55 (1.85)
24Unknown1286NDTh25 $E$ -4,8-Dimethyl-1,3,7-nonatriene130113020.11 (0.10)0.15 (0.11)26Chalcogran isomer #11343NDTh27Chalcogran isomer #21348NDTh28Nonanal138713891390, 14000.30 (0.36)NE29Tetradec-1-ene144414461.02 (1.77)NE301-Octen-3-ol145114481428, 14460.28 (0.06)0.42 (0.07)31Bourbonene/benzaldehyde mix150515160.21 (0.07)0.47 (0.07)32trans- $\alpha$ -Bergamotene157715820.19 (0.19)0.13 (0.33)33 $\beta$ -Copaene160616050.11 (0.18)0.24 (0.02)34Aromadendrene160616050.11 (0.18)0.24 (0.02)351-Hexadecene164516470.63 (1.09)NE36Ethyl benzoate165416610.51 (0.60)0.18 (0.02)371-Methyl-2-pyrrolidinone isomer16620.60 (0.09)0.55 (0.28)38Cyclodecanone <sup>6</sup> 1726174415.00 (0.00)15.00 (0.01)39Unknown18700.58 (0.28)0.53 (0.28)402-Phenylethyl alcohol1899191018480.16 (0.02)0.42 (0.21)411-Dodecanol19680.37 (0.13)0.18 (0.21)0.30 (0.21)422-Phenoxyethanol212621420.21 (0.06)0.14 (0.21) <td>23</td> <td>Conophthorin</td> <td>1280</td> <td></td> <td></td> <td>0.34 (0.26)</td> <td>0.79 (0.88)</td>	23	Conophthorin	1280			0.34 (0.26)	0.79 (0.88)
25E-4,8-Dimethyl-1,3,7-nonatriene130113020.11 (0.10)0.15 (t26Chalcogran isomer #11343NDTh27Chalcogran isomer #21348NDTh28Nonanal138713891390, 14000.30 (0.36)ND29Tetradec-1-ene144414461.02 (1.77)ND301-Octen-3-ol145114481428, 14460.28 (0.06)0.42 (t31Bourbonene/benzaldehyde mix150515160.21 (0.07)0.47 (t32trans-α-Bergamotene157715820.19 (0.19)0.13 (t33 $\beta$ -Copaene158215890.64 (0.20)1.04 (t34Aromadendrene160616050.11 (0.18)0.24 (t351-Hexadecene165416470.63 (1.09)NE36Ethyl benzoate165416610.51 (0.60)0.18 (t371-Methyl-2-pyrolidinone isomer16620.74415.00 (0.00)15.00 (t38Cyclodecanone <sup>6</sup> 1726174415.00 (0.00)15.00 (t39Unknown18700.58 (0.28)0.53 (t402-Phenylethyl alcohol1899191018480.16 (0.02)0.42 (t411-Dodecanol19680.37 (0.13)0.18 (t422-Phenoxyethanol212621420.21 (0.06)0.14 (t43Docosane218722000.52 (0.12)0.30 (t)	24	Unknown	1286			ND	Tr
26Chalcogran isomer #11343NDTr27Chalcogran isomer #21348NDTr28Nonanal138713891390, 14000.30 (0.36)NU29Tetradec-1-ene14441446 $1.02 (1.77)$ NU301-Octen-3-ol145114481428, 14460.28 (0.06) $0.42$ (0.07)31Bourbonene/benzaldehyde mix15051516 $0.21 (0.07)$ $0.47$ (0.33)32trans- $\alpha$ -Bergamotene15771582 $0.19 (0.19)$ $0.13 (0.33)$ 33 $\beta$ -Copaene15821589 $0.64 (0.20)$ $1.04 (0.33)$ 34Aromadendrene16061605 $0.11 (0.18)$ $0.24 (0.33)$ 351-Hexadecene16541661 $0.51 (0.60)$ $0.18 (0.33)$ 36Ethyl benzoate16541661 $0.51 (0.60)$ $0.18 (0.33) (0.30)$ 371-Methyl-2-pyrrolidinone isomer1662 $0.60 (0.09)$ $0.55 (0.33) (0.30)$ 38Cyclodecanone <sup>6</sup> 1726174415.00 (0.00)15.00 (0.00)39Unknown1870 $0.58 (0.28)$ $0.53 (0.28)$ $0.53 (0.28)$ 402-Phenylethyl alcohol189919101848 $0.16 (0.02)$ $0.42 (0.44)$ 411-Dodecanol1968 $0.37 (0.13)$ $0.18 (0.37 (0.13))$ $0.18 (0.37 (0.13))$ 422-Phenoxyethanol21262142 $0.21 (0.06)$ $0.14 (0.37 (0.13))$ 43Docosane21872200 $0.52 (0.12)$ $0.30 (0.$	25	E-4,8-Dimethyl-1,3,7-nonatriene	1301	1302		0.11 (0.10)	0.15 (0.04)
27Chalcogran isomer #21348NDTr28Nonanal138713891390, 14000.30 (0.36)NU29Tetradec-1-ene14441446 $1.02 (1.77)$ NU301-Octen-3-ol145114481428, 14460.28 (0.06)0.42 (0.07)31Bourbonene/benzaldehyde mix150515160.21 (0.07)0.47 (0.07)32trans- $\alpha$ -Bergamotene157715820.19 (0.19)0.13 (0.33)33 $\beta$ -Copaene158215890.64 (0.20)1.04 (0.33)34Aromadendrene160616050.11 (0.18)0.24 (0.33)351-Hexadecene165416470.63 (1.09)NU36Ethyl benzoate165416610.51 (0.60)0.18 (0.33)371-Methyl-2-pyrrolidinone isomer16620.60 (0.09)0.55 (0.60)38Cyclodecanone <sup>6</sup> 1726174415.00 (0.00)15.00 (0.00)39Unknown18700.58 (0.28)0.53 (0.42)402-Phenylethyl alcohol1899191018480.16 (0.02)0.42 (0.42)411-Dodecanol19680.37 (0.13)0.18 (0.44)422-Phenoxyethanol212621420.21 (0.06)0.14 (0.44)43Docosane218722000.52 (0.12)0.30 (0.42)	26	Chalcogran isomer #1	1343			ND	Tr
28         Nonanal         1387         1389         1390, 1400         0.30 (0.36)         NI           29         Tetradec-1-ene         1444         1446         1.02 (1.77)         NI           30         1-Octen-3-ol         1451         1448         1428, 1446         0.28 (0.06)         0.42 (0.07)           31         Bourbonene/benzaldehyde mix         1505         1516         0.21 (0.07)         0.47 (0.07)           32         trans-α-Bergamotene         1577         1582         0.19 (0.19)         0.13 (0.33)           33         β-Copaene         1582         1589         0.64 (0.20)         1.04 (0.33)           34         Aromadendrene         1606         1605         0.11 (0.18)         0.24 (0.33)           35         1-Hexadecene         1654         1661         0.63 (1.09)         NI           36         Ethyl benzoate         1654         1661         0.51 (0.60)         0.18 (0.33)           37         1-Methyl-2-pyrrolidinone isomer         1662         0.60 (0.09)         0.55 (0.28)         0.53 (0.33)           38         Cyclodecanone <sup>6</sup> 1726         1744         15.00 (0.00)         15.00 (0.00)           39         Unknown         1870	27	Chalcogran isomer #2	1348			ND	Tr
29Tetradec-1-ene144414461.02 (1.77)NI301-Octen-3-ol145114481428, 14460.28 (0.06)0.42 (0.07)31Bourbonene/benzaldehyde mix150515160.21 (0.07)0.47 (0.07)32trans- $\alpha$ -Bergamotene157715820.19 (0.19)0.13 (0.07)33 $\beta$ -Copaene158215890.64 (0.20)1.04 (0.07)34Aromadendrene160616050.11 (0.18)0.24 (0.07)351-Hexadecene164516470.63 (1.09)NI36Ethyl benzoate165416610.51 (0.60)0.18 (0.02)371-Methyl-2-pyrrolidinone isomer16620.60 (0.09)0.55 (0.00)38Cyclodecanone <sup>9</sup> 1726174415.00 (0.00)15.00 (0.00)39Unknown18700.58 (0.28)0.53 (0.28)0.53 (0.28)402-Phenylethyl alcohol1899191018480.16 (0.02)0.42 (0.01)411-Dodecanol19680.37 (0.13)0.18 (0.02)0.42 (0.01)422-Phenoxyethanol212621420.21 (0.06)0.14 (0.02)43Docosane218722000.52 (0.12)0.30 (0.02)	28	Nonanal	1387	1389	1390, 1400	0.30 (0.36)	ND
301-Octen-3-ol145114481428, 14460.28 (0.06)0.42 (031Bourbonene/benzaldehyde mix150515160.21 (0.07)0.47 (032trans- $\alpha$ -Bergamotene157715820.19 (0.19)0.13 (033 $\beta$ -Copaene158215890.64 (0.20)1.04 (034Aromadendrene160616050.11 (0.18)0.24 (0351-Hexadecene164516470.63 (1.09)NE36Ethyl benzoate165416610.51 (0.60)0.18 (0371-Methyl-2-pyrrolidinone isomer16620.60 (0.09)0.55 (038Cyclodecanone <sup>e</sup> 1726174415.00 (0.00)15.00 (039Unknown18700.58 (0.28)0.53 (0402-Phenylethyl alcohol1899191018480.16 (0.02)0.42 (0411-Dodecanol19680.37 (0.13)0.18 (0422-Phenoxyethanol212621420.21 (0.06)0.14 (043Docosane218722000.52 (0.12)0.30 (0	29	Tetradec-1-ene	1444	1446		1.02 (1.77)	ND
31Bourbonene/benzaldehyde mix15051516 $0.21$ (0.07) $0.47$ (0.07)32trans- $\alpha$ -Bergamotene15771582 $0.19$ (0.19) $0.13$ (0.37)33 $\beta$ -Copaene15821589 $0.64$ (0.20) $1.04$ (0.32)34Aromadendrene16061605 $0.11$ (0.18) $0.24$ (0.33)351-Hexadecene16451647 $0.63$ (1.09)NE36Ethyl benzoate16541661 $0.51$ (0.60) $0.18$ (0.33)371-Methyl-2-pyrrolidinone isomer1662 $0.60$ (0.09) $0.55$ (0.33)38Cyclodecanone <sup>e</sup> 1726174415.00 (0.00)15.00 (0.33)39Unknown1870 $0.58$ (0.28) $0.53$ (0.42)402-Phenylethyl alcohol189919101848 $0.16$ (0.02) $0.42$ (0.42)411-Dodecanol1968 $0.37$ (0.13) $0.18$ (0.43)422-Phenoxyethanol21262142 $0.21$ (0.06) $0.14$ (0.43)	30	1-Octen-3-ol	1451	1448	1428, 1446	0.28 (0.06)	0.42 (0.12)
32trans- $\alpha$ -Bergamotene157715820.19 (0.19)0.13 (0.19)33 $\beta$ -Copaene158215890.64 (0.20)1.04 (0.20)34Aromadendrene160616050.11 (0.18)0.24 (0.20)351-Hexadecene164516470.63 (1.09)NE36Ethyl benzoate165416610.51 (0.60)0.18 (0.20)371-Methyl-2-pyrrolidinone isomer16620.60 (0.09)0.55 (0.28)38Cyclodecanone <sup>6</sup> 1726174415.00 (0.00)15.00 (0.20)39Unknown18700.58 (0.28)0.53 (0.40)402-Phenylethyl alcohol1899191018480.16 (0.02)0.42 (0.42)411-Dodecanol19680.37 (0.13)0.18 (0.41)0.18 (0.42)422-Phenoxyethanol212621420.21 (0.06)0.14 (0.42)43Docosane218722000.52 (0.12)0.30 (0.42)	31	Bourbonene/benzaldehyde mix	1505	1516		0.21 (0.07)	0.47 (0.66)
33 $\beta$ -Copaene158215890.64 (0.20)1.04 (0.20)34Aromadendrene160616050.11 (0.18)0.24 (0.20)351-Hexadecene164516470.63 (1.09)NE36Ethyl benzoate165416610.51 (0.60)0.18 (0.20)371-Methyl-2-pyrrolidinone isomer16620.60 (0.09)0.55 (0.28)38Cyclodecanone <sup>6</sup> 1726174415.00 (0.00)15.00 (0.28)39Unknown18700.58 (0.28)0.53 (0.28)0.53 (0.28)402-Phenylethyl alcohol1899191018480.16 (0.02)0.42 (0.42)411-Dodecanol19680.37 (0.13)0.18 (0.43)0.18 (0.43)422-Phenoxyethanol212621420.21 (0.06)0.14 (0.43)43Docosane218722000.52 (0.12)0.30 (0.42)	32	trans-α-Bergamotene	1577	1582		0.19 (0.19)	0.13 (0.02)
34       Aromadendrene       1606       1605       0.11 (0.18)       0.24 (0.35)         35       1-Hexadecene       1645       1647       0.63 (1.09)       NE         36       Ethyl benzoate       1654       1661       0.51 (0.60)       0.18 (0.37)         37       1-Methyl-2-pyrrolidinone isomer       1662       0.60 (0.09)       0.55 (0.38)         38       Cyclodecanone <sup>6</sup> 1726       1744       15.00 (0.00)       15.00 (0.00)         39       Unknown       1870       0.58 (0.28)       0.53 (0.40)         40       2-Phenylethyl alcohol       1899       1910       1848       0.16 (0.02)       0.42 (0.42)         41       1-Dodecanol       1968       0.37 (0.13)       0.18 (0.40)       0.18 (0.40)         42       2-Phenoxyethanol       2126       2142       0.21 (0.06)       0.14 (0.42)         43       Docosane       2187       2200       0.52 (0.12)       0.30 (0.42)	33	$\beta$ -Copaene	1582	1589		0.64 (0.20)	1.04 (0.27)
35       1-Hexadecene       1645       1647       0.63 (1.09)       NI         36       Ethyl benzoate       1654       1661       0.51 (0.60)       0.18 (0.33)         37       1-Methyl-2-pyrrolidinone isomer       1662       0.60 (0.09)       0.55 (0.33)         38       Cyclodecanone <sup>e</sup> 1726       1744       15.00 (0.00)       15.00 (0.00)         39       Unknown       1870       0.58 (0.28)       0.53 (0.40)         40       2-Phenylethyl alcohol       1899       1910       1848       0.16 (0.02)       0.42 (0.42)         41       1-Dodecanol       1968       0.37 (0.13)       0.18 (0.40)       0.18 (0.40)         42       2-Phenoxyethanol       2126       2142       0.21 (0.06)       0.14 (0.42)         43       Docosane       2187       2200       0.52 (0.12)       0.30 (0.42)	34	Aromadendrene	1606		1605	0.11 (0.18)	0.24 (0.12)
36       Ethyl benzoate       1654       1661       0.51 (0.60)       0.18 (0.30)         37       1-Methyl-2-pyrrolidinone isomer       1662       0.60 (0.09)       0.55 (0.30)         38       Cyclodecanone <sup>e</sup> 1726       1744       15.00 (0.00)       15.00 (0.30)         39       Unknown       1870       0.58 (0.28)       0.53 (0.40)         40       2-Phenylethyl alcohol       1899       1910       1848       0.16 (0.02)       0.42 (0.42)         41       1-Dodecanol       1968       0.37 (0.13)       0.18 (0.42)       0.21 (0.06)       0.14 (0.42)         42       2-Phenoxyethanol       2126       2142       0.21 (0.06)       0.14 (0.42)         43       Docosane       2187       2200       0.52 (0.12)       0.30 (0.30)	35	1-Hexadecene	1645	1647		0.63 (1.09)	ND
37       1-Methyl-2-pyrrolidinone isomer       1662       0.60 (0.09)       0.55 (0.28)         38       Cyclodecanone <sup>e</sup> 1726       1744       15.00 (0.00)       15.00 (0.00)         39       Unknown       1870       0.58 (0.28)       0.53 (0.28)       0.53 (0.28)         40       2-Phenylethyl alcohol       1899       1910       1848       0.16 (0.02)       0.42 (0.42)         41       1-Dodecanol       1968       0.37 (0.13)       0.18 (0.28)       0.51 (0.28)         42       2-Phenoxyethanol       2126       2142       0.21 (0.06)       0.14 (0.22)         43       Docosane       2187       2200       0.52 (0.12)       0.30 (0.28)	36	Ethyl benzoate	1654	1661		0.51 (0.60)	0.18 (0.20)
38       Cyclodecanone <sup>e</sup> 1726       1744       15.00 (0.00)       15.00 (         39       Unknown       1870       0.58 (0.28)       0.53 (         40       2-Phenylethyl alcohol       1899       1910       1848       0.16 (0.02)       0.42 (         41       1-Dodecanol       1968       0.37 (0.13)       0.18 (         42       2-Phenoxyethanol       2126       2142       0.21 (0.06)       0.14 (         43       Docosane       2187       2200       0.52 (0.12)       0.30 (	37	1-Methyl-2-pyrrolidinone isomer	1662			0.60 (0.09)	0.55 (0.22)
39       Unknown       1870       0.58 (0.28)       0.53 (0.28)         40       2-Phenylethyl alcohol       1899       1910       1848       0.16 (0.02)       0.42 (0.42)         41       1-Dodecanol       1968       0.37 (0.13)       0.18 (0.42)       0.42 (0.42)         42       2-Phenoxyethanol       2126       2142       0.21 (0.06)       0.14 (0.42)         43       Docosane       2187       2200       0.52 (0.12)       0.30 (0.42)	38	Cyclodecanone <sup>e</sup>	1726	1744		15.00 (0.00)	15.00 (0.00)
40       2-Phenylethyl alcohol       1899       1910       1848       0.16 (0.02)       0.42 (0.13)         41       1-Dodecanol       1968       0.37 (0.13)       0.18 (0.02)         42       2-Phenoxyethanol       2126       2142       0.21 (0.06)       0.14 (0.02)         43       Docosane       2187       2200       0.52 (0.12)       0.30 (0.13)	39	Unknown	1870			0.58 (0.28)	0.53 (0.21)
41       1-Dodecanol       1968       0.37 (0.13)       0.18 (0.13)         42       2-Phenoxyethanol       2126       2142       0.21 (0.06)       0.14 (0.43)         43       Docosane       2187       2200       0.52 (0.12)       0.30 (0.13)	40	2-Phenylethyl alcohol	1899	1910	1848	0.16 (0.02)	0.42 (0.62)
42         2-Phenoxyethanol         2126         2142         0.21 (0.06)         0.14 (0.40)           43         Docosane         2187         2200         0.52 (0.12)         0.30 (0.12)	41	1-Dodecanol	1968			0.37 (0.13)	0.18 (0.17)
43 Docosane 2187 2200 0.52 (0.12) 0.30 (	42	2-Phenoxyethanol	2126	2142		0.21 (0.06)	0.14 (0.12)
	43	Docosane	2187	2200		0.52 (0.12)	0.30 (0.29)
44 Unknown 2264 0.17 (0.03) Tr	44	Unknown	2264			0.17 (0.03)	Tr
45 Unknown 2279 0.56 (0.28) 0.53 (	45	Unknown	2279			0.56 (0.28)	0.53 (0.33)
46 Unknown 2471 0.18 (0.04) 0.21 (	46	Unknown	2471			0.18 (0.04)	0.21 (0.21)
47 Vanillin 2541 2585 1.88 (0.57) 0.72 (	47	Vanillin	2541	2585		1.88 (0.57)	0.72 (0.68)

<sup>a</sup> Almonds collected on three different days.

<sup>b</sup> Volatile amounts reported as mean, normalised to 15 µg of internal standard, with standard deviation in parentheses; ND, not detected; Tr, trace amount (<0.10 µg).

<sup>c</sup> Retention index relative to *n*-alkanes on DB-Wax column.

<sup>d</sup> RI of volatile compounds based on in-house database.

<sup>e</sup> Internal standard.

VOCs increased in amounts from CTRL to DMG, while 2-pentylfuran decreased between these two experiments. The VOCs noted to occur during fungal growth, particularly *Aspergillus* species, are 2-methyland 3-methyl-1-butanol, 2-pentylfuran, 1-octen-3-ol and 3-octanone.<sup>25,26</sup> In addition to its previously reported occurrence in almonds,<sup>11,19</sup> it should be noted that 1-octen-3-ol is also a plant volatile of numerous plants, including genera of the Orchidaceae, as well as a semiochemical for several different

insects. 2-Pentylfuran is also an Orchidaceae plant volatile, but to a much lesser extent (The Pherobase, www.pherobase.com, accessed 22 August 2007). The eight-carbon VOCs, in addition to the sesquiterpenes myrcene, limonene and copaene, have been reported to be produced by *Penicillium* species.<sup>27</sup> Moreover, sesquiterpene VOCs unique to *A. flavus*<sup>28</sup> were not found in this study, thus indicating the possible presence of *Penicillium* more so than *Aspergillus*, yet this information did not provide enough evidence to exclusively implicate one particular microbe. Both *Aspergillus* and *Penicillium* are known to be present on almonds.<sup>29</sup>

The compounds unique, albeit only present in trace amounts, to the DMG almond VOCs were 3-pentanol, two chalcogran isomers (Fig. 4) and one unknown compound (No. 24, Table 1). 3-Pentanol is relatively new as a semiochemical, with only one study that demonstrated its ability to provoke a response in the male sugarcane weevil.<sup>30</sup> Interestingly, the same study reported ethyl butyrate, among other esters, as eliciting an antennal response in the female sugarcane weevil. The chalcogran isomers, however, have a long history of semiochemical activity, primarily with the European spruce bark beetle Pityogenes chalcographus.<sup>31,32</sup> The (2S,5R) and (2S,5S) configurations of chalcogran are found in P. chalcographus, and as two isomers with unknown configurations in the bark beetle Pityogenes quadridens.<sup>33</sup> It is interesting to note that Byers et al.34 used combinations of chalcogran, camphene and  $\alpha$ - and  $\beta$ -pinene, all VOCs detected in DMG almond VOCs, along with the compound methyl-E,Z-2,4-decadienoate to enable host recognition of the bark beetle. Other correlations between DMG almond VOCs and semiochemicals from bark beetles are similar VOCs, among others, emitted from Ips typographus males under stress, namely  $\alpha$ - and  $\beta$ pinene, camphene, myrcene, limonene and cymene, and similar VOCs from Pityogenes species, namely limonene, chalcogran, 1-octen-3-ol and 2-phenylethyl alcohol.<sup>35</sup> The occurrence of the chalcogran isomers in this and the one associated previous study<sup>6</sup> does not conclusively determine whether the spiroketals are emitted as a result of damage to the almonds or formed by fungal growth. The detection of several VOCs indicative of fungal growth brings into question whether or not the method of removing the DMG almonds after several days on the tree and subsequent transportation to the laboratory allows ambient fungi to initiate growth on the almonds. Investigations into this matter are ongoing.



**Figure 4.** Stereoisomers of chalcogran (2-ethyl-1,6-dioxaspiro [4.4]nonane).

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## CONCLUSION

The VOC emissions of control and damaged almonds were investigated. VOCs unique to damaged almonds include 3-pentanol and two isomers of the spiroketal chalcogran (unknown configuration) in trace amounts. Other VOCs that increased in relative quantity include the spiroketal conophthorin (unknown configuration), numerous four-carbon ester and ketone as well as alcohol derivatives, in addition to two eightcarbon chain compounds. VOCs suggestive of fungal growth were noted and brought to question whether the chalcogran isomers are damage-induced or a result of fungal growth. Also notable was the apparent correlation between several bark beetle semiochemicals and VOCs from the CTRL and DMG almonds. The detection of the VOCs noted above provides evidence that further investigation into their role in NOW response to damaged almonds is required.

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#### REFERENCES

- Almond Board of California, The Foundation for a Pest Management Strategic Plan in Almond Production: Summary of a Workshop Held December 12, 2002. California Pest Management Center, Modesto, CA (2003).
- 2 Campbell BC, Molyneux RJ and Schatzki TF, Current research on reducing pre- and post-harvest aflatoxin contamination of U.S. almond, pistachio, and walnut. *J Toxicol Toxin Rev* 22:225–266 (2003).
- 3 Robens J and Cardwell W, The costs of mycotoxin management to the USA: management of aflatoxins in the United States. *J Toxicol Toxin Rev* 22:143–156 (2003).
- 4 Epstein L, Bassein S, Zalom FG and Wilhoit LR, Changes in pest management practice in almond orchards during the rainy season in California, USA. *Agric Ecosyst Environ* 83:111-120 (2001).
- 5 Wu F, Mycotoxin risk assessment for the purpose of setting international regulatory standards. *Environ Sci Technol* 38:4049-4055 (2004).
- 6 Roitman JN, Merrill GB and Higbee BS, What attracts navel orangeworm to oviposit preferentially on wounded almonds rather than unblemished fruit? The search for a volatile attractant. Proc 4th Annu Fungal Genomics, 5th Annu Multicrop Fumonisin, 17th Annu Multi-crop Aflatoxin Elimination Workshops, pp. 27 (2004).
- 7 Coffelt JA, Vick KW, Sonnet PE and Doolittle RE, Isolation, identification, and synthesis of a female sex pheromone of the navel orangeworm, *Amyelois transitella* (Lepidoptera: Pyralidae). J Chem Ecol 5:955-966 (1979).
- 8 Leal WS, Parra-Pedrazzoli AL, Kaissling K-E, Morgan TI, Zalom FG, Pesak DJ, *et al*, Unusual pheromone chemistry in the navel orangeworm: novel sex attractants and a behavioral antagonist. *Naturwissenschaften* **92**:139–146 (2005).
- 9 Phelan PL, Roelofs CJ, Youngman RR and Baker TC, Characterization of chemicals mediating ovipositional hostplant finding by *Amyelois transitella* females. *J Chem Ecol* 17:599-613 (1991).

- 10 Buttery RG, Seifert RM, Haddon WF and Lundin RE, 2-Hexyl-3-methylmaleic anhydride: an unusual volatile component of raisins and almond hulls. *J Agric Food Chem* 28:1336-1338 (1980).
- 11 Buttery RG, Soderstrom EL, Seifert RM, Ling LC and Haddon WF, Components of almond hulls: possible navel orangeworm attractants and growth inhibitors. *J Agric Food Chem* 28:353–356 (1980).
- 12 Choi H-S, Lipolytic effects of citrus peel oils and their components. J Agric Food Chem 54:3254–3258 (2006).
- 13 Byers JA, Birgersson G, Lofqvist J, Appelgren M and Bergstrom G, Isolation of pheromone synergists of bark beetle, *Pityogenes chalcographus*, from complex insect-plant odors by fractionation and subtractive-combination bioassay. *J Chem Ecol* 16:861–876 (1990).
- 14 Whitehead AT, Electroantennogram responses by mountain pine beetles, *Dendroctonus ponderosae* Hopkins, exposed to selected semiochemicals. *J Chem Ecol* 12:1603–1621 (1986).
- 15 Batista-Pereira LG, Fernandes JB, Correa AG, Da Silva MFGF and Vierira PC, Electrophysiological responses of eucalyptus brown looper *Thyrinteina arnobia* to essential oils of seven *Eucalyptus* species. *J Braz Chem Soc* 17:555–561 (2006).
- 16 Zhang Q-H, Rolasch T, Schlyter F and Francke W, Enantiospecific antennal response of bark beetles to spiroacetal (*E*)-conophthorin. *J Chem Ecol* 28:1839–1852 (2002).
- 17 Khalilov LM, Khalilova AZ, Odinokov VN, Baltaev UA, Paramonov EA and Dzhemilev UM, Identification and biological activity of the volatile organic substances emitted by plants and insects. II. Sesquiterpene compostion of the native scent of leaves of the potato *Solanum tuberosum*. *Chem Nat Comp* 35:422–426 (1999).
- 18 Francis F, Vandermoten S, Verheggen F, Lognay G and Haubruge E, Is (E)-β-farnesene the only volatile terpenoid in aphids? J Appl Entomol 129:6–11 (2005).
- 19 Caja MM, Ruiz del Castillo ML, Martinez Alvarez R, Herraiz M and Blanch GP, Analysis of volatile compounds in edible oils using simultaneous distillation-solvent extraction and direct coupling of liquid chromatography with gas chromatography. *Eur Food Res Technol* 211:45–51 (2000).
- 20 Marti MP, Mestres M, Sala C, Busto O and Guasch J, Solidphase microextraction and gas chromatography olfactometry analysis of successively diluted samples. A new approach of the aroma extract dilution analysis applied to the characterization of wine aroma. J Agric Food Chem 51:7861–7865 (2003).
- 21 Joulain D, Casazza A, Laurent R, Portier D, Guillamon N, Pandya R, et al, Volatile flavor constituents of fruits from southern Africa: mobola plum (*Parinari curatellifolia*). J Agric Food Chem 52:2322–2325 (2004).
- 22 Bartelt RJ, Schaner AM and Jackson LL, Aggregation pheromones in *Drosophila borealis* and *Drosophila littoralis*. *J Chem Ecol* 14:1319-1327 (1988).

- 23 Pinero JC and Prokopy RJ, Field evaluation of plant odor and pheromonal combinations for attracting plum curculios. *J Chem Ecol* 29:2735-2748 (2003).
- 24 Malo EA, Cruz-Lopez L, Toledo J, Del Mazo A, Virgen A and Rojas JC, Behavioral and electrophysiological responses of the Mexican fruit fly (Diptera: Tephritidae) to guava VOCs. *Fla Entomol* 88:364–371 (2005).
- 25 Magan N and Evans P, VOCs as an indicator of fungal activity and differentiation between species, and the potential use of electronic nose technology for early detection of grain spoilage. *J Stored Prod Res* **36**:319–340 (2000).
- 26 Gao P, Korley F, Martin J and Chen BT, Determination of unique microbial volatile organic compounds produced by five Aspergillus species commonly found in problem buildings. Am Ind Hyg Assoc J 63:135–140 (2002).
- 27 Nilsson T, Larsen TO, Montanarella L and Madsen JO, Application of head-space solid-phase microextraction for the analysis of volatile metabolites emitted by *Penicillium* species. *J Microbiol Meth* 25:245–255 (1996).
- 28 Zeringue HJ, Bhatnagar D and Cleveland TE, C15H24 volatile compounds unique to aflatoxigenic strains of *Aspergillus flavus*. *Appl Environ Microbiol* 59:2264–2270 (1993).
- 29 Bayman P, Baker JL and Mahoney ME, Aspergillus on tree nuts: incidence and associations. Mycopathologia 155:161–169 (2002).
- 30 Perez AL, Campos Y, Chinchilla CM, Oehlschlager AC, Gries G, Gries R, et al, Aggregation pheromones and host kairomones of West Indian sugarcane weevil, *Metamasius* hemipterus sericeus. J Chem Ecol 23:869-888 (1997).
- 31 Francke W, Heemann V, Gerken V, Renwick JAA and Vite JP, 2-Ethyl-1,6-dioxaspiro[4.4]nonane, principal aggregation pheromone of *Pityogenes chalcographus* (L.). *Naturwissenschaften* 64:590-591 (1977).
- 32 Byers JA, Hogberg H-E, Unelius CR, Birgersson G and Lofqvist J, Structure-activity studies on aggregation pheromone components of *Pityogenes chalcographus* (Coleoptera: Scolytidae), all stereoisomers of chalcogran and methyl 2,4-decadienoate. J Chem Ecol 15:685-695 (1989).
- 33 Francke W and Kitching W, Spiroketals in insects. Curr Org Chem 5:233-251 (2001).
- 34 Byers JA, Birgersson G, Lofqvist J and Bergstrom G, Synergistic pheromones and monoterpenes enable aggregation and host recognition by a bark beetle. *Naturwissenschaften* 75:153–155 (1988).
- 35 Francke W, Bartels J, Meyer H, Schroder F, Kohnle U, Baader E, et al, Semiochemicals from bark beetles: new results, remarks, and reflections. J Chem Ecol 21:1043–1063 (1995).