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# Sulfur Volatiles in Guava (*Psidium guajava* L.) Leaves: Possible Defense Mechanism

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Volatiles from crushed and intact guava leaves (*Psidium guajava* L.) were collected using static headspace SPME and determined using GC-PFPD, pulsed flame photometric detection, and GC-MS. Leaf volatiles from four common citrus culitvars were examined similarly to determine the potential component(s) responsible for guava's protective effect against the Asian citrus psyllid (*Diaphorina citri* Kuwayama), which is the insect vector of Huanglongbing (HLB) or citrus greening disease. Seven sulfur volatiles were detected: hydrogen sulfide, sulfur dioxide, methanethiol, dimethyl sulfide (DMS), dimethyl disulfide (DMDS), methional, and dimethyl trisulfide (DMTS). Identifications were based on matching linear retention index values on ZB-5, DB-Wax, and PLOT columns and MS spectra in the case of DMDS and DMS. DMDS is an insect toxic, defensive volatile produced only by wounded guava but not citrus leaves and, thus, may be the component responsible for the protective effect of guava against the HLB vector. DMDS is formed immediately after crushing, becoming the major headspace volatile within 10 min. Forty-seven additional leaf volatiles were identified from LRI and MS data in the crushed guava leaf headspace.

**KEYWORDS: Citrus; allomone; dimethyl disulfide; wound response**

## INTRODUCTION

The volatiles released by the common guava (*Psidium guajava* L.) have been of intense recent interest since it was reported that in Vietnam guava grown in proximity to or intercropped with citrus had a protectant or repellant effect against the Asian citrus psyllid (*Diaphorina citri* Kuwayama) (*1*). Citrus groves interplanted with guava are devoid of *D. citri* infestation compared with heavily infested nearby citrus groves without guava (*1*). This insect is the vector of the devastating Huanglongbing (HLB) or citrus greening disease. HLB, which means in Chinese "yellow dragon disease", was first reported in southern China in 1919 (*2*) but is thought to have originated in Africa. It is now known to occur in approximately 40 different Asian, African, and North and South American countries including both Florida and Brazil, the two major citrus-producing regions in the world. HLB is caused by the phloem-limited fastidious prokaryotic α-proteobacterium *Candidatus* Liberibacter spp. Infected citrus trees go into decline, producing misshapened, off-flavor fruit and die within a few years. There is no cure for this disease. The $1.4 billion annual Florida citrus industry (*3*) is severely threatened by this vector-disease pathosystem. Given that guava appears to reduce *D. citri* populations and incidence of associated HLB disease, its protective effect requires investigation.

Because guava fruit is edible and possesses a unique aroma profile, guava fruit volatiles from different cultivars grown throughout the tropical and subtropical areas of the world have been extensively examined (*4*−*9*). The protective effect of interplanting guava and citrus is likely due to volatiles produced from the guava leaves and not the fruit because the protective effect is present year round. There are, however, few published studies on guava leaf volatiles. Early leaf volatile studies (*10*) examined terpene concentrations in leaf essential oils for chemotaxonomy purposes. Other leaf oil samples prepared from solvent extracts found menthol and α-terpenyl acetate along with ethanol and propanol (*11*). Fifty-seven components including 27 terpenes (or sesquiterpenes) along with 14 alcohols and 4 esters were identified in guava leaf oil using GC-MS obtained from a hydrodistillation of the leaves (*12*). The major volatiles consisted of $\beta$-caryophyllene (21.6%) and (*E*)-nerolidol (19.2%). Forty-two constituents including 29 hydrocarbon terpenes were observed in the air-dried and steam-distilled guava leaf oil from Nigeria (*13*). Chief among the terpenes were limonene (42.1%) and $\beta$-caryophyllene (21.3%). Given that almost all of the above volatiles are also common to citrus, they may lack insect activity and cannot account for the repellent effect against the Asian citrus psyllid.

The composition of plant leaves is known to change with age, exposure to sunlight, and other environmental factors (*14*). Some plants produce chemicals not directly related to their metabolic systems but which reduce or inhibit the palatability

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**Table 1.** Linear Retention Index (LRI) Values of Guava Sulfur Volatiles on Three Dissimilar Columns[a]

| sulfur volatile | LRI values ZB-5 | Wax | PLOT |
|---|---|---|---|
| hydrogen sulfide | <400 | 528 | <400 |
| sulfur dioxide | <400 | 831 | 414 |
| methanethiol | 423 | 675 | 414 |
| dimethyl sulfide (DMS) | 519 | 736 | 718 |
| dimethyl disulfide (DMDS)* | 744 | 1064 | 860 |
| methional | 914 | 1450 | |
| dimethyl trisulfide (DMTS)* | 978 | 1355 | |

[a] Sulfur volatiles denoted with an asterisk (*) were observed from only crushed guava leaves.

of the plant to herbivorous organisms. Other plants have developed systems that produce defense chemicals in response to wounding/insect attack (*15, 16*). For example, leeks (*Allium porrum* L.) produce sulfur-based defense chemicals in response to insect attack (*17*). Sulfur volatiles are extremely potent in terms of aroma and physiological effects. Balandrin and coworkers (*18*) reported finding a range of sulfur volatiles in neem seeds (*Azadirachta indica*). The major volatile component was reported to be di-*n*-propyl disulfide, which is larvicidal to *Aedes aegypti* (L.) (Diptera: Culicidae) (yellow fever mosquito), *Heliothis virescens* (Fab.) (Lepidoptera: Noctuidae) (tobacco budworm), and *Heliothis zea* (Boddie) (Lepidoptera: Noctuidae) (corn earworm). The active components in garlic (*Allium sativum* L.), which have long been used as a natural insect repellent and insecticide, are also sulfur compounds (*19*). Because none of the previous guava leaf studies have determined sulfur volatiles, the objective of this work was to determine if there are sulfur volatiles in guava leaves that could be responsible for the repellent effects against the Asian citrus psyllid. A secondary objective was to identify headspace volatiles in guava leaves using GC-MS as previous leaf volatile results were solely based on leaf oils from solvent extraction or steam distillation.

## MATERIALS AND METHODS

**Leaf Samples.** Leaf flush from 'white guava' (*P. guajava* L.; Myrtaceae), two cultivars of sweet orange, 'Hamlin' and 'Valencia' (*Citrus sinensis* L. Rutaceae), Ray Ruby grapefruit (*C. paradisi* Macf.), and rough lemon (*Citrus limon* Burm.) were harvested, weighed on a Mettler AE 160 (Greifensee, Switzerland) balance, and immediately placed in 40 mL septum-sealed glass vials. Approximately 3.5 g of leaves from each plant was placed in the vial and equilibrated at room temperature for ~30 min. *P. guajava* and *C. limon* were obtained from Cee Jay Nursery, Lakeland, FL, and a managed citrus grove at the Citrus Research and Education Center (CREC) in Lake Alfred, FL, respectively, in 2007, and their seedlings have been maintained in a screen house since then. Seedlings of *C. sinensis* and *Citrus paradisi* were obtained from Southern Citrus Nurseries LLC, Dundee, FL, in 2007. These citrus cultivars were selected for analysis because Hamlin and Valencia oranges as well as Ray Ruby grapefruit are the most commonly cultivated citrus varieties in Florida (*20*).

**SPME Headspace Sampling.** A 50/30 $\mu$m divinylbenzene/Carboxen/polydimethylsiloxane (DVB/Carboxen/PDMS) Stable Flex solid phase microextraction (SPME) fiber (Supelco, Bellefonte, PA) was manually inserted into the septum-sealed glass vial for 1 (GC-PFPD) or 15 (GC-MS) min to collect emanated static head space volatiles from the uncrushed guava or citrus leaf flush after it had equilibrated with ambient room conditions. Collected volatiles were eluted in the injector port of the GC and separated and analyzed as indicated in the following sections. Subsequently, the vial was opened to crush the leaf samples using the blunt end of a glass stirring rod and rapidly closed to minimize volatile loss. Thereafter, the SPME fiber was again exposed to static volatiles within the vial immediately after it was closed for 1 min (time 0). The vial was repeatedly sampled (for 1 min) after 10, 30, and 60 min from crushing to investigate kinetics of volatile production from crushed leaves. Following each volatile collection, the volatile-impregnated fiber was transferred to the injector of the GC-PFPD or GC-MS and desorbed for ~5 min at 200 °C.

**GC-Pulse Flame Photometric Detector (PFPD).** Sulfur compounds were analyzed using a pulsed flame photometric detector (PFPD) (model 5380, OI Analytical Co., College Station, TX) set up in the sulfur mode coupled to a HP-5890 series II GC. Separation and tentative identification were accomplished using three different capillary columns, ZB-5 (30 m × 0.32 mm. i.d. × 0.5 $\mu$m, (Zebron ZB-5, Phenomenex, Torrance, CA), DB-Wax (30 m × 0.32 mm. i.d. × 0.5 $\mu$m, J&W Scientificm Folsom, CA) and Gas Pro PLOT (30 m × 0.32 mm. i.d., Agilent, Palo Alto, CA). The ZB-5 column oven temperature was programmed from 40 to 265 °C and from 40 to 240 °C for DB-Wax at 7 °C/min, with a 5 min hold at the maximum temperature. Helium was used as carrier gas at a flow rate of 1.5 mL/min. Injector and detector temperatures were 200 and 250 °C, respectively. A 0.75 mm

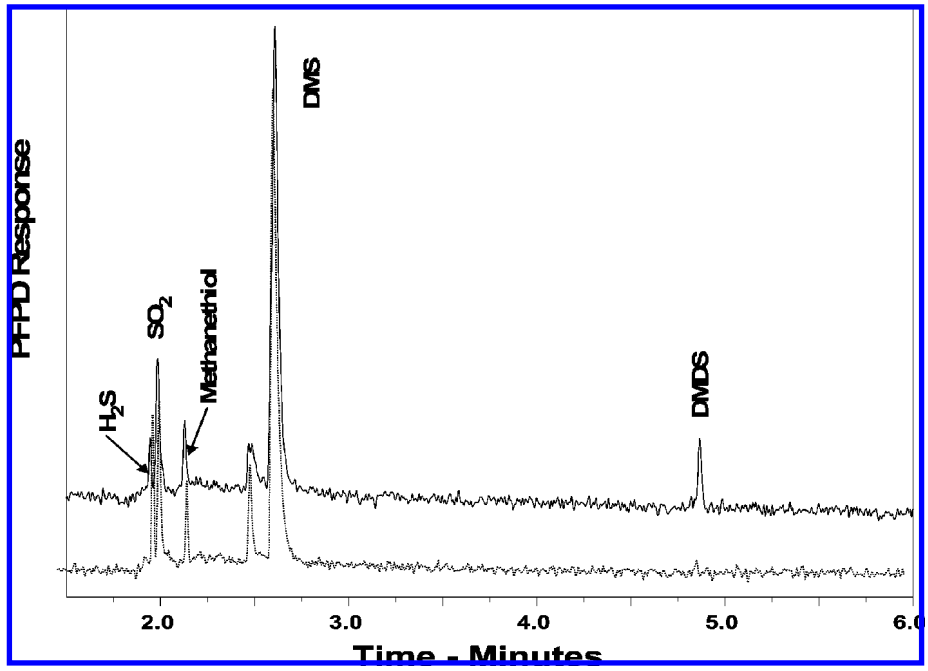


**Figure 1.** Comparison of sulfur chromatograms from intact (lower trace) and crushed (upper trace) guava leaves.

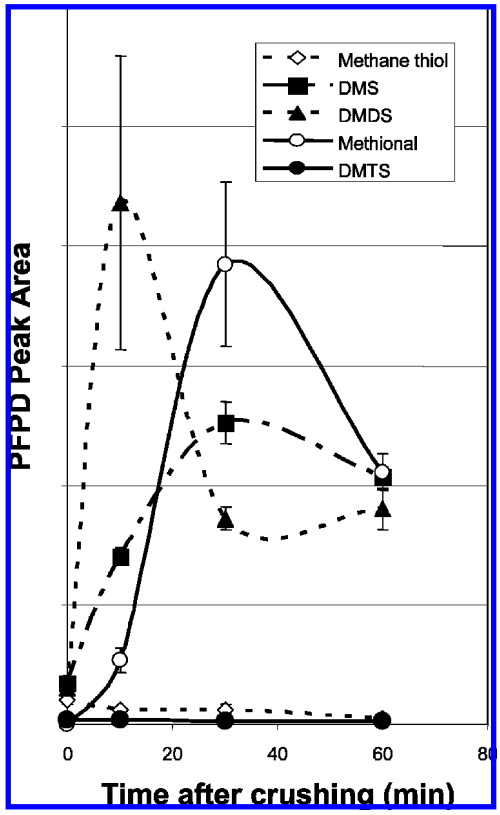


**Figure 2.** Formation kinetics of sulfur volatiles in crushed guava leaves. Volatiles were collected using SPME and separated on a ZB-5 column. Peak areas are from a PFPD detector. Other experimental details are given in the text.

injector liner was employed to improve peak shape and chromatographic efficiency. Injections were splitless. Identification of sulfur volatiles was determined by matching the linear retention index (LRI) values with authentic standards on both polar and nonpolar columns.

**GC-MS.** Analyses were performed with a Perkin-Elmer Claris 500 quadrupole mass spectrometer equipped with Turbo Mass software (Perkin-Elmer, Shelton, CT) and an RTX-5 capillary column (Restek; 60 m × 0.25 mm. i.d. × 0.50 $\mu$m). Helium was used as the carrier gas in the constant flow mode of 2 mL/min. The source was kept at 200 °C, and the transfer line and injector were maintained at 260 °C. The oven temperature program consisted of a linear gradient from 40 to 260 at 7 °C/min. Electron impact ionization in the positive ion mode was used (70 eV), either scanning a mass range from 25 to 300 *m/z* or acquiring data in the selected ion mode (see **Table 1** for the selected ions used of the specific compounds). Mass spectra matches were made by comparison of NIST 2005 version 2.0 standard spectra (NIST, Gaithersburg, MD). Only those compounds with spectral fit values ≥800 and appropriate LRI values were considered to be positive identifications. Authentic standards were used to confirm identifications when available.

## RESULTS AND DISCUSSION

**Identification of Sulfur Volatiles.** As shown in **Table 1**, the preliminary identifications of the seven identified guava leaf sulfur volatiles were based on matching standardized retention index values from three dissimilar columns with those of authentic standards. These values were obtained using the pulsed flame photometric detector, which is highly selective for detecting sulfur volatiles only. On some column types, the sulfur volatiles are not sufficiently different to provide unambiguous identification. However, when the LRI values from all three columns are used, a unique set of values can be determined for unambiguous identification. Even the relative elution order is different on some columns as exemplified by methional and dimethyl trisulfide on ZB-5 and DB-Wax columns, which provides additional unique information in terms of peak identification. For example, hydrogen sulfide is the first peak in all three chromatographic systems. Although its retention time is very close to that of methanethiol on a ZB-5 column, it is well resolved on both Wax and PLOT columns. Furthermore, methanethiol is not resolved from sulfur dioxide on the PLOT

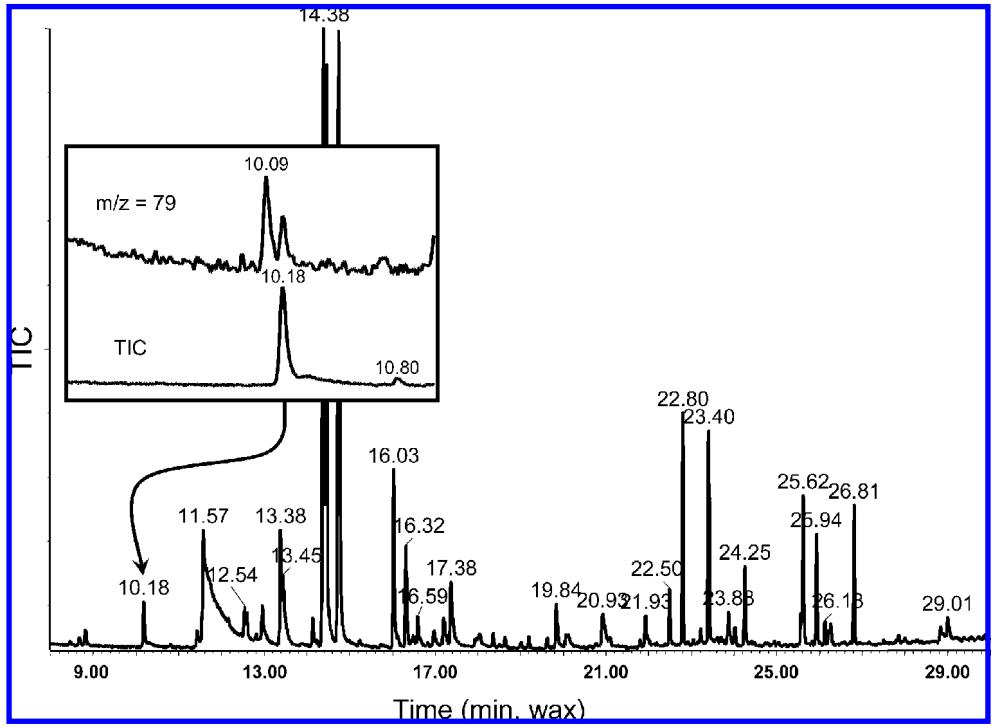


**Figure 3.** GC-MS total ion current (TIC) chromatogram of wounded guava leaves, 1 min after wounding. (Inset) Comparison of the TIC response with an extracted ion chromatogram (EIC) of *m/z* 79 at 1000× greater scale. Hexanal (RT 10.18) is shown in both inset chromatograms as a point of reference. The peak at 10.09 corresponds to DMDS. See text for chromatographic details.

**Table 2.** GC-MS Identifications from Guava Total Ion Chromatograms[a]

| RT | LRI obsd | LRI ref | % | name (fit) | CAS Registry No. | prior report |
|---|---|---|---|---|---|---|
| 7.28 | 970 | 969 | 0.08 | 2-ethylfuran (926) | 3208-16-0 | |
| 7.84 | 997 | 996 | 0.08 | 3-pentanone (879) | 99-22-0 | |
| 8.45 | 1025 | 1024 | 0.09 | methyl 2-methylbutyrate (919) | 868-57-5 | |
| 8.67 | 1035 | 1034 | 0.23 | methyl 3-methylbutyrate (880) | 556-24-1 | |
| | | 1038 | | **α-pinene** (925) | 7785-70-8 | *11*, *13* |
| 8.82 | 1041 | 1040 | 0.36 | ethyl vinyl ketone (927) | 1629-58-9 | |
| 10.18 | 1101 | 1101 | 1.15 | hexanal (939) | 66-25-1 | |
| 10.80 | 1129 | 1131 | 0.1 | *E*-2-pentenal (897) | 1576-87-0 | |
| 11.57 | 1163 | 1151 | 14.55 | *Z*-3-hexenal (880) | 6789-80-6 | |
| 12.16 | 1189 | 1180 | 1.16 | isobutyl 2-methylbutyrate (916) | 2445-67-2 | |
| 12.46 | 1203 | 1202 | 0.05 | isobutyl 3-methylbutyrate (850) | 589-59-3 | |
| 12.54 | 1207 | 1206 | 0.54 | isoamyl butyrate | 106-27-4 | |
| 12.59 | 1209 | 1208 | 0.56 | isoamyl isobutyrate | 2050-01-3 | |
| 12.81 | 1219 | 1220 | 0.14 | **limonene** (927) | 138-86-3 | *11*−*13* |
| 13.38 | 1245 | 1236 | 2.41 | *E*-2-hexenal (940) | 6728-26-3 | |
| 13.45 | 1248 | 1249 | 2.88 | **(*Z*)-β-ocimene** (897) | 3338-55-4 | *12* |
| 14.38 | 1293 | 1304 | 10.68 | isoamyl 2-methylbutyrate (962) | 27625-35-0 | |
| 14.46 | 1296 | 1294 | 8.21 | 2-methylbutyl 2-methylbutyrate (951) | 2445-78-5 | |
| 14.74 | 1310 | 1308 | 19.33 | isoamyl 3-methylbutyrate (917) | 659-70-1 | |
| 15.23 | 1334 | 1334 | 0.15 | *Z*-2-pentenol (838) | 1576-95-0 | |
| 16.03 | 1373 | | 3.95 | unidentified | | |
| 16.32 | 1388 | | 2.1 | 3-methyl-3-butenyl 3-methylbutyrate (948) | 54410-94-5 | |
| 16.59 | 1401 | 1400 | 0.64 | *Z*-3-hexenol (924) | 928-96-1 | |
| 16.98 | 1421 | 1419 | 0.54 | *neo-allo*-ocimene (909) | 673-84-7 | |
| 17.20 | 1432 | | 0.78 | (*E*,*Z*)-2,4-hexadienal (931) | 53398-76-8 | |
| 17.38 | 1441 | 1440 | 2.45 | (*E*,*E*)-2,4-hexadienal (927) | 142-83-6 | |
| 17.95 | 1471 | 1469 | 0.25 | α-*p*-dimethylstyrene (949) | 1195-32-0 | |
| 18.36 | 1492 | 1492 | 0.29 | *Z*-3-hexenyl 2-methylbutyrate (930) | 53398-85-9 | |
| 18.52 | 1501 | 1500 | 0.07 | pentadecane (869) | 629-62-9 | |
| 18.64 | 1508 | 1507 | 0.23 | *Z*-3-hexenyl 3-methylbutyrate (927) | 35154-45-1 | |
| 19.01 | 1527 | 1505 | 0.14 | **α-copaene** (908) | 3856-25-5 | *12*, *13* |
| 19.63 | 1560 | 1560 | 0.27 | cyclohexyl 3-methylbutyrate (894) | 7774-44-9 | |
| 19.84 | 1572 | 1571 | 1.11 | **benzaldehyde** (968) | 100-52-7 | *12* |
| 20.77 | 1623 | 1600 | 0.12 | β-elemene (922) | 515-13-9 | |
| 20.93 | 1632 | | 1.67 | unidentified | | |
| 21.09 | 1641 | 1641 | 0.3 | **β-caryophyllene** (913) | 87-44-5 | *10*, *12*, *13* |
| 21.80 | 1681 | 1666 | 0.16 | **β-farnesene** (890) | 18794-84-8 | *12* |
| 21.93 | 1689 | 1690 | 1.05 | 3-methylbutyric acid (882) | 503-74-2 | |
| 22.50 | 1722 | 1711 | 1.14 | methyl geranate (928) | 2349-14-6 | |
| 22.80 | 1740 | 1745 | 4.16 | (*Z*,*E*)-α-farnesene (931) | 26560-14-5 | |
| 23.05 | 1755 | 1753 | 0.12 | **β-bisabolene** (913) | 495-61-4 | *10*, *12*, *13* |
| 23.22 | 1765 | 1765 | 0.32 | (*E*,*E*)-α-farnesene (925) | 502-61-4 | |
| 23.40 | 1776 | 1767 | 4.14 | geranyl acetate (955) | 105-87-3 | |
| 23.88 | 1804 | 1786 | 0.84 | **curcumene** (912) | 644-30-4 | *12* |
| 24.03 | 1814 | | 0.31 | 5-ethyl-2(5*H*)-furanone (927) | 2407-43-4 | |
| 24.25 | 1828 | 1837 | 1.7 | geranyl propionate (913) | 105-90-8 | |
| 25.63 | 1915 | 1912 | 2.92 | geranyl butyrate (944) | 106-29-6 | |
| 25.94 | 1936 | | 2.12 | geranyl isovalerate (947) | 109-20-6 | |
| 26.27 | 1958 | 1957 | 0.44 | isoamyl benzoate (909) | 94-46-2 | |
| 26.81 | 1994 | | 2.24 | unidentified | | |

[a] Compounds in bold have been previously identified. Observed LRI values are compared with references from standards or literature values.

column and is only slightly resolved on the ZB-5 column, but is well resolved on the Wax column. Methional and dimethyl trisulfide values were not obtained for the PLOT column as they were too highly retained and were not required as ZB-5 and Wax LRI values were sufficiently unique as to provide satisfactory identification.

**Sulfur Volatiles in Crushed and Intact Guava Leaves.** As shown in the lower sulfur chromatogram in **Figure 1**, there are five sulfur volatiles in undamaged guava leaves. It should be pointed out that the sulfur chromatograms were obtained using a PFPD detector in the sulfur (square root mode) that is both highly selective and sensitive for sulfur volatiles. The output from the same sample detected using the PFPD carbon mode is much more complex. The sulfur volatiles have been identified as hydrogen sulfide, sulfur dioxide, methanethiol, and dimethyl sulfide. The small peak between methanethiol and dimethyl sulfide remains unidentified.

The upper chromatogram shows the sulfur volatiles produced immediately after the leaves are crushed. This chromatogram contains all of the previously identified sulfur volatiles plus dimethyl disulfide (DMDS). (Also produced as a result of crushing but not shown are methional and traces of dimethyl trisulfide.) The wound response production of DMDS is particularly interesting as it can be considered to be a plant defense response. Defensive sulfur compounds such as DMDS are highly toxic for most insect species. The toxicity of DMDS in these insects is due to disruption of the cytochrome oxidase system of their mitochondria (*21*). It is one of an emerging group of botanically produced insecticides that offers an alternative approach to established chemical pesticides and their mode of action.

Preliminary testing suggests that this compound is highly repellent to Asian citrus psyllid, and these data will be reported elsewhere. Furthermore, other preliminary tests have shown that crushed guava leaves were more repellant than the uncrushed leaves.

**Sulfur Volatiles in Crushed and Intact Citrus Leaves.** Because citrus leaves are highly susceptible to psyllid attack and guava leaves seem to induce a repellent effect, the volatile(s) responsible for the repellency must be present only in the guava

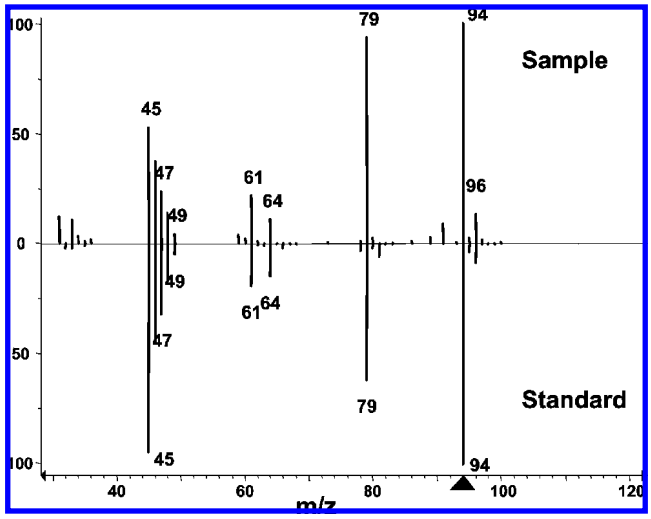


**Figure 4.** Comparison of TIC fragmentation pattern of background-corrected peak at 10.09 min (see inset in **Figure 3**) with reference standard DMDS.

leaves. Therefore, citrus leaves were crushed and analyzed in the same manner as the guava leaves. Leaves from a total of four different citrus cultivars were evaluated for sulfur volatiles. Evaluated cultivars included both 'Valencia' and 'Hamlin' sweet orange (*C. sinensis*), rough lemon (*C. limon*), and grapefruit (*C. paradisi*). All of the citrus leaves produced dimethyl sulfide at low levels in uncrushed leaves, and the relative concentration of this sulfide increased over 10-fold when the leaves were crushed. Although the injury response elevated concentrations of dimethyl sulfide, dimethyl disulfide was not produced in any of the citrus cultivars evaluated either wounded or unwounded. Therefore, citrus leaves appear to lack the ability to produce the potent defensive chemical dimethyl disulfide, which may explain guava's unique repellent properties, which are not shared with citrus.

**Formation Kinetics of Dimethyl Disulfide in Crushed Guava Leaves.** As shown in **Figure 2**, dimethyl disulfide is formed rapidly once guava leaves are crushed. The precursor of DMDS in the intact leaves has yet to be identified. However, Tulio and co-workers (*22*) found that DMDS and methanethiol were produced from crushed broccoli florets through the enzymatic degradation of the nonvolatile substrate *S*-methyl-L-cysteine sulfoxide. A similar reaction may be occurring in crushed guava leaves, but in the case of crushed broccoli, methanethiol was produced in much higher amounts that DMDS. As shown in **Figure 2**, DMDS is the major sulfur volatile product and very little methanethiol was formed. Although DMDS starts out from undetectable levels, it becomes the most prominent headspace sulfur volatile within 10 min after crushing, but then its concentration diminishes just as rapidly as it was formed. The eventual decline in DMDS concentration may be due in part to its well-known disproportionation into dimenthyl sulfide and dimethyl trisulfide as dimethyl sulfide levels increase as DMDS levels decrease. Because dimethyl trisulfide levels remained low, it must be assumed that it undergoes further reactions as soon as it is formed. Methional [3-(methylthio)propanal] levels also increase, but not as rapidly as those of DMDS, reaching maximum concentrations at about 25 min after crushing and then slowly diminishing thereafter. It is presumably produced from the degradation of the sulfur-containing amino acid, methionine.

**GC-MS Identification of Guava Leaf Volatiles.** The TIC chromatogram from crushed guava leaves is shown in **Figure 3**, and the corresponding peak identifications are listed in **Table 2**. Forty-seven volatiles are identified, of which 9 had been previously reported. Over 100 peaks were observed in the high-resolution capillary chromatogram, but only the 50 largest peaks are included in **Table 2**. These 50 peaks account for 92% of the total peak area, whereas the remaining peaks comprised only 8% of the remaining total peak area. As one might expect in complex samples such as guava volatiles, there was some coelution. It can be seen from **Table 2** that the front half of the peak at 8.67 min is composed of methyl 3-methylbutyrate and the back portion is composed of α-pinene. α-Copaene, which was found in the crushed guava leaves in this study at 19.01 min, has also been reported to be a component in guava fruit (*8, 23*) and is a reported attractant to the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (*24*). α-Copaene is also found in citrus.

Unlike previous studies that had reported terpenes as major volatiles, the major volatiles in crushed fresh leaves are composed of esters [isoamyl 3-methylbutyrate (19.33%), isoamyl 2-methylbutyrate (10.68%), 2-methylbutyl 2-methylbutyrate (8.21%)] and aldehydes [*Z*-3-hexenal (14.6%)]. Prior GC-MS leaf volatile studies were based on solvent extractions or steam distillations of the dried leaves. Many of the volatiles observed from the fresh leaves in this study were undoubtedly lost during the drying process in the prior studies. As seen in **Table 2**, only 9 of the 47 identified guava volatiles have been previously reported. Both Pino et al. (*12*) and Ogunwande and co-workers (*13*) reported about the same number of volatiles as this study; the major difference was that this study examined fresh, new growth leaves at room temperature, whereas the other two studies employed dried leaves and hydrodistilled them for 4 h in a Clevenger-type apparatus. It is not known if prior studies used intact or crushed leaves.

Guava fruit volatiles have been extensively studied for two major reasons, first, to determine which volatiles are responsible for their unique aroma, and, second, because guava fruit is a known host and possible attractant for the Mediterranean fruit fly, *C. capitata* (*25*), and the Oriental fruit fly, *B. dorsalis* Hendel (*26*). Guava fruit is also the preferred host for the Caribbean fruit fly, *A. suspensa* (Loew) (*27*). Many insects find their hosts by locating the odorant trails emitted by specific plants, and guava fruit volatiles have been examined to find such a fruit fly attractant (*28, 29*).

**GC-MS Confirmation of DMDS in Crushed Guava Leaves.** As seen in **Figure 3** and the expanded inset, DMDS cannot be readily observed in the TIC mode. Dimethyl disulfide can be detected using MS in the extracted ion mode using *m/z* 79 (corresponding to $CH_3S_2+$, DMDS minus methyl group) as shown for the peak at 10.09 min in the inset. The background-corrected spectrum for the peak at 10.09 min is shown in **Figure 4** and compared with standard DMDS (inverted). It can be readily seen that the spectrum of the suspected DMDS in the leaf sample is essentially identical to that of standard DMDS. Although DMDS has a strong $M^+$ ion at *m/z* 94, it cannot be used for quantitation as another compound with a fragment at this same *m/z* elutes on the back half of the DMDS peak; therefore, the ion at *m/z* 79 is the next best choice and used in **Figure 3**. The *m/z* 79 peak at 10.09 min is completely absent in uncrushed guava leaves, which substantiates the findings from the sulfur-specific GC-PFPD chromatograms shown in **Figure 1**. Therefore, it is confirmed that DMDS is formed in the crushed guava leaves and was not detected in the intact leaves sampled under similar circumstances. DMDS appears to be produced in

response to wounding/mechanical injury and is a highly potent defensive plant volatile known to affect insect behavior.

## ABBREVIATIONS USED

LRI, linear retention indices; GC-PFPD, gas chromatography−pulsed flame photometric detection; MS, mass spectrometry; TIC, total ion current; PLOT, porous layer open tubular; DMS, dimethyl sulfide; DMDS, dimethyl disulfide; DMTS, dimethyl trisulfide.

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