

- Haga, H.; Imanari, T.; Tamura, Z.; Momose, A. Gas Chromatography and Its Combination with Mass Spectrometry of Urinary Sugar Alcohols. *Chem. Pharm. Bull.* 1972, 20, 1805-1808.
- Huneck, S.; Follmann, G. Lichen Component. 48. Components of *Combea Mollusca*, *Rocella Vicentina*, *Rocella Gayana* and *Rocella Fucoides*. *Z. Naturforsch.* 1967, 22, 1369-1370.
- Huneck, S.; Follmann, G.; Weber, W. A.; Trotet, G. Lichen Constituents. 37. Components of Some Species of *Rocella*. *Z. Naturforsch.* 1967, 22, 671-673.
- Imanari, T.; Matsunaga, I.; Miura, K.; Tamura, Z. Urinary Excretion of Sugar-Alcohols in Man. *Rinsho Kagaku* 1975, 4, 220-224.
- Komiyama, Y.; Harakawa, M.; Tsuji, M. Characteristics of Sugar Composition of Various Fruits in Relation to Maturity and Storage. *Nippon Shokuhin Kogyo Gakkaishi* 1985, 32, 522-529.
- Masuda, Y.; Sasaki, S.; Yamashita, K.; Hattori, K.; Ito, Y. Sugar Composition of Sake. II Variation in Sugar and Sugar Alcohol Compositions During Brewing and Storage of Sake. *Nippon Jozo Kyokai Zasshi* 1967, 62, 1242-1244.
- Matsushita, A. Variation of Free Sugar Contents During the Ripening Period of Seeds and Fruits. *Nippon Nogei Kagaku Kaishi* 1971, 45, 49-53.
- Onishi, H.; Saito, S. Polyols in Soy Sauce. *Hakko Kogaku Zasshi* 1959, 37, 457-461.
- Onishi, H.; Saito, N. Polyols in Sake. *Hakko Kogaku Zasshi* 1960, 38, 536-538.
- Roboz, J.; Kappatos, D. C.; Greaves, J.; Holland, J. F. Determination of Polyols in Serum by Selected Ion Monitoring. *Clin. Chem.* 1984, 30, 1611-1615.
- Sasaki, T. Search and Improvement of a High Activated Microorganism. Development of Production Technology of Erythritol As a New Sweetener. *B. R. A. IN. Techno News* 1988, 6, 6-8.
- Shindou, T.; Sasaki, Y.; Miki, H.; Eguchi, T.; Hagiwara, K.; Ichikawa, T. Determination of Erythritol in Fermented Foods By HPLC. *Shokuhin Eiseigaku Zasshi* 1988, 29, 419-422.
- Sponholz, W. R.; Dittrich, H. H. Alditols and Myo-inositol in Wines and Sherries. *Vitis* 1985, 24, 97-105.
- Storset, P.; Stokke, O.; Jellum, E. Monosaccharides and Monosaccharide Derivatives in Human Seminal Plasma. *J. Chromatogr.* 1978, 145, 351-357.
- Yoshida, H.; Sugahara, T.; Hayashi, J. Studies on Free Sugars and Free Sugaralcohols of Mushrooms. *Nippon Shokuhin Kogyo Gakkaishi* 1984, 31, 765-771.
- Yoshida, H.; Sugahara, T.; Hayashi, J. Studies on Free Sugars and Free Sugaralcohols of Mushrooms. *Nippon Shokuhin Kogyo Gakkaishi* 1986, 33, 426-433.

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## Volatile Components from Bartlett and Bradford Pear Leaves

Robert L. Miller,\* Donald D. Bills,† and Ron G. Buttery‡

Eastern Regional Research Center, U.S. Department of Agriculture—Agricultural Research Service,  
600 East Mermaid Lane, Philadelphia, Pennsylvania 19118

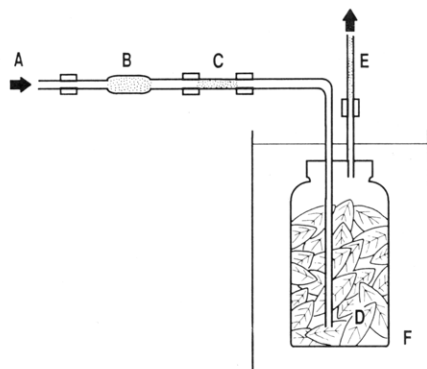
Volatile components from whole leaves of Bartlett and Bradford pear were collected in a glass tube packed with Tenax and Carbotrap by passing a stream of air through a container of pear leaves and then into the trap. The trap was thermally desorbed onto a capillary GC column, and the compounds were identified by means of mass spectral data and Kovats retention indices. The compounds that were identified in all samples include (*E*)- $\beta$ -ocimene,  $\beta$ -caryophyllene, and (*E,E*)- $\alpha$ -farnesene. Perillene and (*Z,E*)- $\alpha$ -farnesene were also found in all samples but were only tentatively identified.  $\alpha$ -Copaene was a major component found in the Bartlett samples but not in the Bradford samples. Linalool (tentatively identified) was a medium to large peak and was found in the Bradford samples but not in the Bartlett samples. Compounds listed in this publication have not been reported previously as volatiles from pear leaves.

*Psylla pyricola* Forester (pear psylla) is an oligophagous insect that is quite specific in the selection of its host (Burts, 1970). It is known to infest only pear, quince, and chess grass (Madsen et al., 1962; Glass, 1969). Pear psylla exists in two distinct forms, and the summer form is one of the most destructive insect pests attacking pear orchards in the United States and Europe (Butt and Stuart, 1986). Losses in pear production are caused by direct

defoliation, lower quality of the fruit, and "psylla shock", i.e., reduced quantity of fruit the year following an infestation. Psylla attacks most species of pear but with different levels of success (Chang and Philogene, 1976). Some experiments indicate that certain cultivars of pear are preferred targets for adult feeding (Chang and Philogene, 1978; Westigard et al., 1970), while other studies suggest an ovipositional preference for susceptible pear trees and nymphal antibiosis (mortality) on resistant pear trees (Harris, 1973). Although nonvolatile components from pear leaves have been studied (Chalice and Williams, 1968a,b) and the volatiles from pear fruit have been investigated (Jennings et al., 1960), volatile organic compounds emanating from the foliage of pear trees have not been examined yet. These may, in fact, play a role

\* Present address: Beltsville Agricultural Research Center, U.S. Department of Agriculture—Agricultural Research Service, Beltsville, MD 20705.

† Present address: Western Regional Research Center, U.S. Department of Agriculture—Agricultural Research Service, Albany, CA 94710.



**Figure 1.** Apparatus for collection of volatiles from pear leaves. Key: A, compressed air tank; B, Supelpure HC2-2445 trap; C, Tenax TA (2,6-diphenyl-1-*p*-phenylene oxide) trap; D, 2-quart jar containing leaves; E, Tenax TA trap; F, constant-temperature (29 °C) bath.

in the insect's identification mechanism. The objective of this study was to isolate and identify volatile components from Bartlett and Bradford pear leaves. Compared to other pear species, Bartlett pears are produced in the largest volume in the United States and they are subject to psylla infestation. Bradford pear leaves, on the other hand, are resistant to pear psylla (Schalk and Ratcliffe, 1976).

#### EXPERIMENTAL SECTION

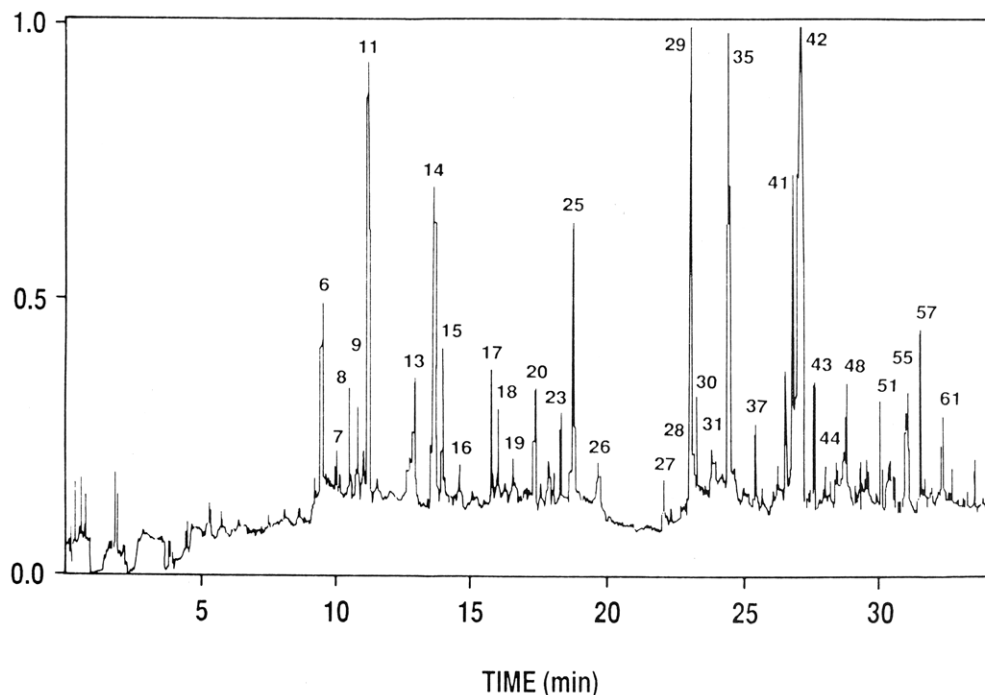
**Materials and Reagents.** Bartlett pear (*Pyrus communis*) and Bradford pear (*Pyrus calleryana*) leaves were obtained locally.

**Methods.** The leaves were cut midstem, weighed, counted, and placed in a 2-quart, wide-mouth, glass Mason jar fitted with a specially designed Teflon lid (Berghoff America, Raymond, NH). The lid had two 6-mm ports: one for introducing and one for exhausting a stream of air. The inlet air stream was directed to the bottom of the jar through a tube that extended from the inlet port in the lid (see Figure 1). The inlet line was connected to a tank of zero-grade compressed air through an inline Supelpure HC2-2445 trap (Supelco, Bellefonte, PA) and a Pyrex glass tube 6-mm o.d. × 10-cm length packed with Tenax TA (a porous polymer based upon 2,6-diphenyl-1-*p*-

phenylene oxide) to assure the purity of incoming air. The outlet port on the lid was fitted with an adapter for connecting a trap. The entire system was constructed of glass, Teflon, and stainless steel. Volatiles from the pear leaves were trapped in a Sylon-CT-treated Pyrex glass tube 6-mm o.d. × 8-cm length packed with 50 mg of Tenax TA (35/60 mesh) adsorbent (Alltech Applied Science Laboratory, Deerfield Park, IL) and 160 mg of Carbotrap (20/40-mesh) adsorbent (Supelco) plugged with approximately 5 mm of silanized glass wool (Supelco) at each end (Zlatkis, 1973). Traps were activated by purging them with helium at 20 mL/min while being heated for 24 h at 322 °C. After conditioning, the tubes were sealed with graphitized vespel ferrules and brass end caps until use. Traps prepared in this manner exhibited excellent absorptive capacity, and gas chromatography (GC) analysis showed no organic background.

**Isolation of Volatiles.** Collection of volatile compounds by purging was initiated within 1 h of removal of leaves from trees. The 2-quart jar held an average of 309 Bartlett leaves weighing an average of 145 g/jar or 209 Bradford leaves weighing an average of 157 g. The system was purged with purified air at a rate of 25 mL/min for 72 h with three changes of leaves for Bartlett I, five changes for Bartlett II and Bradford I, and four changes for Bradford II at 24-h intervals. The jar containing the leaves was immersed in a constant-temperature bath at 29 °C.

**Sample Analysis.** For GC-mass spectral (GC-MS) analysis, the volatile compounds collected on Tenax-Carbotrap tubes were thermally desorbed directly into the GC-MS system with a specially designed introduction device interfaced to the GC injection port (Hartman, 1987). The device allowed the glass trap to be heated rapidly to 250 °C. Bartlett I was desorbed at 250 °C, while the other samples were desorbed at 200 °C with a helium flow rate of 20 mL/min. The thermally desorbed volatiles were swept directly from the desorption heating zone into the capillary column injection port. Desorption was carried out for 20 min during which time the volatiles were cryofocused onto the head of the capillary column. To achieve the desired temperature of -80 °C for cryofocusing, a dry ice-acetone bath was used to immerse a few loops of the anterior part of the GC capillary column. After the desorption period, the GC oven was heated rapidly to 50 °C for Bartlett I (30 °C for the other samples) and normal temperature programming was initiated. All experiments were conducted with a Varian 3400 series gas chromatograph interfaced to a Finnigan 8230 high-resolution mass spectrometer. Data were recorded and processed with a Finnigan SS-300 data system. Computerized mass spectral library



**Figure 2.** Reconstructed ion chromatogram from the capillary GC analysis of the Tenax-Carbotrap trapped volatiles from Bartlett pear leaves.

**Table I. Volatile Compounds Identified in the Atmosphere Associated with Bartlett I Pear Leaves Using Tenax-Carbotrap Trapping**

peak no.	compound	characteristic MS	Kovats	authentic	rel %
	myrcene <sup>c</sup>	41, 93, 69, 79, 53, 121, 136		980	1.0 <sup>e</sup>
6	(Z)-3-hexenyl acetate <sup>b</sup>	43, 67, 82, 54, 69	986	985	4.6
8	limonene	68, 41, 93, 136, 53, 79, 107	1020	1020	1.4
9	(Z)- $\beta$ -ocimene	93, 79, 53, 67, 121	1025	1026	1.7
11	(E)- $\beta$ -ocimene	93, 79, 53, 121, 67	1040	1040	9.2
	linalool <sup>d</sup>	93, 71, 41, 55, 80, 121, 136		1080	6.0 <sup>e</sup>
14	perillene <sup>a</sup>	69, 41, 81, 150, 79, 107	1110		6.5
25	MW 164; 69	69, 79, 67, 121, 164			3.1
27	$\alpha$ -cubebene	161, 119, 105, 120, 91	1350	1347	0.5
29	$\alpha$ -copaene	105, 119, 161, 93, 81	1377	1374	9.2
30	$\beta$ -bourbonene	81, 105, 119, 133, 161	1385	1381	0.8
31	$\beta$ -cubebene	161, 105, 91, 119, 93, 79	1390	1385	0.6
35	$\beta$ -caryophyllene <sup>b</sup>	69, 93, 79, 133, 55	1415	1415	6.5
37	humulene	93, 121, 80, 147, 107, 204	1450	1448	1.0
41	(Z,E)- $\alpha$ -farnesene <sup>c</sup>	93, 69, 55, 107, 79, 119	1490		5.0
42	(E,E)- $\alpha$ -farnesene	93, 69, 107, 55, 79, 119	1498	1495	16.1
43	$\Delta$ -cadinene <sup>b</sup>	161, 134, 105, 119, 204, 91	1513	1513	1.9
44	calacorene	157, 142, 200, 67, 77	1520	1525	0.4

<sup>a</sup> Identity tentative. <sup>b</sup> Used as internal standards to determine Kovats index. <sup>c</sup> Identity tentative in Bradford I only. <sup>d</sup> Identity tentative in Bradford I and Bradford II. <sup>e</sup> Approximate.

searches were compared to the National Bureau of Standards reference spectral library. Gas chromatography was performed on a 60 m  $\times$  0.32 mm (i.d.) DB-1 methyl silicone capillary column with a 0.25- $\mu$ m film thickness for all samples. The injector temperature was 250 °C for Bartlett I and 200 °C for the others. Carrier gas flow rate was 20 linear cm/s (helium). The sample was not split. Column program temperature was -80 °C for 20 min (during thermal desorption), then 50–200 °C at 4 °C/min for Bartlett I (2 °C/min for the others), and then 10 °C/min to 320 °C (final temperature). No components emerged beyond 200 °C.

The MS was used in the electron ionization mode with an ion source temperature of 250 °C, filament current 1.0 mA, and electron multiplier voltage 1.2 kV. The mass range was 50–300 amu for Bartlett I and 40–300 amu for the others; scan rate was 1 s/decade, and interscan time was 0.8 s.

**Reference Samples.** MS and GC retention data had already been obtained on authentic samples of sesquiterpene hydrocarbons obtained from known essential oil sources (Buttery and Ling, 1984; Buttery et al., 1982). Myrcene, limonene, linalool, and 3-hexenyl acetate were obtained from commercial sources.

## RESULTS AND DISCUSSION

Combined capillary GC-MS analyses were carried out on three to five batches of pear leaves harvested at daily intervals in early and late summer 1987. Identification was made if mass spectra and GC Kovats indices (Kovats, 1965) were identical with those of authentic samples. Kovats GC indices were measured relative to three other components present in the mixture and readily identified by GC-MS. These were (Z)-3-hexenyl acetate,  $\beta$ -caryophyllene, and  $\Delta$ -cadinene.

A representative chromatogram for the volatiles (Bartlett I) collected at 29 °C is shown in Figure 2. Sixty-one peaks are labeled. Fourteen components were identified, two tentatively identified, and forty-three peaks could not be conclusively identified in Bartlett I. Although Bartlett I was desorbed at 250 °C, the other samples were desorbed at 200 °C to provide a more moderate environmental temperature.

On the basis of GC peak area (see Table I) of Bartlett I, the seven largest components were (E,E)- $\alpha$ -farnesene (16.1%), (E)- $\beta$ -ocimene (9.2%),  $\alpha$ -copaene (9.2%),  $\beta$ -caryophyllene (6.5%), perillene (6.5%), (Z,E)- $\alpha$ -farnesene (5.0%), and (Z)-3-hexenyl acetate (4.6%). These components comprised 57% of the volatiles collected.

(E)- $\beta$ -Ocimene,  $\beta$ -caryophyllene, and (E,E)- $\alpha$ -farnesene were identified in all four samples (see Table II).

**Table II. Comparison of Volatiles Identified in Bartlett and Bradford Pear Leaves<sup>a</sup>**

	Bartlett I	Bartlett II	Bradford I	Bradford II
myrcene			s <sup>b</sup>	
(Z)-3-hexenyl acetate	m		m	m
limonene	s		s	
(Z)- $\beta$ -ocimene	s		s	
(E)- $\beta$ -ocimene	l	l	vl	l
linalool			l	m
perillene	l	vl	vl	l
MW 164; 69	l	m	l	m
$\alpha$ -copaene	vl	l		
$\beta$ -caryophyllene	l	m	m	m
humulene	s		s	
(Z,E)- $\alpha$ -farnesene	s	s	s	s
(E,E)- $\alpha$ -farnesene	vl	vl	vl	vl

<sup>a</sup> Sesquiterpenes that were found only in Bartlett I are not all included unless they occurred in one other sample. <sup>b</sup> Key: s = small; m = medium; l = large; vl = very large.

Perillene and (Z,E)- $\alpha$ -farnesene, which were tentatively identified, also were found in all the samples. (Z)-3-Hexenyl acetate was found in three of the four samples. A large component with an apparent molecular ion of 164 and a base ion of 69 was found in all samples.  $\alpha$ -Copaene was a major component found in the Bartlett samples, but not in the Bradford samples. Linalool was a medium to large peak and was found in the Bradford samples, but not in the Bartlett samples.

This trapping method might be expected to give a profile of volatile components similar to what an insect would encounter in the atmosphere near a pear tree (Buttery et al., 1982). Disadvantages of this trapping method include the small amounts of volatiles obtained, the possibility of chemical changes occurring on the Tenax/Carbotrap surface, and desorption in the metal-lined injector port of the GC under high-temperature conditions (200–250 °C) (e.g., germacrene was not found under these conditions). Care was taken in handling the pear leaves to avoid further damage after being cut at midstem. Although there was some damage at the point where the stem was cut, very little of the usual compounds, e.g., unsaturated aldehydes, etc., that result from tissue damage was observed.

Terpene and sesquiterpene hydrocarbon type compounds may be important as semiochemicals that influence insect behavior (Buttery and Ling, 1984; Buttery et

al., 1982; Metcalf, 1987). Although there were differences in the chromatographic patterns of the volatiles from psylla-susceptible and psylla-resistant species of pear leaves, gross differences that might account for psylla recognition were not obvious. Establishment of any relationship of these volatile components to the resistance or susceptibility of pear species to pear psylla infestation and damage will require further study including appropriate bioassays.

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**Registry No.** Myrcene, 123-35-3; (Z)-3-hexenyl acetate, 3681-71-8; limonene, 5989-27-5; (Z)- $\beta$ -ocimene, 3338-55-4; (E)- $\beta$ -ocimene, 3779-61-1; linalool, 78-70-6; perillene, 539-52-6;  $\alpha$ -cubebene, 17699-14-8;  $\alpha$ -copaene, 3856-25-5;  $\beta$ -bourbonene, 5208-59-3;  $\beta$ -cubebene, 13744-15-5;  $\beta$ -caryophyllene, 87-44-5; humulene, 6753-98-6; (Z,E)- $\alpha$ -farnesene, 26560-14-5; (E,E)- $\alpha$ -farnesene, 502-61-4;  $\Delta$ -cadinene, 483-76-1; calacorene, 38599-17-6.

#### LITERATURE CITED

- Burts, E. C. The Pear Psylla in Central Washington. *Wash., Agric. Exp. Stn., Stn. Circ.* 1970, 516.
- Butt, B. A.; Stuart, C. Oviposition by Summer and Winter Forms of Pear Psylla (Homoptera: Psyllidae) on Dormant Pear Budwood. *Environ. Entomol.* 1986, 15(5), 1109.
- Buttery, R. G.; Ling, L. C. Corn Leaf Volatiles: Identification Using Tenax Trapping for Possible Insect Attractants. *J. Agric. Food Chem.* 1984, 32, 1104.
- Buttery, R. G.; Kamm, J. A.; Ling, L. C. Volatile Components of Alfalfa Flowers and Pods. *J. Agric. Food Chem.* 1982, 30, 739.
- Challice, J. S.; Williams, A. H. Phenolic Compounds of the Genus *Pyrus*-I. *Phytochemistry* 1968a, 119.

- Challice, J. S.; Williams, A. H. Phenolic Compounds of the Genus *Pyrus*-II. *Phytochemistry* 1968b, 1781.
- Chang, J. F.; Philogene, B. J. R. The Development and Behavior of the Pear Psylla, *Psylla pyricola* (Homoptera: Psyllidae) on Different Pear Rootstocks and Cultivars. *Phytoprotection* 1976, 57(3), 137.
- Chang, J. F.; Philogene, B. J. R. Response of *Psylla pyricola* (Homoptera: Psyllidae) to and Characterization of Polar and Lipid Fractions of *Pyrus* S.P. Leaves. *Phytoprotection* 1978, 59(1), 28.
- Glass, E. H. Pear psylla control studies. *NY State Agric. Exp. Stn. Res. Circ.* 1969, 16.
- Harris, M. K. Host Resistance to the Pear Psylla in a *Pyrus communis*  $\times$  *P. ussuriensis* Hybrid. *Environ. Entomol.* 1973, 2(5), 883.
- Hartman, T. G. *Characterization of Undesirable Volatile Compounds in PVC Films*; Charalambous, G., Ed.; Elsevier: Amsterdam, 1987.
- Jennings, W. G.; Leonard, S.; Panghorn, R. M. Volatiles Contributing to the Flavor of Bartlett Pears. *Food Technol.* 1960, 587.
- Kovats, E. Gas Chromatographic Characterization of Organic Substances in the Retention Index System. *Adv. Chromatogr.* 1965, 1, 229.
- Madsen, H. F.; Sisson, R. L.; Bethell, R. S. The Pear Psylla in California. *Circ.—Calif. Agric. Exp. Stn.* 1962, 510.
- Metcalf, R. L. Plant Volatiles as Insect Attractants. *CRC Crit. Rev. Plant Sci.* 1987, 5(3), 251.
- Schalk, J. M.; Ratcliffe, R. H. Evaluation of ARS Program on Alternative Methods of Insect Control: Host Plant Resistance to Insects. *Bull. Entomol. Soc. Am.* 1976, 22(1), 7.
- Westigard, P. H.; Westwood, M. N.; Lombard, P. B. Host Preference and Resistance of *Pyrus* Species to the Pear Psylla *Psylla pyricola* Forester. *J. Am. Soc. Hort. Sci.* 1970, 95(1), 34.
- Zlatkis, A.; Lichtenstein, H. A.; Tishbee, A. Concentration and Analysis of Trace Volatile Organics in Gases and Biological Fluids with a New Solid Adsorbent. *Chromatographia* 1973, 6(2), 67.

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