Oat Leaf Volatiles: Possible Insect Attractants

The volatile components of oat leaves have been isolated by both a Tenax trapping method and vacuum steam distillation continuous extraction. A total of 26 components was identified by capillary gas chromatography–mass spectrometry analysis. (*Z*)-3-Hexenyl acetate formed 94% of the volatiles when the Tenax trapping method was used. Other major components included (*Z*)-3-hexenol, (*E*)-β-ocimene, β-ionone, and nonanal. Unusual volatile components included β-cyclocitral, 2,2,6-trimethylcyclohexanone, caryophyllene, and γ-muurolene.

Many insects feed and oviposit upon host plants that are closely related. This is true for such diverse insects as the cereal leaf beetle, *Oulema melanopus* (L.), the English grain aphid, *Marosiphum avenae* (Fab.), the armyworm, *Pseudaletia unipuncta* (Haworth), and the Hessian fly, *Mayetiola destructor* (Say), which attack the leaves and stems of small grains and native grasses (Everson and Gallun, 1980). A question often posed is, how do these insects find their specific hosts amid all of the other plants in the environment? One possible way for these small grain insects to locate their hosts would be to orient toward volatile chemicals arising from these hosts. There is very little data in the literature on cereal leaf volatiles. The alkaloid gramine, 3-[(*N*-dimethylamino)-methyl]indole, is a known component of sprouting barley (Bowen and Marion, 1951). This compound can, however, have very little volatility. (*Z*)-3-Hexenol, (*Z*)-3-hexenal, (*Z*)-3-hexenyl acetate, methyl salicylate, benzyl alcohol, nonanal, and some other compounds have been identified in grass silage (Kibe and Kagura, 1976). Some of the authors had previously studied volatile compounds associated with corn (Buttery et al., 1978) and alfalfa (Buttery and Kamm, 1980) and in the present work have used similar methods to identify the volatile constituents of oat leaves.

EXPERIMENTAL SECTION

Materials. Oats (*Avena sativa*; Montezuma variety) were grown in California on an experimental plot on the grounds of the authors' laboratory. The plants were cut off at the base when they had grown to about 8–12 in. high before any seed had developed. Except for the point at which they were cut from the roots, care was taken not to damage the plants, and they were placed whole in the isolation flasks.

Isolation Using Tenax Traps. The traps as described previously (Buttery et al., 1982) were made from Pyrex glass and contained a 1.3 cm diameter by 7 cm long (1.7 g) column of Tenax GC adsorbent (60–80 mesh, Applied Science Laboratory). The whole oat plants (excluding roots, 100 g), were placed in a 5-L flask, and the activated trap was attached to the neck of the flask with standard taper joints. Purified air (500 mL/min) was drawn through the flask and then through the Tenax trap by applying reduced pressure to the outlet of the trap. The isolation was carried out for 24 h at room temperature (25 °C). The trap was then removed and the trapped material eluted with freshly distilled diethyl ether. The ether extract was then concentrated to a small volume by using a warm water bath and low hold up distillation columns.

Isolation Using Vacuum Steam Distillation Continuous Extraction. This was carried out on the whole oat plant (excluding roots) by using a Likens-Nickerson type steam distillation continuous extraction apparatus under reduced pressure (100 mmHg) in essentially the same way as described previously for whole alfalfa leaves (Buttery and Kamm, 1980) except that 1500-g quantities of whole oat leaves were used. The hexane extract obtained was concentrated as described previously for alfalfa.

Capillary Gas–Liquid Chromatography–Mass Spectrometry Analysis (GLC–MS). This was carried out on the concentrates from the Tenax trap and vacuum steam distillation. The GLC column was a 150 m long by 0.64 mm i.d. Pyrex glass capillary coated with Carbowax 20M. The GLC column was temperature programmed by holding at 50 °C for 30 min after injection and then increasing the temperature from 50 to 170 °C at 1 °C/min and holding at the upper limit. The column inlet pressure was 16 psi He. The coupling of the GLC column to the mass spectrometer (a modified Consolidated 21-620 cycloidal type) was made using a Lewellyn-Littlejohn single-stage silicone rubber membrane molecular separator. Electron ionization was 70 eV. Separate GLC–MS analyses were made on samples from four different lots of oat leaves.

Authentic chemical compounds for comparison (mass spectra and GLC Kovats indices) were generally obtained from reliable commercial sources (e.g., Aldrich Chemical Co.) or synthesized by established methods. Authentic sesquiterpenes were isolated from hop oil as described previously (Buttery et al., 1982). All compounds were repurified by gas–liquid chromatography (GLC) separation and their identities verified by spectral methods.

RESULTS AND DISCUSSION

Two different methods of isolating the volatiles from the oat leaves were used. The first involved sweeping purified air over the whole leaves and trapping the volatiles in the exit stream on a Tenax adsorbent trap. The volatiles were then eluted from the Tenax with diethyl ether, and the concentrate was used for GLC–MS analysis. The second method used vacuum steam distillation continuous extraction with hexane as the solvent, and the hexane concentrate was used for GLC–MS analysis. A discussion of the two isolation methods has already been made by some of the authors (Buttery et al., 1982).

Table I lists the results of the analysis. The present study was intended to be mainly only qualitative. Relative percentages, based on GLC peak areas, are also shown in Table I. There was some variation between samples, and these figures are only meant to give a general idea for a typical sample. With the Tenax trapping method (*Z*)-3-hexenyl acetate is by far the major constituent, forming 94% of the volatiles. Calculations showed that the (*Z*)-3-hexenyl acetate trapped amounted to 0.2 parts per million (ppm) of the oat leaves used. (*Z*)-3-Hexenol, (*E*)-β-ocimene, and β-ionone were the next major volatiles found by the Tenax trapping method. These compounds were also prominent when the vacuum steam distillation method was used. Nonanal, 1-octen-3-ol, and methyl salicylate were additional major components of the vacuum steam volatile material.

The method of isolation using Tenax trapping probably gives the best picture of what the insect is exposed to in the atmosphere around the plant. The reduced pressure

Table I. Volatile Constituents of Oat Leaves

compound[a]	Kovats GLC index[b]	rel % Tenax trap	rel % vac steam distill.
Aliphatic Aldehydes			
heptanal	1190		0.3
octanal	1290		0.3
nonanal	1390	0.1	6-25
(*E*)-2-nonenal	1530		0.3
(*E*)-2-decenal	1630		1
(*E,E*)-2,4-decadienal	1790		0.7
Aliphatic Alcohols			
(*Z*)-3-hexenol	1370	0.4	22
1-octen-3-ol	1460		11-16
octanol	1530		2
(*E*)-2-octenol	1590		0.3
nonanol	1630		3-5
Aliphatic Esters			
hexyl acetate	1270	0.3	
(*Z*)-3-hexenyl acetate	1310	94	26
Terpenoids			
myrcene	1160	0.1	
limonene	1180	0.1	0.2
(*E*)-β-ocimene	1250	0.7	1
2,2,6-trimethylcyclohexanone	1320		0.2
2-methyl-2-hepten-6-one	1340		0.3
caryophyllene	1570	0.2	
β-cyclocitral	1600		2
γ-muurolene	1655	0.05	
geranylacetone	1850		0.8
β-ionone	1920	0.5	3-5
Aromatic Compounds			
benzaldehyde	1520	0.02	
methyl salicylate	1730	0.05	11-16
p-vinylguaiacol	2160		0.2

[a] The mass spectrum (complete spectrum) and the Kovats GLC retention index of all compounds listed are consistent with that of authentic samples. Characteristic mass spectral ions were essentially the same as that reported by the authors for other products in recent publications (Buttery and Kamm, 1980; Buttery et al., 1978, 1981). [b] The Kovats GLC index for the Carbowax 20M Pyrex capillary described under Experimental Section.

used in the vacuum steam distillation isolation method may cause damage to the plant cells. This would initiate enzyme action which could give rise to some of the additional components found by using this method such as nonanol and 1-octen-3-ol. It is interesting that nonanal and nonanol have been found to stimulate germination of uredospores of *Puccinia coronata* F. sp. *avenae* which causes crown rust diseases in oats [cf. French et al. (1975)].

(*Z*)-3-Hexenyl acetate, (*Z*)-3-hexenol, and related C_6 compounds seem to be common in the green leaves of many plants [cf. Buttery and Kamm (1980), Visser and Ave (1978), and Wallbank and Wheatley (1976)]. The compounds are apparently formed from the plants' controlled oxidative breakdown of linolenic acid. The presence of the terpenes and sesquiterpenes (*E*)-β-ocimene, myrcene, caryophyllene, and γ-muurolene, as green leaf components, seems to have been much less reported although some of the authors (Buttery et al., 1982) had also identified these compounds in alfalfa leaves. The terpenes seem to be readily lost when purified air is passed over the leaves. It seems reasonable that the terpenes and sesquiterpenes are associated with the waxy layer on the surface of the leaves. Plant waxes frequently contain triterpenoids, and the above compounds may be intermediates or byproducts of the plant's synthesis of these triterpenoids.

The authors intend to eventually extend their studies to the leaves of the other cereal grain plants wheat and barley. We have already examined (GLC–MS) the vacuum steam volatile oil from barley leaves (Norwin variety) of about the same maturity as the oat leaves used in the present work. The barley leaf volatiles identified were essentually the same as those found for the oats in the present work. Benzyl alcohol, previously reported in barley leaves (Juneja et al., 1972), was not detected in the Norwin barley variety or in any of the oat leaf samples examined, although the authors had previously detected benzyl alcohol in alfalfa flowers (Buttery et al., 1982).

Tests with Insects. Electroantennogram comparisons, with the volatile compounds identified in the oat leaves, are in progress using the armyworm and cereal leaf beatle as test insects.

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Ron G. Buttery*[1]
Louisa C. Ling[1]
Stanley G. Wellso[2]

[1]Western Regional Research Laboratory
Agricultural Research Service
U.S. Department of Agriculture
Berkeley, California 94710
[2]Agricultural Research Service
U.S. Department of Agriculture
East Lansing, Michigan 48823

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