Volatiles of Wild Blueberry, *Vaccinium angustifolium*: Possible Attractants for the Blueberry Maggot Fruit Fly, *Rhagoletis mendax*

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By Tenax trapping and GC–MS procedures, 23 volatiles of the ripe fruit of the wild blueberry, *Vaccinium angustifolium*, have been identified. Electroantennograms (EAGs) have been measured for each of these compounds on the female blueberry maggot fruit fly, *Rhagoletis mendax*. Most intense EAGs were observed for benzaldehyde, ethyl 2-methylbutanoate, 2-heptanone, benzyl alcohol, and 2-nonanone. Intermediate responses were evoked by 3-octanone, methyl (*E*)-2-pentenoate, and *p*-cymene.

The wild blueberry, *Vaccinium angustifolium*, is an important commercial fruit in the northeastern United States and eastern Canada. Among the factors limiting fruit production is the blueberry maggot (blueberry fruit fly), *Rhagoletis mendax* Curran, the major insect pest of this crop. The female fly oviposits a single egg in the blueberry, and the hatched larva feed and grow within the berry, thus destroying the fruit. Since blueberry volatiles may play a role in host location by the gravid female, identification of these compounds is an essential component of elucidating the mechanisms of oviposition site selection by this insect.

In an early study utilizing only gas chromatography, it was reported that acetaldehyde, ethylene, ethanol, ethyl acetate, and methyl acetate occur as volatiles of ripening *V. angustifolium* (Hall et al., 1970). There are recent reports on the volatiles of the related high-bush blueberry *Vaccinium corymbosum* (Hirvi and Honkanen, 1983; Parliment, and Kolor, 1975), rabbiteye blueberry, *Vaccinium ashei* (Horvat and Senter, 1985), bilberry, *Vaccinium myrtillus* (Hirvi and Honkanen, 1983), and the bog blueberry, *Vaccinium uliginosum* (Hirvi and Honkanen, 1983).

In the present study, we have utilized a Tenax trapping procedure and GC–MS to identify volatiles of ripe fruit of *V. angustifolium*. Authentic samples of the identified compounds have been assayed with *R. mendax* by an electroantennogram procedure.

MATERIALS AND METHODS

Ripe fruit of *V. angustifolium* was collected in Beddington, ME, chilled over ice, and used within 2 h of collection. Charcoal-filtered air was drawn over the berries (250 g) in a glass chamber (23 cm × 9.5 cm (diameter)) and subsequently through a Tenax trap at a flow rate of 150 mL/min for 15 h. The Tenax trap consisted of a glass tube (7.5 cm × 0.4 cm (i.d.)) packed with Tenax GC (70 mg, 60/80 mesh; Alltech Industries, Inc.) and preconditioned at 350 °C with helium flow for 2 h.

GC–MS was performed on a Hewlett-Packard 5970 MSD equipped with a Hewlett-Packard 5890 gas chromatograph. A fused silica capillary column (Hewlett-Packard; 12 m × 0.2 mm (i.d.)) coated with methyl silicone (0.33-μm film) and He carrier gas at a linear velocity of 12.5 cm/s were used. EI-MS spectra of the column effluent were recorded at 70 eV and at a source temperature of 200 °C.

The Tenax trap was placed in the cooled injection port (30 °C) of the gas chromatograph in the splitless mode. The injection port was heated to 250 °C for 20 min to desorb the volatiles from the trap. The desorbed volatiles were trapped in a section of the capillary column (approximately 10 cm in length and 25 cm from the beginning of the column) immersed in a dry ice–acetone mixture. Immediately after the desorption period, the dry ice–acetone mixture was removed and the column was programmed from 30 to 250 °C at 5 °C/min.

To test for the possibility of breakthrough of volatiles, a second Tenax trap was placed in tandem with the above described trap and the same purge and desorption procedure was applied to the tandem trap. No significant breakthrough (<10%) was observed.

Identification of the effluent components was accomplished by comparison of mass spectra and retention times with those of authentic samples. Ethyl 3-methylbutanoate, ethyl 3-methyl-2-butenoate, ethyl 2-methylbutanoate, and methyl (*E*)-2-pentenoate were prepared by acid-catalyzed esterification of the appropriate acids and alcohols. The remaining compounds were obtained from Aldrich Chemical Co.

Field-collected *R. mendax* pupae were held in the laboratory in cold storage (4 °C) for 5 months before they were placed in an environmental chamber (24 °C, 16 h light:8 h dark regime) for adult emergence. Flies were held until they were 7–10 days old (sexually mature) before electroantennograms (EAGs) were conducted. Only female flies were tested.

EAGs were conducted as described by Fein et al. (1982). The entire head was excised, and a glass–Ag/AgCl electrode was inserted into the head cavity; the other electrode was slipped over the antennal tip. Test compounds were applied in pentane solutions to filter paper slips for an application dosage of 10 μg/slip for each compound. The solvent was allowed to evaporate, and the slips were placed in disposable glass Pasteur pipets (9 in.). Stimulation of antennae was performed by puffing 2 cm^3 of air, driven by syringe, over the loaded filter paper onto the antenna, a distance of 3 cm. Pentane-treated (10 μL) filter paper slips served as the control treatment.

Each compound was tested in random order, preceded and followed by antennal exposures to reference treatments. The reference compound (2-nonanone) was used to judge the acceptable responsiveness of the antennal preparation: When succeeding reference response was <50% of the preceding reference response, the preparation was discarded. Treatment responses were expressed as a ratio of the average response of preceding and succeeding reference exposures. A total of 29 flies was tested.

RESULTS AND DISCUSSION

Headspace volatiles of ripe fruit of *V. angustifolium* were trapped on Tenax, and 23 volatiles were identified by GC–MS through comparison of mass spectra and re-

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Table I. Volatiles of *V. angustifolium* and *R. mendax* EAG Responses

compound	rel RT, min	% rel	rel EAG resp ± SE[a]
ethanol	0.06	<1.0	0.03 ± 0.03
ethyl acetate	0.12	3.7	0.04 ± 0.04
ethyl propanoate	0.21	<1.0	0.04 ± 0.04
methyl 2-methylpropanoate	0.23	1.7	0.17 ± 0.09
ethyl 2-methylpropanoate	0.25	1.9	0.14 ± 0.07
methyl butanoate	0.28	1.3	0.19 ± 0.08
methyl 3-methylbutanoate	0.35	45.2	0.06 ± 0.03
methyl (*E*)-2-pentenoate	0.37	4.3	0.70 ± 0.13
ethyl butanoate	0.41	4.5	0.17 ± 0.09
methyl 3-methyl-2-butenoate	0.42	2.5	0.01 ± 0.01
ethyl 2-methylbutanoate	0.55	7.0	1.45 ± 0.55
ethyl 3-methylbutanoate	0.56	3.4	0.01 ± 0.01
2-heptanone	0.58	<1.0	1.09 ± 0.20
ethyl 3-methyl-2-butenoate	0.65	<1.0	0.32 ± 0.07
benzaldehyde	0.73	1.7	1.42 ± 0.18
3-octanone	0.76	<1.0	0.84 ± 0.12
benzyl alcohol	0.95	>1.0	1.06 ± 0.24
p-cymene	0.99	<1.0	0.62 ± 0.11
limonene	1.00	9.5	0.36 ± 0.07
2-nonanone	1.06	7.0	1.00[b]
methyl benzoate	1.08	1.3	0.37 ± 0.14
undecane	1.26	<1.0	0.40 ± 0.14
tridecane	1.70	<1.0	0.22 ± 0.09
pentane control			0.05 ± 0.03

[a] Relative to the reference compound, 2-nonanone, where a value of 1.0 indicates equal treatment response to average reference response. [b] Mean response 1.4 mV, $n = 29$ insects.

tention times with those of authentic samples. The results are presented in Table I. Of the 23 compounds identified, 13 were esters, with methyl 3-methylbutanoate (methyl isovalerate) by far the most abundant at 45.3% of the total volatiles detected. In their comparative investigation of volatiles of the fruit of bog blueberry, bilberry, and highbush blueberry, Hirvi and Honkanen (1983) found many compounds in common among the three species, with benzyl alcohol most abundant in each. We have found that benzyl alcohol appears as a minor component of the volatiles of *V. angustifolium*, but none of the esters we have observed in the present work were reported by Hirvi and Honkanen. This disparity may be due at least in part to the considerable difference in methods. Our volatiles were trapped from intact berries on Tenax and thermally desorbed, while those of Hirvi and Honkanen were obtained by concentration of an ether–pentane extract of the crushed fruit. It is also of interest that an earlier study of the *V. corymbosum*, utilizing ether extraction of the vacuum distillate of the macerated berries, demonstrated the major volatile component to be *trans*-2-hexenal and no benzyl alcohol was reported (Parliment and Kolor, 1975). Horvat and Senter (1985) reported 51 volatiles in the steam distillate of the rabbiteye blueberry (*V. ashei*), with 15 of those being the same as those found in the Hirvi and Honkanen (1983) study. Benzyl alcohol was not reported as a volatile of the rabbiteye blueberry.

Four volatiles elicited EAGs equal to or greater than the reference compound, 2-nonanone (Table I): benzaldehyde, ethyl 2-methylbutanoate, 2-heptanone, and benzyl alcohol. An intermediate level of response was evoked by 3-octanone, methyl (*E*)-2-pentenoate, and *p*-cymene; the remaining compounds induced weak or no EAGs.

In a study of the relationship between apple volatiles and apple maggot (*Rhagoletis pomonella*) fly attraction, Fein et al. (1982) determined the major volatile components eliciting directed upwind movement toward and arrival at the source. Their active compounds included hexyl acetate, (*E*)-2-hexen-1-yl acetate, propyl hexanoate, hexyl propanoate, butyl hexanoate, and hexyl butanoate in a 35:2:8:12:5:28:10 ratio. Although we did not design our study for the identification of only biologically active volatiles, it is interesting to compare the compounds of Fein et al. (1982) with ours that elicited strong EAGs, especially since the two insect species are thought to be closely related and overlap in their distribution and phenology (Diehl and Bush, 1984; Diehl and Prokopy, 1986). Fein et al. (1982) identified only esters as being biologically active. Although we found several esters as components of the blueberry effluent, only two evoked significant EAGs, and only one (ethyl 2-methylbutanoate) was identified as an apple volatile by Carle et al. (1987). Our antennally active compounds included esters, ketones, an alcohol, an aldehyde, and an aromatic, of which most are generally referred to as plant "green odor" components used by many insect species in host location (Visser, 1986).

Antennal responses alone cannot be used for umambiguous prediction of biological activity, and we are currently developing behavioral assays to determine the volatiles utilized by the female blueberry fruit fly in host location. Our reported results provide the basis for more detailed studies of the possible correlation between fruit phenology, volatile composition, and insect oviposition site selection.

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Registry No. Ethanol, 64-17-5; ethyl acetate, 141-78-6; ethyl propanoate, 105-37-3; methyl 2-methylpropanoate, 547-63-7; ethyl 2-methylpropanoate, 97-62-1; methyl butanoate, 623-42-7; methyl 3-methylbutanoate, 556-24-1; methyl (*E*)-2-pentenoate, 15790-88-2; ethyl butanoate, 105-54-4; methyl 3-methyl-2-butenoate, 924-50-5; ethyl 2-methylbutanoate, 7452-79-1; ethyl 3-methylbutanoate, 108-64-5; 2-heptanone, 110-43-0; ethyl 3-methyl-2-butenoate, 638-10-8; benzaldehyde, 100-52-7; 3-octanone, 106-68-3; benzyl alcohol, 100-51-6; *p*-cymene, 99-87-6; limonene, 138-86-3; 2-nonanone, 821-55-6; methyl benzoate, 93-58-3; undecane, 1120-21-4; tridecane, 629-50-5.

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