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OLFACTOMETER-ASSESSED RESPONSES OF APHID *Rhopalosiphum padi* TO WHEAT AND OAT VOLATILES

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Abstract—Volatiles from wheat and oat seedlings elicited attraction in apterae and alatae *Rhopalosiphum padi*. Cereal volatiles were identified by GC-MS and olfactometric tests were performed with each compound. Attraction of aphids was elicited by (*E*)-2-hexenyl acetate, (*Z*)-3-hexenol, (*E*)-2-hexenyl acetate, (*E*)-2-hexenol, (*Z*)-2-hexenol, *n*-heptanal, *n*-octanal, *n*-nonanal, *n*-decanal, benzaldehyde, and linalool. The difference between the sensory capacity of alatae and apterae is discussed in relation to migrations between hosts during their life cycle.

Key Words—Odors, semiochemicals, green leaf volatiles, cereals, Hemiptera, Aphididae.

INTRODUCTION

Several studies have demonstrated a role for olfactory cues in host-plant selection by aphids prior to landing. Thus, attraction by host-plant odors was shown in *Aphis fabae* (Alikhan, 1960; Nottingham *et al.*, 1991), *A. gossypii* (Pospisil, 1972), and *Brevicoryne brassicae* (Pettersson, 1973) and *Cryptomyzus korschelti* showed positive upwind anemotaxis mediated by host-plant odors (Visser and Taanman, 1987). The chemicals responsible for the effects reported were not identified.

The bird-cherry-oat aphid, *Rhopalosiphum padi* (L.) (Hemiptera, Aphididae), is a holocyclic host-alternating species in temperate climates: bird-cherry, *Prunus padus* L. (Rosaceae), is the winter or primary host and a wide range of cereals and grasses (Poaceae = Gramineae) are its summer or secondary hosts.

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Odors play a role in this host alternating behavior. Thus, autumn migrants from the secondary host are attracted by odors from the winter host (Pettersson, 1993), the aggregation of *R. padi* colonies on *P. padus* is disrupted during the spring by methyl salicylate produced by the host (Pettersson *et al*, 1994), and parthenogenetic generations on the secondary host are attracted by host-plant odors (Quiroz *et al.*, 1997).

This report enquires into the composition of volatiles emitted by two summer hosts of *R. padi*, wheat and oat, and the behavioral responses they elicit in alatae and apterae *R. padi* in an olfactometer.

METHODS AND MATERIALS

Aphids. Aphids to start the cultures were collected in grass fields near the Laboratorio de Química Ecológica in Santiago, Chile. Cultures were kept on oat (*Avena sativa* L. cv. Nahuén) in a growth room at 18–22°C and light regime of 18L:6D. Winged forms were produced by wilting the oat plants by dehydration. In order to get individuals in an homogenous "searching behavioral mood," both alate and apterous morphs of similar size were collected from the cage walls and removed from the culture one hour before the experiment.

Plant Materials. Wheat (*Triticum aestivum* L. cv. Ciko) and oat seedlings were grown in a growth room at 18–22°C, light regime of 12H:12D, light intensity of 200 μmol photons/cm^{-2}/min and 45–65% relative humidity. Seedlings at growth stage 12 (Zadoks et al., 1974) were used for air entrainment experiments.

Chemicals. Samples used as stimulus in the olfactometer were purchased from Aldrich Chem. Co. and diluted in hexane before use.

Olfactometry. Behavioral studies were performed in an olfactometer as described by Pettersson (1970). One aphid was enclosed in an observation quadratic arena permeated by air (250 ml/min) coming from each of its four stretched-out corners and drawn out through a hole above its center. The treatment chemical was placed inside a test tube connected to the end of one of the arms; tubes with the control odors were connected to the other three arms. The pot with treatment seedlings was placed inside a bell jar connected to one of the olfactometer arms, and an empty control bell jar was connected to the other three arms. The carrier air was purified by passage through a device containing activated charcoal. The observation arena was divided into four arm zones and one indifferent zone in the center. The time the aphid spent in each arm was recorded during 15 min with the olfactometer being rotated every minute. Each experiment was replicated 10 times and results were analyzed using nonparametric statistics (Wilcoxon one-tailed rank-sum test for two groups), the total time spent in the treatment arm being compared with the mean time spent in the control

arms. An index was defined (equal to the ratio between time spent by the test aphid in the stimulus arm over time spent in the control arm) that reflected the behavioral effect of the stimulus, an index higher than 1 indicating attractiveness and an index lower than 1 indicating repellence.

Entrainment of Volatiles. Air was dried and purified by passage through activated 5 molecular sieves and charcoal, and drawn for 48 hr at 1 liter/min through two bell jars containing the odor sources (treatment and control). The treatment consisted of an intact plant in a plastic pot with soil and the control only a pot with soil. Volatiles were absorbed onto Porapak Q inside containers placed at the outlets of each bell jar and were desorbed by elution with freshly distilled ethyl ether (Blight, 1990). The resulting extract was concentrated under a stream of nitrogen and stored in seal ampoules at −20°C prior to analysis. Extracts or pure compounds (10 ng) diluted in hexane were applied onto pieces of Whatman No. 1 filter paper (2 cm^2), which were then placed inside the test tubes to be connected to the olfactometer arms.

Coupled Gas Chromatography—Mass Spectrometry. Two capillary GLC columns were used at different times: 25 m × 0.25 mm ID CBP20 and 25 m × 0.20 mm ID HP-1. The columns were directly coupled to a mass detector and an integrated data system (GC model HP-5890, MD model HP-5972). Ionization was electron impact at 70 eV and 280°C. The GC oven was maintained at 35°C for 5 min and then programmed to increase to 5°C/min to 200°C.

Each identified compound was quantified by interpolation from calibration curves obtained using four different dilutions (1, 25, 50, and 100 ng/μl) and docosane as internal standard. Quantitations were repeated three times on three different plant volatile extracts (standard errors below 10%).

Volatility of Pure Compounds in Olfactometer. Two series of samples of pure compounds (*ca.* 1 and 10 mg) were studied. Samples were applied onto pieces of Whatman No. 1 filter paper (2 cm^2) which were then placed inside test tubes to be connected to one of the arms of the olfactometer. Air was allowed to flow at 250 ml/min for 15 min. The filter paper was then removed and weighed. Three independent determinations were performed for each compound in each series. Average percentage vaporization did not differ between the series for every compound tested (Student's test). The values reported in Table 2 below correspond to those obtained using 1 mg of each compound.

RESULTS

The volatiles released from a group of 20 intact wheat seedlings and 20 intact oat seedlings elicited attraction in both alatae and apterae of *R. padi* in the olfactometer (Table 1, entries 1 and 5). Air entrainments of wheat and oat seedlings produced extracts whose constituents were identified by gas chroma-

TABLE 1. RESPONSE OF *Rhopalosiphum padi* APTERAE AND ALATAE TOWARDS OAT AND WHEAT VOLATILES IN OLFACTOMETER TESTS

	Apterae			Alatae		
Stimulus applied	Mean time spent in each arm (min)	Ratio stim./control	*P*	Mean time spent in each arm (min)	Ratio stim./control	*P*
20 Oat seedlings	4.71 ± 0.76	2.03	0.011	4.55 ± 0.86	2.26	0.0081
Blank	2.31 ± 0.83			2.01 ± 0.97		
Identified volatiles combined in the same ratio as released by oat (6.7 ng)[a]	3.56 ± 0.48	1.47	0.037	3.97 ± 0.69	1.31	0.045
Hexane	2.41 ± 0.63			3.02 ± 0.17		
Volatiles attractive to apterae combined in the same ratio as released by oat (4.5 ng)[a]	4.23 ± 0.54	1.20	0.047			
Hexane	3.53 ± 0.14					
Volatiles attractive to alatae combined in the same ratio as released by oat (1.5 ng)[a]				3.90 ± 0.44	1.31	0.045
Hexane				2.98 ± 0.42		

20 Wheat seedlings	4.30 ± 0.91			3.91 ± 0.81		
		1.72	0.007		1.82	0.049
Blank	2.50 ± 0.75			2.15 ± 0.85		
Identified volatiles combined in the same ratio as released by wheat (3.4 ng)[a]	3.95 ± 0.38			3.65 ± 0.41		
		1.24	0.045		1.20	0.045
Hexane	3.18 ± 0.21			3.04 ± 0.16		
Volatiles attractive to apterae combined in the same ratio as released by wheat (2.6 ng)[a]	3.83 ± 0.19					
		1.22	0.045			
Hexane	3.15 ± 0.51					
Volatiles attractive to alatae combined in the same ratio as released by wheat (0.6 ng)[a]				3.49 ± 0.86		
					1.16	0.151
Hexane				3.02 ± 0.26		

[a] Amount of volatiles released during 15 min from 20 seedlings of wheat or oat.

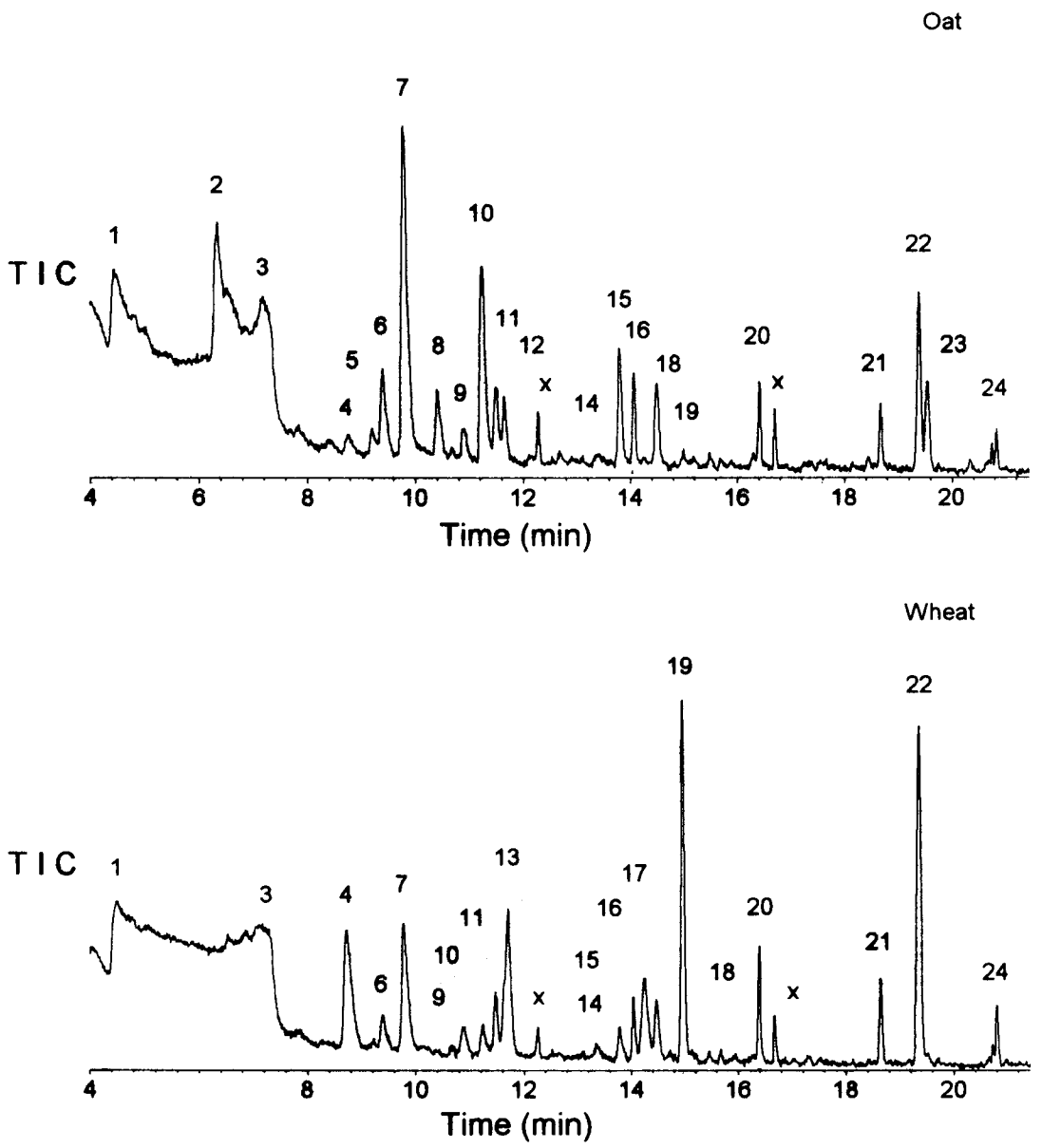

FIG. 1. Gas chromatographic analysis of Porapak-trapped volatiles from 20 intact wheat or oat seedlings using a 25-m × 0.25-mm ID CBP20 capillary column. Conditions are described in the Methods and Materials section. Peaks are identified in Table 1. *X* = compounds bleeding from the column.

tography coupled to mass spectrometry (Figure 1 and Table 2). Compounds were considered positively identified if peak enhancement occurred by coinjections with standard compounds, and their mass spectra and GLC Kovats indices were consistent with those from authentic samples analyzed under the same conditions. The average total release rates of volatiles were 330 and 641 ng/day/20 seedlings, for wheat and oat, respectively. Olfactory responses of apterae and alatae towards each of the volatile chemicals identified in the extract were

assessed in an olfactometer (Table 2). Volatile compounds that elicited attraction in apterae were (*Z*)-3-hexenyl acetate, (*E*)-2-hexenyl acetate, (*E*)-2-hexenol, (*Z*)-2-hexenol, *n*-heptanal, *n*-octanal, *n*-nonanal, *n*-decanal, benzaldehyde, and linalool, while volatile compounds which elicited attraction in alatae were (*E*)-2-hexenyl acetate, (*Z*)-3-hexenol, (*E*)-2-hexenol, and benzaldehyde. While most green leaf volatiles (GLV) and aldehydes were active, alkenes were inactive. All compounds showed volatilities above 40% in the olfactometer. Mixtures were produced in order to emulate natural host odors. In them, either all identified compounds or those active in the olfactometer were mixed in the average proportion found in the air entrainments from cereal seedlings. An amount of each mixture equivalent to that released by 20 wheat seedlings or 20 oat seedlings over a 15-min period elicited attraction in the olfactometer (Table 1, entries 2 and 6). Mixtures of volatiles individually eliciting attraction in alatae or apterae combined in the same ratio as released by the respective host plant elicited, with one exception (Table 1, entry 8), attraction in olfactometer tests (Table 1, entries, 3, 4 and 7).

DISCUSSION

The compounds identified belonged to five different categories: (1) green leaf volatiles, C-6 compounds arising from enzymic transformations of linolenic and linoleic acids (Hatanaka, 1993); (2) long-chain alkanes commonly present in plant epicuticular waxes; (3) common terpenoid plant constituents, such as linalool and camphor, (4) benzaldehydes, also common plant constituents, and (5) indene and naphthalene, two common environmental pollutants (Manahan, 1994). The present composition of cereal volatiles differs from those given in earlier reports for oat (Buttery et al., 1982) and wheat volatiles (Hamilton-Kemp and Andersen, 1984; Buttery et al., 1985). Since severed plants were used in the above investigations, some of the compounds reported may correspond to enzymic breakdown products of plant constituents (Schwimmer, 1981). The total amount of volatiles collected in the present work is on the order of 330 and 640 ng/day from ca. 10 g of fresh wheat and oat material respectively (Table 1). These amounts are comparable to those reported previously in the case of wheat (100–500 ng/day from 10 g of wheat leaves) (Buttery et al., 1985), but smaller than in the case of oat (ca. 2000 ng/day from 10 g of oat leaves) (Buttery et al., 1982). This latter differences may be ascribed to the age of the plants used; lower volatile production is expected from the younger plants used in the present work.

Most of the compounds identified were inactive in the olfactometer. Since the lack of activity of a given compound may be ascribed to a lack of volatility, the rate of vaporization of each compound was determined under the olfac-

TABLE 2. VOLATILES RELEASED FROM *Triticum aestivum* CV. CIKO AND *Avena sativa* CV. NAHUÉN AND THEIR EFFECT IN AN OLFACTOMETER TOWARDS *Rhopalosiphum padi* APTERAE AND ALATAE

Peak No.[a]	Compound	GLC Kováts index		Release rate (ng/day/20 seedlings)		Vaporization percentage in 15 min in the olfactometer	Olfactometer response (stimulus/control)[b]		Ratio of active volatiles released (oat/wheat)[c]
		CBP20	HP-1	Wheat	Oat		Alatae	Apterae	
GLVs									
1	*n*-Hexanal	1074	773	60.1	166.5	97	ns	1.3***	2.8
3	(*E*)-2-Hexenal	1214	809	32.1	75.0	97	ns	ns	
6	(*Z*)-3-Hexenyl ac.	1305	988	7.3	23.1	99	ns	2.0*	3.2
7	(*E*)-2-Hexenyl ac.	1323	995	24.0	81.6	83	2.3***	2.5*	3.4
8	(*E*)-3-Hexen-1-ol	1351	825		26.6	96	ns	ns	
9	(*Z*)-3-Hexen-1-ol	1371	831	6.4	11.1	98	1.8**	ns	1.7
11	(*E*)-2-Hexen-1-ol	1393	850	14.3	25.0	71	1.8**	1.9*	1.7
13	(*Z*)-2-Hexen-1-ol	1401	855	38.7		88	ns	1.7*	
Other components									
2	*n*-Heptanal	1172	902		40.3	95	ns	1.7***	
4	*n*-Octanal	1280	982	34.6	3.0	99	ns	1.5*	0.09

5	Tridecane	1301	1302		6.4	85	ns	ns	
10	*n*-Nonanal	1382	1081	5.3	44.6	60	ns	1.7*	8.4
12	Tetradecane	1402	1400		21.2	70	ns	ns	
15	*n*-Decanal	1489	1181	4.5	17.9	68	ns	1.4*	3.9
16	Pentadecane	1502	1501	7.3	22.1	60	ns	ns	
17	Camphor	1507	1121	9.4		81	ns	ns	
18	Benzaldehyde	1519	940	13.4	21.9	96	1.5*	1.3***	1.6
19	Linalool	1536	1083	43.4	4.0	74	ns	1.6***	0.09
20	Hexadecane	1605	1601	11.9	16.9	50	ns	ns	
21	Heptadecane	1702	1700	8.9	16.0	48	ns	ns	
23	4-Ethybenzaldehyde	1739	1147		9.6		ns	ns	
24	Octadecane	1801	1805	5.6	8.2	50	ns	ns	
Contaminants									
14	Indene	1472	1025	5.1	2.7	94	ns	ns	
22	Naphthalene	1731	1159	52.1	36.7	62	ns	ns	

[a] Peak numbers as in Figure 1.
[b] $P < 0.05$** $P < 0.01$*** $P < 0.001$ ns = non significant differences.
[c] These values were calculated from data in columns 5 and 6.

tometer conditions. All values were above 40% (Table 1), indicating that all compounds were sufficiently volatile to be present in the airstream of the olfactometer.

The activities in the olfactometer of artificial mixtures of volatile compounds (complete set of identified compounds or mixtures of compounds eliciting attraction in alatae and apterae) in the same quantities and proportions as released by oat and/or wheat seedlings were qualitatively the same as the plant headspace extracts (Table 1). However, the attraction elicited by the headspace extracts tended to be higher than the artificial mixtures (with the possible exception of Table 1, entry 8, which did not elicit a significant response). This suggests that the natural sources of volatiles (wheat and oat seedlings) emit other volatile(s) in quantities that were not detected by the method used and that either synergize the effect of other compounds present or elicit by themselves an attraction response.

Alatae and apterae showed different sensitivities toward volatiles from their host plants (Table 2). Electroantennographic studies on other aphid species have shown that different morphs may differ in their capacity to detect volatile semiochemicals. For example, while the antennal receptor system of alatae and apterae of *Sitobion avenae* (Fabr.) responded to alcohols containing six to seven carbon atoms, green leaf volatiles, and benzaldehyde, higher electroantennnographic responses were found for alatae (Yan and Visser, 1982), and in the aphid *Nasonovia ribis-nigris* different electroantennographic activities between alatae and apterae were found when the stimulus was hexanol or (*Z*)-3-hexen-1-ol (van Giessen et al., 1994).

The fact that attraction in *R. padi* alatae was elicited by four compounds and by 11 compounds in apterae suggests a basic difference in olfactory responses of the two morphs. We suggest that this olfactory specialization is in part related with the type of migration performed by each morph. Following take-off from a plant, flying alatae would need to be able to discriminate between a greater range of plant species than apterae, which tend to migrate locally within a reduced range of hosts (Wiktelius, 1989). Hence, alatae would improve their host-finding efficiency either by being tuned to specific volatile cues or, alternatively, to a set of common volatile cues in a given proportion. The results in Table 1 support this later alternative. Thus, while the ratios of ''alatae-active'' compounds in oat and wheat vary within a rather narrow range (between 1.5 and 3.4), the range of variation of ratios for ''apterae-active'' compounds is considerably larger [from 0.09 to 0.84 or more, as in the case of (*Z*)-2-hexen-1-ol and *n*-heptanal which were not even found in wheat extracts].

The results presented point to the involvement of olfactory cues in the early stages of host location by *R. padi*. The final choice of host will depend on the specificity of internal plant constituents assessed during probing (Niemeyer, 1990; Tjallingii, 1995).

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