

SEMIOCHEMICALS MEDIATING SPACING BEHAVIOR
OF BIRD CHERRY-OAT APHID, *Rhopalosiphum padi*
FEEDING ON CEREALS

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Abstract—Olfactometry using an apterous individual of *Rhopalosiphum padi* (L.) showed an arresting effect by volatiles from a wheat seedling and a repellent effect by volatiles from a wheat seedling infested with aphids at a high population density (ca. 9 aphids/cm²). Four compounds, 6-methyl-5-hepten-2-one, (–)- and (+)-6-methyl-5-hepten-2-ol, and 2-tridecanone, were identified by GC-MS in air entrainments from the wheat seedlings with high aphid density but not from the wheat seedlings alone. The mixture of the four compounds in the natural proportion counteracted the attractivity of the volatiles from the intact uninfested wheat seedling. The likely role of these compounds in the spacing behavior of this aphid species, when present in high densities on wheat, is discussed.

Key Words—Spacing pheromones, olfactometry, aphids, wheat.

INTRODUCTION

The bird cherry-oat aphid, *Rhopalosiphum padi* (L.) (Homoptera: Aphididae), behaves as a holocyclic species with alternating winter and summer hosts in the most temperate areas of its distribution. The winter primary host is *Prunus padus* L. (Rosaceae), and several Gramineae (= Poaceae) may serve as secondary

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summer hosts. Volatile semiochemicals have been demonstrated as mediating several key processes in the behavioral ecology of *R. padi*. Thus, during the autumn migration from Gramineae, the gynoparae react positively to primary host-plant semiochemicals (Pettersson, 1970a), and a pheromone associated with the gynoparae is possibly involved in the settling of this morph in the canopy of *P. padus* (Pettersson, 1993). The sex pheromone isolated from several aphid species (Pickett et al., 1992) is attractive towards males of *R. padi* and is synergized by benzaldehyde, a major volatile in *P. padus*. Furthermore, the volatile primary host-plant component, methyl salicylate, switches off during the spring, with the aggregation odor stimulus keeping the colonies of fundatrices together on *P. padus* (Pettersson, 1994).

Laboratory experiments have shown that the attraction of *R. padi* to oat, one of its graminaceous summer hosts, is mediated by plant volatiles (Quiroz and Niemeyer, 1998a). Mobility of apterae of *R. padi* within the secondary summer hosts has been examined, and a density dependent deaggregation behavior was attributed to volatiles emitted by the aphid-plant complex (Pettersson et al., 1994, 1995). This work extends the preliminary study on the relationship between density and mobility of *R. padi* (Pettersson et al., 1995), with the aim of identifying compounds involved in the mutual repellence of aphids present at high densities on wheat seedlings.

METHODS AND MATERIALS

Aphids. Aphids for starting the cultures were collected in grass fields near the Laboratorio de Química Ecológica in Santiago, Chile, and maintained on wheat or oats for at least five generations in a greenhouse at 18–22°C, a 18L:6D photoperiod, and 50–70% relative humidity. All individuals used in the experiments had recently molted to adults and were in an active food site-searching mood. Additionally, experiments were performed in the middle of the day since a diurnal rhythm in the readiness of an aphid to respond to olfactory stimuli may also give rise to variation among individuals.

Olfactometry. Behavioral assays were performed in an olfactometer originally described by Pettersson (1970b). One aphid was enclosed in an arena permeated by air coming from each of its four outstretched arms and drawn out through a hole above the center of the arena. The test stimulus was applied to a filter paper that was placed in the stimulus arm of the olfactometer. Equivalent amounts of solvent were placed on filter papers in the control arms. The aphid was constantly under observation for 15 min, and the time spent in each arm was noted. The olfactometer rotated every minute. Each experiment was replicated 10 times, and results were analyzed using nonparametric statistics (Wil-

coxon 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.

Entrainment of Volatiles. Volatiles were entrained from two different sources: wheat (*Triticum aestivum* L. cv. Ciko) seedlings at growth stage 12 (Zadoks et al., 1974), and wheat seedlings with apterae of *R. padi* at high density (9 aphids/cm²). Air, dried and purified by passage through activated 5 Å molecular sieves and charcoal, was drawn at 1 liter/min for 48 hr through two bell jars containing the odor sources. Volatiles were adsorbed onto Porapak Q and were desorbed by elution with freshly distilled diethyl ether (Pettersson et al., 1994). The resulting extract was concentrated under a stream of nitrogen and stored in sealed glass ampoules at -20°C prior to analysis. Aliquots of extract used in olfactometry contained between 10 and 60 ng of the repellent semiochemicals identified.

Coupled Gas Chromatography-Mass Spectrometry (GC-MS). Volatile extracts obtained as above were injected into a capillary GC column (CBP20, 25 m × 0.25 mm ID) directly coupled to a mass detector with an integrated data system (GC model HP5890, MD model HP5972). Ionization was by electron impact at 70 eV and 280°C. The GC oven was maintained at 35°C for 5 min and then programmed to increase at 5°C/min to 200°C. Tentative identifications by GC-MS were confirmed using Kovats indices and coinjection of authentic samples when available. The pure chemicals (>99% purity) were obtained from Aldrich and diluted in hexane for behavioral assays. Chromatograms were recorded as total ion current (TIC) in time (minutes).

Determination of Enantiomeric Composition of 6-Methyl-5-hepten-2-ol. One of the compounds identified in entrainments from wheat seedlings infested with a high density of aphids was 6-methyl-5-hepten-2-ol. The proportion of enantiomers present in the extract was determined by esterification of the commercial racemate of the alcohol with (1*S*)-(-)-camphanic chloride (Aldrich Chem. Co.) to obtain the diastereomeric esters, microesterification of the extract with (1*S*)-(-)-camphanic chloride, and quantification of the peaks generated by calibration lines produced with the pure diastereomeric esters. Pure enantiomeric alcohols were generated by hydrolysis of the corresponding diastereomeric esters (Quiroz and Niemeyer, 1998b).

RESULTS

Summer generation apterae of *R. padi* were attracted in the olfactometer towards volatiles from an undamaged wheat seedling and were repelled by a wheat seedling with a high aphid density (Table 1). Entrainment volatiles produced by these systems were attractive and repellent towards apterae of *R. padi*, respectively (Table 1).

TABLE 1. RESPONSE OF APTERAE OF *R. padi* INTRODUCED SINGLY INTO AN OLFACTOMETER TO UNINFESTED WHEAT SEEDLINGS, WHEAT SEEDLINGS INFESTED WITH APHIDS, AND ENTRAINMENTS OF AIR ABOVE WHEAT SEEDLING AND WHEAT SEEDLING INFESTED WITH APHIDS

Stimulus applied	Average time spent in each arm (min)	P
Wheat seedling	5.11 ± 0.16	0.0014
Blank	2.78 ± 0.13	
Wheat seedling with a high density of apterous <i>R. padi</i> (9 aphid/cm ²)	2.13 ± 0.20	0.0018
Wheat seedling	4.24 ± 0.06	
Volatiles from wheat seedling (10 μl)	5.35 ± 0.15	0.0015
Solvent (10 μl)	2.78 ± 0.05	
Volatiles (10 μl) from wheat seedling with high density of apterous <i>R. padi</i> (9 aphids/cm ²)	2.17 ± 0.21	0.0018
Volatiles from wheat seedling (10 μl)	4.17 ± 0.07	

Figure 1 shows the GC-MS analysis of an entrainment sample from a wheat seedling with high aphid density and the identity of compounds present. Peaks labeled A, B, and C, were present only in entrainments from wheat seedlings with aphids at high population density. These compounds were identified by

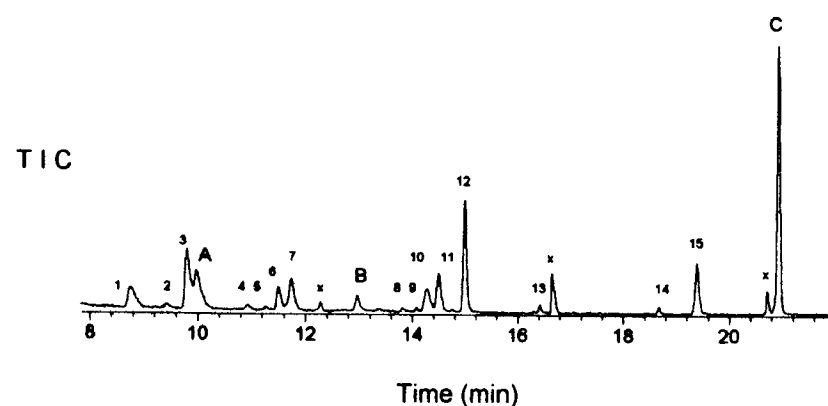


FIG. 1. Trace from the gas chromatographic run of air entrainments from a wheat seedling infested with a high density of aphids. Identification of peaks is as follows: 1, octanal; 2, (*Z*)-3-hexenyl acetate; 3, (*E*)-2-hexenyl acetate; A, 6-methyl-5-hepten-2-one; 4, (*Z*)-3-hexenol; 5, nonanal; 6, (*E*)-2-hexenol; 7, (*Z*)-2-hexenol; B, 6-methyl-5-hepten-2-ol; 8, decanal; 9, pentadecane; 10, camphor; 11, benzaldehyde; 12, linalool; 13, hexadecane; 14, heptadecane; 15, naphthalene; 16, octadecane; C, 2-tridecanone.

coupled GC-MS as 6-methyl-5-hepten-2-one (peak A, MHO), 6-methyl-5-hepten-2-ol (peak B, MHOH), and 2-tridecanone (peak C, 2-T). MHOH consisted of the (+) and the (-) enantiomers in a 1:3 ratio.

Identifications were confirmed with Kovats indices and peak enhancement on coinjection with authentic commercial samples. The absence of these compounds in entrainments from uninfested wheat seedlings was confirmed by single ion monitoring (SIM) in the corresponding regions of the chromatogram using characteristic or abundant ions from MHO (111, 83, 69, 55), MHOH (110, 69, 55), and 2-T (183, 85, 71). These ions were absent from the SIM runs.

In the olfactometer, MHO, MHOH [1:3 mixture of (+) and (-) enantiomers] and 2-T were repellent to apterae of *R. padi* either as pure compounds or as a mixture in the proportions occurring in the natural samples (Table 2) or when combined as the stimulus with an undamaged wheat seedling (Table 3). The compounds also exhibited dose-dependent repellent activity with respect to a blank (Figure 2).

DISCUSSION

Previous investigations have demonstrated that walking apterae of *R. padi* avoid oat plants with aphid colonies above a certain density (Pettersson et al., 1995). Olfactometer experiments were designed to confirm these observations using wheat as the aphid host plant. Odors released from a wheat seedling

TABLE 2. RESPONSE OF APTERAE OF *R. padi* INTRODUCED SINGLY INTO AN OLFACTOMETER AND EXPOSED TO CHEMICALS PRESENT IN AIR ENTRAINMENT FROM WHEAT SEEDLINGS WITH APHIDS IN HIGH DENSITY

Stimulus applied*	Average time spent in each arm (min)	P
0.01 μ g of MHO Hexane	2.48 \pm 0.77 3.73 \pm 0.60	0.0375
0.01 μ g of MHOH [1:3 mixture of (+) and (-) enantiomers] Hexane	2.71 \pm 0.16 3.52 \pm 0.26	0.008
0.01 μ g of 2-T [†] Hexane	2.61 \pm 0.07 3.85 \pm 0.21	0.045
0.01 μ g of MHO, MHOH [1:3 mixture of (+) and (-)-enantiomers], and 2-T in ratio 3:1:10 Hexane	2.11 \pm 0.44 3.97 \pm 0.48	0.005

*MHO = 6-methyl-5-hepten-2-one; MHOH = 6-methyl-5-hepten-2-ol; 2-T = 2-tridecanone.

TABLE 3. RESPONSE OF APTERAE OF *R. padi* INTRODUCED SINGLY INTO AN OLFACTOMETER TO WHEAT SEEDLING AND WHEAT SEEDLING PLUS DIFFERENT CHEMICALS CONTAINED IN A GLASS CAPILLARY TUBE PLACED INSIDE BELL JAR CONTAINING WHEAT SEEDLING

Stimulus applied ^a	Average time spent in each arm (min)	P
Undamaged wheat	5.22 ± 0.23	0.0013
Blank	2.49 ± 0.11	
Undamaged wheat + 10 µg of MHO	2.17 ± 0.09	0.0051
Undamaged wheat	4.46 ± 0.14	
Undamaged wheat + 10 µg of MHOH [1:3 mixture of (+) and (-) enantiomers]	2.36 ± 0.08	0.015
Undamaged wheat	5.01 ± 0.85	
Undamaged wheat + 10 µg 2-T	2.30 ± 0.20	0.045
Undamaged wheat	4.11 ± 0.18	
Undamaged wheat + 10 µg of MHO, MHOH [1:3 mixture of (+) and (-) enantiomers], and 2-T in ratio 3:1:10	2.16 ± 0.09	0.0015
Hexane	5.15 ± 0.85	

^aMHO = 6-methyl-5-hepten-2-one; MHOH = 6-methyl-5-hepten-2-ol; 2-T = 2-tridecanone.

without aphids attracted apterae of *R. padi*, and the odors released by the plant infested with a high density (9 aphids/cm²) of apterous *R. padi* repelled apterae of *R. padi*. Similar effects were obtained with air entrained from the systems MHO, MHOH [1:3 mixture of (+) and (-) enantiomers], and 2-T were found in entrainments from infested wheat but not from wheat alone. These compounds, either alone or in the natural mixture, elicited a repellent response similar to that shown by the volatiles released from wheat with a high aphid density, suggesting that they are the main active compounds in the volatile extract.

The present work does not establish the origin of the active compounds, i.e., whether they are released by the plant or by the aphid. Neither are the causes of the release addressed, i.e., a defense of the plant against high aphid infestation or a reaction of aphids to overcrowding and hence to overexploitation of its food source. The presence of any of these three compounds is rather frequent both in plants as well as in some animals, although seldom have their roles as semiochemicals been demonstrated. For example, MHO has been

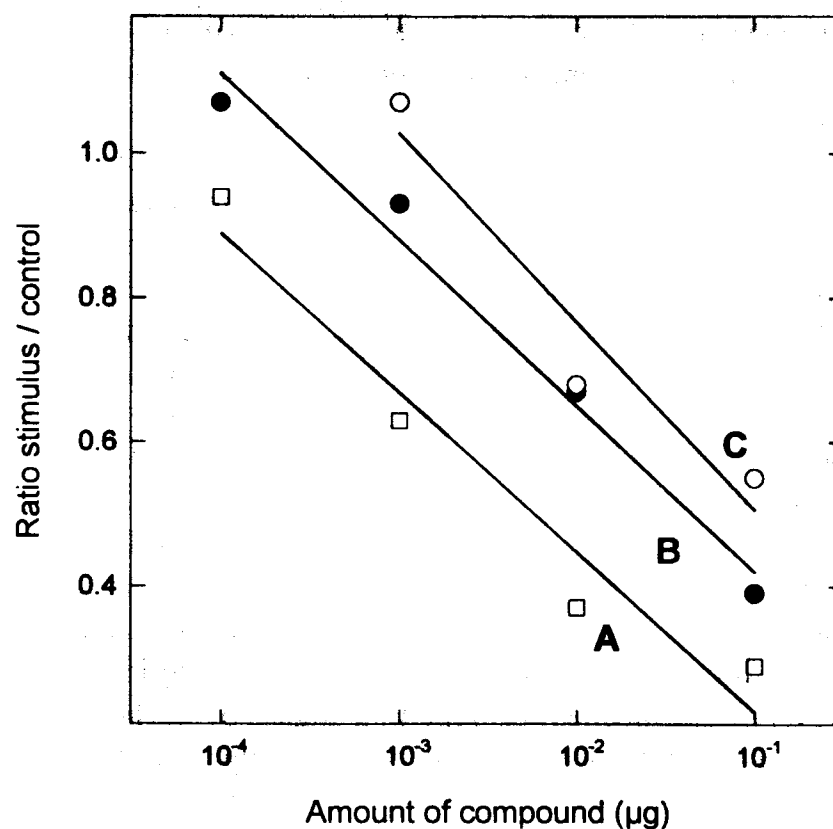


FIG. 2. Dose-response curves for the olfactometric activity of 6-methyl-5-hepten-2-one (A), 6-methyl-5-hepten-2-ol [1:3 mixture of (+) and (-) enantiomers] (B), and 2-tridecanone (C). Ratios smaller than 1 indicate a repellent effect of the stimulus.

reported as a defense compound released by bees (Davies and Madden, 1985; Hefetz et al., 1982; Tomalski et al., 1987; Wittmann et al., 1990; Voelkl et al., 1994), as an ant alarm pheromone that can be used by spiders as a kairomone (Allan et al., 1996), and as a sexual and spacing pheromone in the aphid hyperparasitoid *Alloxysta victrix* (Micha et al., 1993). MHOH has been reported as a population aggregation pheromone released by the beetle *Gnathotrichus sulcatus* (Byrne et al., 1974). The third substance, 2-T, an insect repellent of wide activity (Eisner et al., 1972), is present in secretions of several species of ants (do Nascimento et al., 1993) and bees (Fisher et al., 1993) but is also secreted by the glandular hairs of the wild tomato, *Lycopersicon hirsutum* f. *glabratum* (Solanaceae), and is toxic towards the aphid *Aphis gossypii* (Williams et al., 1980). It seems likely that in the present study the heptenone and the heptenol are emitted by the aphid and the 2-tridecanone is released by the plant. However, further studies are necessary to determine the origin of these substances.

The ecological effect of this spacing semiochemical complex is likely to be important as a colony density regulation mechanism in *R. padi*. The behavioral expression of this complex is defined in the present laboratory system as an increase in mobility (walking) by aphid individuals. In a natural environment,

other related consequences may be a higher sensitivity to disturbance (colony mates, wind, etc.) and prolonged time between probing impulses.

Application of the semiochemicals identified here in crop fields may have various effects. The first might be to repel aphids towards places where the semiochemicals have not been sprayed, which in turn would reduce the aphid population in the crop. A second is that increased mobility could increase exposure of affected aphids to natural enemies and the risks of disorientation and accidents. Another is that time spent on walking as an adult could lead to a reduction in the time available for producing offspring on a plant. Finally, the aphid population could be distributed more evenly between plants in the stand, leading to a dilution of the stress on individual plants.

It is interesting to note that MHO has been described in the aphid hyperparasitoid *Alloxysta victrix* (Westwood) (Hymenoptera: Alloxystidae) as a sex and spacing pheromone (Micha et al., 1993) and as a semiochemical that disperses females of the aphid parasitoid *Aphidius uzbekistanicus* (Luzhetski) (Hymenoptera: Aphidiidae) (Höller et al., 1994) and also in the related species *Alloxysta brevis* as a semiochemical repelling attacks by the honeydew-collecting ant *Lasius niger* (Voelkl et al., 1994). Our finding that this compound also constitutes a spacing semiochemical in aphids points to it as a key element in the regulation of the predator-prey relationship in this multitrophic chain. The different sensitivity to this compound of the members of this chain may provide a unique mechanism for pest control in the sustaining crop.

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