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SEX ATTRACTANT PHEROMONE OF DAMSON-HOP APHID *Phorodon humuli* (HOMOPTERA, APHIDIDAE)

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Abstract—Behavioral studies using an olfactometer demonstrated that sexual females (oviparae) of the damson-hop aphid, *Phorodon humuli*, release a pheromone to which males respond. Volatiles produced by the oviparae were analyzed by coupled gas chromatography–single cell recording from the secondary rhinaria on the male antenna and showed the presence of one peak with major activity. Coupled gas chromatography–mass spectrometry suggested a nepetalactol, which was shown to have the 4a*R*, 7*S*, 7a*S* stereochemistry by synthesis from the corresponding nepetalactone isolated from the labiate plant *Nepeta mussinii*. Although the stereochemistry at carbon-1 is not yet established, a synthetic sample comprising ca. 70% 1*S* and 30% 1*R* attracted highly significant numbers of males to water traps placed within and adjacent to a hop garden. Initial studies also indicated attraction of males in both the olfactometer and in the field by volatiles from the primary host.

Key Words—Pheromone, sex, attractant, aphid, *Phorodon humuli*, Homoptera, Aphididae, nepetalactol, monoterpenoid, electrophysiology, single cell recording, mass spectrometry.

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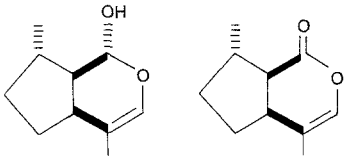
INTRODUCTION

Aphids reproduce asexually on their host plants during the summer but many species migrate to winter, or primary, hosts where sexual reproduction occurs. Pettersson (1970, 1971) demonstrated that sexual females (oviparae) of *Schizaphis* spp. attract males by mean of a sex pheromone released from the hind tibiae. Marsh (1972) also demonstrated a sex pheromone for the vetch aphid *Megoura viciae*, and this was recently identified as a synergistic mixture of (−)-(1*R*,4a*S*,7*S*,7a*R*)-nepetalactol (I) and (+)-(4a*S*,7*S*,7a*R*)-nepetalactone (II) (Dawson et al., 1987, 1989).



I II

SCHEME 1.

Electrophysiological recordings from single olfactory cells (SCR) within the secondary rhinaria on the third antennal segments of the male, coupled with gas chromatography (GC), facilitated identification. Two cell types could be distinguished as responding preferentially to compound I or II. The same compounds were found for the sex pheromones of the pea aphid (*Acyrthosiphon pisum*), the black bean aphid (*Aphis fabae*), and the peach-potato aphid (*Myzus persicae*), but in different ratios (Dawson et al., 1990; Hardie et al., 1990). The nepetalactol I alone comprised the pheromone of the greenbug, *Schizaphis graminum* (Dawson et al., 1988).

Aphid sex pheromones have not been shown previously to influence behavior under field conditions. For such investigations, the damson-hop aphid, *Phorodon humuli*, was chosen as a model. The sex pheromone, and chemical components of the host, would be expected to play an important role for this species, since its host alternation is obligate within a narrow range of plants (Eppler, 1986): *Prunus* spp., Rosaceae (winter or primary host) and hops, *Humulus lupulus*, Cannabaceae (summer or secondary host). As the summer host is a valuable crop and control of *P. humuli* by insecticides is now failing because of resistance, use of semiochemicals against this pest should be considered. The sex pheromone of *P. humuli* therefore was characterized chemically and its activity in the field investigated.

METHODS AND MATERIALS

Aphids. Virginoparous *P. humuli* (Badsey strain) from East Malling were maintained on hops (hop progeny 64/84/15) at 20°C with a 16:8 hr day–night regime. Males and gynoparae were produced by transferring virginoparae from the stock culture to 16°C with an 11:13 hr day–night regime. Oviparous females then were obtained by placing gynoparae onto leaves of myrobalan (cherry-plum), *P. cerasifera.*

Pheromone Entrainment. Methods have been described previously (Dawson et al., 1988) and involved entraining volatiles from "calling" oviparae onto Porapak Q and eluting with freshly distilled ether.

Primary Host Volatiles. Twigs or leaves from myrobalan, *P. cerasifera*, were employed in the olfactometer. An ether extract with a concentration of 35 g fresh bark per milliliter was used in laboratory and field studies.

Behavior. Behavioral assays were done in a Perspex olfactometer (100 mm diam.) similar to that described by Pettersson (1970), having a weak airstream directed towards the center from each of four side arms. The odor stimulus (aphids, plant material, or solutions applied to filter paper) was placed at the end of one of the arms. When the stimulus comprised fresh plant material, moistened filter paper was used as a control. Male *P. humuli* (10) were introduced into the center of the arena, and the numbers present in each arm were recorded every two minutes for 20 min.

Coupled GC-SCR. Methods of recording from cells on aphid antennae have been described previously (Wadhams, 1990). For this work, cells associated with the secondary rhinaria on the third antennal segment of the male were employed. GC was done on a 50 m × 0.32 mm id HP-1 fused silica capillary column equipped with a cold on-column injector and with hydrogen as the carrier gas.

Coupled GC-Mass Spectrometry (MS). The methods have been described (Pickett, 1990) and employed the same GC conditions as for GC-SCR. MS was by electron impact at 70 eV, 250°C.

*(4a*R, 7*S*, 7*a*S*)-Nepetalactol (III).* The labiate plant *Nepeta mussinii* was cultivated under glass and, when mature, fresh plants (1,123 g) were macerated in a blender with water (2 liters) and the mixture steam distilled. The distillate was extracted with diethyl ether (2 × 500 ml), the extract dried ($MgSO_4$), filtered, and concentrated to leave a pale yellow oil (1.03 g). This was purified by HPLC using a Jobin Yvon Chromatospac Prep with Lichroprep as the support, eluting with diethyl ether–hexane (12:88), leaving, after concentration, the lactone (570 mg). This was reduced with DIBAH to the (4a*R*,7*S*,7a*S*)-nepetalactol (III) (510 mg, 90%) and shown to comprise ca. 70% 1*S* and 30% 1*R* isomers by nuclear magnetic resonance spectroscopy (Dawson et al., 1989).

The nepetalactol III was stored in glass ampoules sealed under nitrogen and diluted in hexane for laboratory behavioral assays.

Lures. For field trials, pheromone lures were prepared by placing an ether solution of (4a*R*,7*S*,7a*S*)-nepetalactol (10 mg) in closed polyethylene vials (WP/5, Fisons) allowing diffusion through the walls. Myrobalan bark extract (1 ml) was prepared similarly and control lures contained solvent only. Separate vials were used for each test material.

Field Trials. Traps were made from 15-cm-diam. plastic Petri dishes that had been painted on the outside with yellow paint (BS 381C: 1980 Color 309 Canary Yellow), and then with black paint so that the yellow coloring was visible only through the inside. Water containing 0.2% of a nonionic detergent was placed in each dish, and the lures were suspended above the surface of the water by wires. The traps were set on canes 0.6 m above ground level and arranged 2 m apart in six separate randomized blocks. Traps were emptied and the treatments rerandomized daily. Numbers of all morphs of *P. humuli* in the daily trap catches were counted and the data analyzed using a chi-square procedure.

Trapping began on September 12, and initially the blocks were situated outside the hop garden, at least 20 m from the nearest hops. During the week of October 6 to 11, the traps were transferred to within the hop garden (ca. 1 m from the hop bines), where they remained until the end of the trial on November 3. Fresh lures were provided on October 6 and October 17. During the trial, a suction trap (Burkard VI/9, 23 cm fan diam.) sampling approximately 510 m^3 of air per hour (Taylor, 1951) was operated in the hop garden. The inlet was 1.5 m above ground level and the trap was emptied daily.

RESULTS AND DISCUSSION

Results from initial behavioral studies using the olfactometer are given in Table 1. Male *P. humuli* clearly were attracted (or arrested) by a volatile pheromone released by oviparous females, but showed no response to gynoparae. As interspecific attraction has been demonstrated for a number of aphid species (Dawson et al., 1990), the males were also tested against oviparae of *M. viciae*. There was no response, suggesting that the pheromone of *P. humuli* did not comprise either the nepetalactol I or the nepetalactone II.

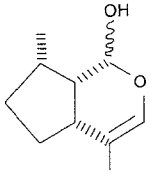
Volatiles entrained from "calling" *P. humuli* oviparae were active on SCR from the male antennae and stimulated only one cell type. The extract was analyzed by coupled GC-SCR and one slightly broadened peak was found to be associated with activity (Figure 1). Coupled GC-MS of the extract showed the compound to have a molecular weight of 168 and a spectrum (Figure 2)

TABLE 1. RESPONSES OF *P. humuli* MALES IN OLFACTOMETER TO FEMALES: CUMULATIVE COUNTS OVER 20 MIN[a]

Stimulus	No. of females	Mean No. males in: Treated arm	Each control arm	Significance[b]
P. humuli oviparae	10	31 ± 1.9	18 ± 1.1	<0.01
	5	33 ± 1.8	17 ± 1.1	<0.01
	1	23 ± 1.2	16 ± 1.0	<0.01
P. humuli gynoparae	3	25 ± 2.7	20 ± 1.1	NS[c]
M. viciae oviparae	10	22 ± 1.7	22 ± 1.0	NS

[a] Number of replicates = 6.
[b] Student's *t* test.
[c] NS = not significantly different at probability level 0.05.

similar to that reported for the nepetalactol I (Dawson et al., 1988). However, the *P. humuli* compound chromatographed later than I, suggesting an isomeric nepetalactol. The stereochemistry at carbon-7 would be fixed early in the biosynthesis and is likely to be the same for all aphids, i.e., 7*S*. Also, since the diastereoisomer of nepetalactol I having the alternative stereochemistry at carbon-1, i.e., (1*S*,4a*S*,7*S*,7a*R*)-nepetalactol, was known to cochromatograph with I (Dawson et al., 1989), then the *P. humuli* compound could only differ from I by having alternative stereochemistry at the ring fusion, i.e., carbons-4a and -7a. The two isomers with *trans*-fused rings would, because of steric strain, open to give iridodials (Dawson et al., 1989), which chromatograph much earlier. This left only one possibility, the all-*cis* (4a*R*,7*S*,7a*S*)-nepetalactol III. This compound previously had been prepared on a very small scale (Dawson et al., 1989) but had not been implicated as a pheromone component for the species studied. Preparation by reduction of the corresponding nepetalactone,



III

SCHEME 2.

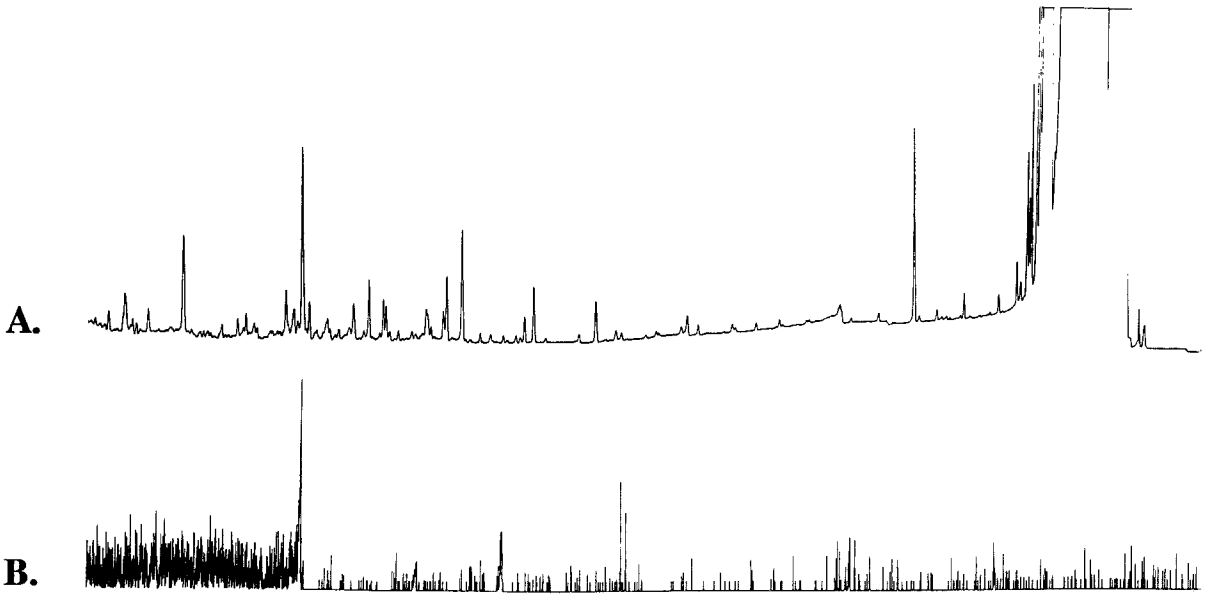


FIG. 1. GC-SCR of volatiles entrained from calling *P. humuli* oviparae. (A) Flame ionization response; (B) action potential summation from cell in secondary rhinarium on third antennal segment of *P. humuli* male.

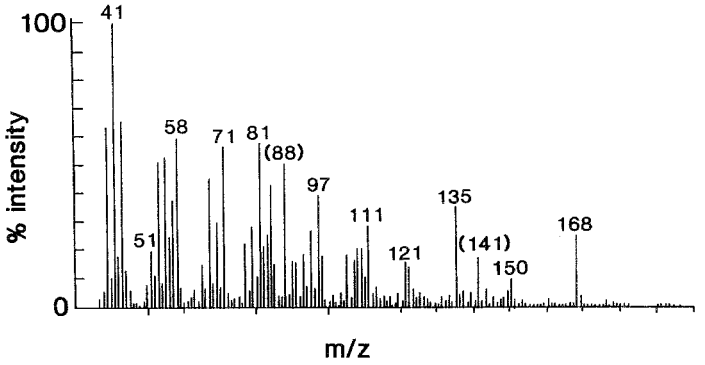


FIG. 2. MS of *P. humuli* sex pheromone; ( ) indicates ions from cochromatographing impurities.

isolated from *N. mussinii*, created a new asymmetric center at carbon-1, with an isomeric composition of 70% 1*S* and 30% 1*R*. The mass spectrum for this product was similar to that obtained by entrainment of *P. humuli*. The isomers were unresolved on GC but, as with the *P. humuli* peak, some peak broadening, indicating partial resolution, was noticed. On coinjection with authentic nepetalactol III, the *P. humuli* peak was enhanced on the HP-1 column and also on a polar column (Carbowax 20 M). This suggested that the pheromone was the nepetalactol III and that the natural material contained both the 1*S* and 1*R* isomers. The nepetalactol III proved to be extremely active in the olfactometer bioassay, attracting males at levels down to 0.15 ng (Table 2).

It has been suggested that sex pheromones of aphids provide only one component of the behavior leading to mate location on the primary host (Steffan, 1987). Since it was considered likely that primary host plant volatiles would

TABLE 2. RESPONSES OF *P. humuli* MALES IN OLFACTOMETER TO NEPETALACTOL III: CUMULATIVE COUNTS OVER 20 MIN[a]

Amount applied (ng)	Mean No. males in: Treated arm	Each control arm	Significance[b]
150	43 ± 2.2	15 ± 1.3	<0.001
15	33 ± 2.5	18 ± 1.0	<0.01
1.5	28 ± 2.3	18 ± 0.9	<0.02
0.15	26 ± 1.0	19 ± 1.1	<0.01

[a] Number of replicates = 6.
[b] Student's *t* test.

TABLE 3. RESPONSES OF *P. humuli* MALES IN OLFACTOMETER TO VOLATILES FROM PRIMARY HOST (MYROBALAN, *P. cerasifera*): CUMULATIVE COUNTS OVER 20 MIN

Stimulus	No. of replicates	Mean No. males in: Treated arm	Each control arm	Significance[a]
Leaf	6	30 ± 2.3	19 ± 0.2	<0.05
Twig	4	35 ± 2.5	18 ± 0.9	<0.001
Bark extract	6	32 ± 2.5	17 ± 1.0	<0.001
Bark extract + nepetalactol III (III as control)	6	36 ± 3.4	14 ± 1.2	<0.01

[a] Student's *t* test.

represent an important olfactory cue in the complex orientation process, some initial investigations were made so that appropriate material would be available for use with pheromone in the intended field trails. Results from olfactometer studies on male response to primary host volatiles are given in Table 3. Positive responses were obtained for myrobalan leaf and twig and an ether extract of bark was shown to be active also. A mixture of bark extract and synthetic pheromone, placed in one arm of the olfactometer, was significantly more attractive than controls comprising the pheromone alone. Other interactions between *P. humuli* and the host plant, and between different morphs, were studied and will be published elsewhere (Pettersson et al., 1990).

In the field trial, when the water traps were placed 20 m outside the hop garden, lures incorporating the nepetalactol III attracted significantly more males than the controls. When the traps were deployed within the crop, the pheromone caught many more males, suggesting that a migratory flight is not essential (Table 4). With fresh lures, the attractancy was enhanced by addition of bark extract. However, synergism was short-lived, which indicated that the compounds involved are either highly volatile or unstable. Daily catches of *P. humuli* in the water traps and in the standard suction trap were also compared (Figure 3). Large numbers of gynoparae were caught in the suction trap in September, but very few were found in the water traps (Table 5). The daily pattern of males caught in both types of trap showed a good correlation. However, each water trap with synthetic pheromone, although having a diameter of only 15 cm, caught as many males as the suction trap which sampled ca. 510 m^3 of air per hour. Indeed, on October 18, water traps incorporating fresh pheromone plus bark extract were three times as effective as the suction trap.

TABLE 4. NUMBERS OF *P. humuli* MALES CAUGHT IN WATER TRAPS IN AUTUMN 1989

	Mean No. males per trap:	
	20 m outside crop	Within the crop (18/10/89)
Nepetalactol III	17.8a	52.0c
Myrobalan bark extract	14.8a	7.8d
III + extract	19.0a	119.5e
Control	2.4b	2.2d

[a] Difference a from b, and c from d from e, $P < 0.05$, based on chi-square test.

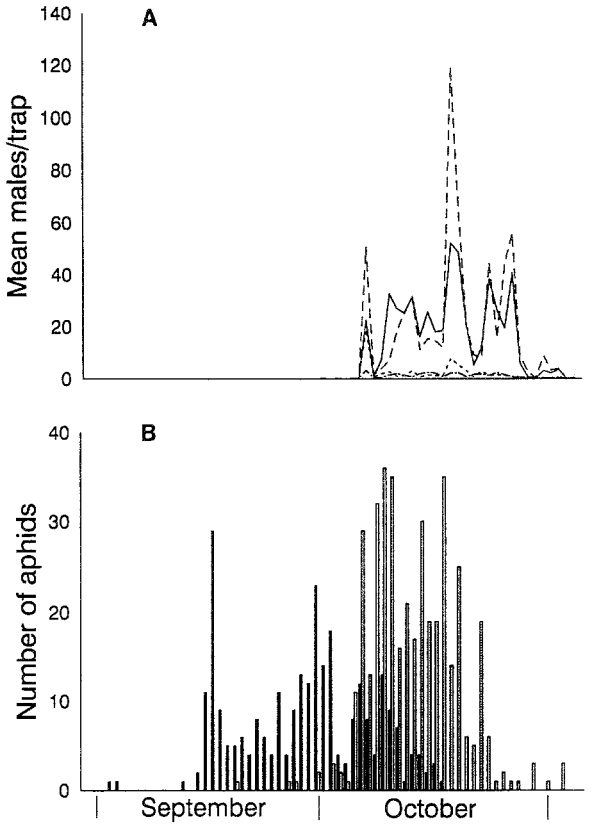


FIG. 3. (A) Daily catches of male *P. humuli* in water traps: ——, nepetalactol III; -----, bark extract; – –, III + bark extract; -·-·-·-, solvent control; (B) daily catches of *P. humuli* in suction trap: ■ gynoparae; ⊡ males.

TABLE 5. TOTAL NUMBERS OF *P. humuli* CAUGHT IN WATER TRAPS IN AUTUMN 1989

	Nepetalactol III	Myrobalan bark extract	III + bark extract	Control
Males	3045	370	3622	210
Gynoparae	74	55	62	53

CONCLUSIONS

The sex pheromone for *P. humuli* is shown to be the (4a*R*,7*S*,7a*S*)-nepetalactol, which we believe has not been detected previously in insects or in plants. The stereochemistry at carbon-1 is not yet defined, but the aphid is thought to produce both 1*S* and 1*R* isomers. A synthetic mixture of these isomers attracted males in the field without the apparent need for other olfactory cues, which is a surprising result in view of previous reports (Steffan, 1987). Nonetheless, there is evidence that primary host volatiles may have a synergistic role that requires further investigation. Now that long-range attraction of male aphids by synthetic sex pheromone has been demonstrated, novel methods of modifying behavior to reduce crop damage can be considered. Although trapping is shown to be feasible, the assertion of Nault and Montgomery (1977) that the pheromone would be more useful in inhibiting mating rather than as a trap lure may yet prove to be correct.

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