Volatile Constituents from Honeysuckle Aphids, *Hyadaphis tataricae*, and the Honeysuckle, *Lonicera* Spp.: Search for Assembling Pheromones

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Volatile fractions of the honeysuckle aphid, *Hyadaphis tataricae* (Aizenberg), and honeysuckle (*Lonicera* spp.) leaves and flowers were analyzed by GLM-MS. The aphids contained (*E*)-β-farnesene, a pheromone known from other aphids. The aphids also contained 18 hydrocarbons and 4 ethyl esters, none of which were present in the plant. It is suggested that the ethyl ester may aid in the assembling of the aphids and the attack on them by their predators, several coccinellid beetles.

The honeysuckle aphid, *Hyadaphis tataricae* (Aizenberg) (Homoptera: Aphididae), originally described from Russia, emigrated to the United States and Quebec in the late 1970s. It is now moving to the west with the expectation that it will soon reach the west coast. It seems to have a northern distribution and has been collected only in the northern half of the United States. This aphid attacks chiefly honeysuckle (Lonicera spp.) on which it builds up to great numbers (Voegtlin and Stoetzel, 1988). In Montana, aphidophagous lady beetles, mainly *Adalia bipunctata* (L.), *Coccinella transversoguttata* Brown, and *Hippadamia convergens* G. M. (Coleoptera: Coccinellidae), are attracted to these aphids from some distance and feed upon them (Dysart, 1990, unpublished data). This sudden appearance of the lady beetles suggests a sequence of events in which the aphide may be attracted by the plant, the aphids increase, and then the lady beetles are attracted to the aphids. One or several chemical signals could be involved; however, no systematic field or laboratory tests have ever been performed to confirm these observations.

No reports could be found of pheromones or related volatile constituents in honeysuckle aphids. Evidently, the first report of an aphid pheromone was from the green peach aphid (*Myzus persicae* Sulzer), which produces an alarm pheromone, (*E*)-β-farnesene (Nault and Bowers, 1974). The turnip aphid, *Hyadaphis erysimi*, also responds weakly to the alarm pheromone (*E*)-β-farnesene, and the response is substantially increased by incorporating plant-derived isothiocyanates (Dawson et al., 1987a). The male vetch aphid, *Megoura viciae*, responds to a mixture of (4a*S*,7*S*,7a*R*)-nepetalactone and (-)-(1*R*,-4a*S*,7*S*,7a*R*)-nepetalactol (Dawson et al., 1987b). Some other volatile constituents have also been found in various aphids. The pea aphid, *Acyrthosiphon pisum*, contains a homologous series of alkanes (both straight and branched), (*E*)-β-farnesene, and some higher esters, aliphatic acids, and alcohols (Stransky et al., 1976).

Flowers of the honeysuckle *L. japonica* were reported to contain linalool, pinene, hexene-1, *cis*-3-hexen-1-ol, geraniol, α-terpineol, benzyl alcohol, β-phenylethanol carvacrol, eugenol, and some tetrahydrofurans and tetrahydropyrans (Wu and Fang, 1980). The fruits of the honeysuckles *L. altaica*, *L. caerulea*, and *L. edulis* were reported to contain triterpenoic acids, β-carotene, ascorbic acid, anthocyanins, catechol, flavonols, chlorogenic acid, and other acids (Fedoseeva and Nauk, 1971; Shapiro et al., 1981).

We report the results of a chemical survey of the volatile fractions of honeysuckle aphids and of honeysuckle leaves and flowers. We also report on one field test in which some of the presumptively identified compounds were tested for attractiveness to the honeysuckle aphid and coccinellid beetles.

METHODS AND MATERIALS

Collection of Insects. Adult winged and wingless honeysuckle aphids were collected from honeysuckle shelter belts in Sidney, MT, on May 22 and 30, 1989. The aphids were covered with hexane and stored at -20 °C until processed. Approximately 3000 aphids, average weight of 0.01 mg, were collected on each date. Additionally, 25 g of honeysuckle leaves and 25 g of flowers were collected on May 30, 1989, covered with hexane, and stored at -20 °C until processed.

Processing and Analysis of Extracts. Each extract was poured without concentration onto a 2.5 × 16 cm silicic acid column (Bio-Sil A, 200-400 mesh, Bio-Rad Laboratories, Richmond, CA) that had been slurried in hexane. The residue was shaken with additional hexane (100 mL), and the hexane extract was poured onto the column. It was subsequently eluted with 100 mL of hexane, 200 mL of methylene chloride/hexane (1/9), 200 mL of methylene chloride, hexane (1/1), and 200 mL of methylene chloride to give four fractions corresponding to the four solvent/solvent mixtures. Each eluate was concentrated under reduced pressure to ca. 10 mL and examined by TLC to confirm that differences existed. Each fraction was analyzed by GLC-EI-MS on a methyl silicone fused silica column (25 m × 0.25 mm, film thickness 0.25 μm) that was interfaced to a Hewlett-Packard 5985-B quadrupole mass spectrometer. An approximation of relative concentrations of components was obtained by comparing the MS data system total abudance count of the ion chromatogram with that of the appropriate standards available from our previous work (Hedin et al., 1988a,b). Identifications were assigned by comparison with available standards and by reference to compiled spectra in the Registry of Mass Spectral Data (Stenhagen et al., 1974).

Additionally, the presence of β-farnesene was confirmed by GLC-MS analysis of an authentic sample obtained from Be-

Table I. Volatile Constituents Found in Honeysuckle Aphid Extracts and in Honeysuckle Leaf and Flower Extracts by GLC–MS Analysis

compound	elemental formula	elution time, min	ng/aphid, May 22	ng/aphid, May 30	leaves, μg/g	flowers, μg/g
hydrocarbons						
Δ-carene	$C_{10}H_{18}$	5.38				52.2
n-undecane	$C_{11}H_{24}$	6.65	4.0	46.8		
1,2-diethylbenzene	$C_{10}H_{14}$	7.47		25.1		
n-dodecane	$C_{12}H_{26}$	7.97	3.3	43.8		
n-tridecane	$C_{13}H_{28}$	9.32	3.4	27.8		
M^+160		10.65	6.0			
(*E*)-β-farnesene	$C_{15}H_{24}$	11.35	10.6	43.2	22.7	
n-pentadecane	$C_{15}H_{32}$	11.95	5.7	20.3		
n-hexadecane	$C_{16}H_{34}$	13.18	6.6	21.5		
n-heptadecane	$C_{17}H_{36}$	14.35	10.9	19.9		
n-octadecane	$C_{18}H_{38}$	15.85		14.5		
n-nonadecane	$C_{19}H_{40}$	16.52	19.5	19.9		
n-unacosane	$C_{21}H_{44}$	18.85		20.1		
n-tricosane	$C_{23}H_{48}$	20.67		48.3		
n-tetracosane	$C_{24}H_{50}$	21.73	45.8			
n-pentacosane	$C_{25}H_{52}$	22.45	15.6	127.5		
n-hexacosane	$C_{26}H_{54}$	22.70	53.5	107.2		
n-heptacosane	$C_{27}H_{56}$	23.98	70.1	73.4		
n-nonacosane	$C_{29}H_{60}$	26.28	67.9	88.0	73.5	
methyl esters						
methyl palmitate	$C_{17}H_{34}O_2$	17.43			35.6	59.8
methyl linoleate	$C_{19}H_{34}O_2$	18.10			108.5	101.0
methyl stearate	$C_{19}H_{36}O_2$	19.05				12.0
methyl docosanate	$C_{23}H_{32}O_2$	21.58				22.1
methyl tetracosanate	$C_{25}H_{50}O_2$	23.17				33.5
ethyl esters						
ethyl caproate	$C_8H_{16}O_2$	5.35	1.9			
ethyl laurate	$C_{14}H_{28}O_2$	13.55		35.0		
ethyl myristate	$C_{16}H_{32}O_2$	15.23	8.2	104.7		
ethyl palmitate	$C_{18}H_{36}O_2$	18.00		20.1		1.2
carboxylic acids						
myristic acid	$C_{14}H_{28}O_2$	14.80	1.2	7.6		
palmitic acid	$C_{16}H_{32}O_2$	16.90	0.8		5.4	
oleic acid	$C_{18}H_{34}O_2$	18.77	1.2			
stearic acid	$C_{18}H_{36}O_2$	18.92	0.8			
ketones						
2-hexanone	$C_6H_{12}O$	4.78	0.7	25.7	20.2	
lupen-3-one	$C_{30}H_{48}O$	23.40	6.4		2.5	
alcohols						
4-methylcyclohexanol	$C_7H_{14}O$	5.18				3.1
p-cresol	$C_7H_{12}O$	5.30				3.0
eugenol	$C_{10}H_{12}O_2$	10.42			8.5	
(*E*,*E*)-farnesol	$C_{16}H_{26}O$	12.47				10.7
1-hexadecanol	$C_{16}H_{34}O$	16.00				15.7
octadeca-9,12,15-trien-1-ol	$C_{18}H_{32}O$	19.35			98.3	10.5
phytol	$C_{20}H_{40}O$	19.52			71.3	5.6

doukian Research Inc., Danbury, CT, and the presence of ethyl caproate, ethyl laurate, ethyl myristate, and ethyl palmitate were confirmed by GLC–MS analysis of authentic samples obtained from Fluka Chemical Corp., Ronkonkoma, NY.

Field Test. Five formulations (treatments) were exposed at two locations (replications), one on each side of a honeysuckle (*Lonicera* spp.) windbreak at the USDA–ARS Research Station at Sidney, MT. The position of traps was randomized weekly from prepared charts (entire trap was moved to a new position) for 6 weeks (May 21–June 25, 1990). The experiment was begun at first bloom. The treatments were as follows: (1) 10 mg of β-farnesene (Bedoukian); (2) 10 mg of 1:1:1:1 mixture of ethyl caproate, ethyl laurate, ethyl myristate, and ethyl palmitate; (3) 5 mg of β-farnesene/5 mg of ethyl ester mixture; (4) blank wicks; (5) live honeysuckle aphids (*H. tataricae*) on a bouquet of honeysuckle foliage with flowers. Each treatment sample was impregnated in a cigarette filter wick and stored at −20 °C prior to use. Wicks were changed at the beginning of each week.

Trap positions were rerandomized at the beginning of each week. Standard boll weevil pheromone traps were placed on metal fence posts at ca. 120 cm above ground surface. All insects were collected from traps twice weekly. Trap collections were made on 12 dates (May 25–July 3, 1990). At these times all trapped insects were labeled and placed in a freezer until they could be stored, identified, and counted.

RESULTS AND DISCUSSION

Among the volatiles present in either aphid or plant extracts were 18 hydrocarbons, 5 methyl esters, 4 ethyl esters, 4 fatty acids, 2 ketones, and 7 alcohols. In addition, of greatest interest was the presence of (*E*)-β-farnesene in aphids from both collection dates and from leaves. While the identification of (*E*)-β-farnesene must be considered presumptive, the ratios of ion fragments were nearly identical with that published for (*E*)-β-farnesene (Stenhagen et al., 1974) and clearly different from those of α-farnesene (*EE* plus *ZZ*) and (*Z*)-β-farnesene. Moreover, its presence is further indicated because (*E*)-β-farnesene has been reported as a pheromone in at least three other aphids (Nault and Bowers, 1974; Stransky et al., 1976; Dawson et al., 1987a). Additionally, (*E*,*E*)-farnesol was found in the flowers. A series of *n*-alkanes from C_{10} to C_{29}, probably cuticular in origin, was found in insects but not in leaves or flowers. Alkanes were previously isolated from

the pea aphid (Dawson et al., 1987a), but they have not been reported as present in *Lonicera* flowers or fruits (Fedoseeva, 1971; Wu and Fang, 1980; Shapiro et al., 1981). Higher quantities of the *n*-alkanes were found in honeysuckle aphids collected on the second date, presumably because they had fed longer.

Ethyl esters were identified from the insects, while methyl esters were identified from the plant material. This metabolic diversity is not evidently unique because ethyl esters also were found by us in lady beetles (Hedin et al., 1988b) and are occasionally found in other insects.

Greenway et al. (1978) reported that C_8–C_{13} fatty acids of green peach aphids were deterrent to settling of other aphids while those longer than C_{16} stimulated settling. In this study, four fatty acids were found, mostly in honeysuckle aphids, and two ketones and seven alcohols were identified from honeysuckle leaves and flowers, several of which had previously been reported (Fedoseeva, 1971; Wu and Fang, 1980; Shapiro et al., 1981). Only three terpenoids, Δ-carene, (*E*)-β-farnesene, and (*E*,*E*)-farnesol, were identified from leaves or flowers. Presumably, additional terpenoids could have been identified had larger quantities of plant material been steam-distilled.

In the field test, no honeysuckle aphids or coccinellid beetles were trapped before June 15. Treatments with live honeysuckle aphids and honeysuckle foliage (see Methods and Materials, test 5) captured only eight coccinellid beetles between June 15 and July 3. Treatments containing β-farnesene and the ethyl esters (tests 1, 2, and 3 combined) captured a total of eight honeysuckle aphids during the same time period. No insects were captured in tests with blanks (test 4).

In summary, evidence has been obtained that one or more (*E*)-β-farnesene isomers are present in the honeysuckle aphid, but these preliminary tests did not determine sex or morph specificity. Considering its known pheromone role in other aphids (Nault and Bowers, 1974; Dawson et al., 1987a), a similar role in this insect is possible. The presence of ethyl esters in the aphid, but not in the plant, indicates there is a somewhat proscribed metabolic capability in the insect that also may provide a chemical marker for assembling of aphids. Considering that some lady beetles also contain relatively large quantities of many of the same ethyl esters (Hedin et al., 1988b), the coccinellids observed in this study conceivably may be aided in the location of honeysuckle aphids by odor familiarity. Attraction of the aphids to the plant may be aided by the plant ketones and alcohols, several of which have established attractancy roles with other insects. In a preliminary field tests, definitive data could not be obtained regarding to attractiveness of these compounds to honeysuckle aphids and beetles.

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