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Attraction of Scavenging Chloropid and Milichiid Flies (Diptera) to Metathoracic Scent Gland Compounds of Plant Bugs (Heteroptera: Miridae)

QING-HE ZHANG[1, 2] AND JEFFREY R. ALDRICH[2, 3]

Environ. Entomol. 33(1): 12–20 (2004)

ABSTRACT Hexyl butyrate and (*E*)-2-hexenyl butyrate, common metathoracic scent gland compounds of plant bugs (Heteroptera: Miridae), attracted large numbers of female chloropid [*Olcella trigramma* (Loew), *O. cinerea*, *Conioscinella* sp.] and milichiid (*Leptometopa latipes* Meigen) flies. Blends of these two butyrates attracted significantly more chloropids than did the compounds individually. The optimal synergistic ratios for *O. trigramma* attraction ranged from 1:1–9:1 hexyl butyrate to hexenyl butyrate. These values are similar to natural ratios of the compounds in the scent gland secretion from tarnished plant bugs, *Lygus lineolaris*, and other mirids. Antennae of female *O. trigramma* gave strong electrophysiological responses to (*E*)-2-hexenyl and hexyl butyrates, whereas electroantennogram responses to butyl butyrate and pentyl butyrate were insignificant. (*E*)-2-octenyl acetate, one of the major sex pheromone components of mirids in the genus *Phytocoris*, was strongly attractive to the milichiid, *L. latipes*, and another pheromone component of *Phytocoris* bugs, hexyl acetate, was inactive alone, yet synergized the attraction of the milichiid and three chloropid species to (*E*)-2-octenyl acetate. Traps baited with (*E*)-2-hexenyl (*E*)-2-hexenoate, a volatile component of various heteropterans, were significantly attractive to both *O. cinerea* and *L. latipes*, whereas addition of γ-caprolactone and green leaf alcohols significantly reduced the numbers of both fly species caught. Our results suggest that females of these chloropid and milichiid flies use volatile defensive and pheromonal compounds from plant bugs as kairomones to find freshly injured or dead bugs on which to feed. The sex-specific attraction might indicate that females of these flies need a protein-rich meal for maximum fecundity, as do anautogenous mosquitoes.

KEY WORDS grass flies, *Olcella*, kairomone, anautogenous, hexyl butanoate

WHILE TESTING POTENTIAL EFFECTS of hexyl butyrate and (*E*)-2-hexenyl butyrate on attraction of the tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois) (Heteroptera: Miridae), tremendous numbers of various chloropid and milichiid flies were caught. These two butyrate esters are commonly produced in the metathoracic scent gland of lygus bugs (Aldrich et al. 1988, Ho and Millar 2002) and other mirids (McBrien and Millar 1999). This finding led to further study of the kairomonal function of mirid allomones and raised the possibility that mirid sex pheromone omponents (also produced in the metathoracic scent gland) are used as kairomones by these kinds of flies.

Most chloropids, called grassflies, lay their eggs on plants or decaying plant material on which the larvae develop. Some members of Chloropidae are pests of wheat, barley, and millet (Sabrosky 1987, Armstrong et al. 1995). Certain chloropids (i.e., *Hippelates* sp.) are known as eye gnats because their females are attracted to and feed on moist areas of animals such as around the eyes and on exposed mucus or wounds; in the process, they sometimes transmit pink eye and other diseases (Harwood and James 1979). Several chloropids and members of the related family, Milichiidae—including some of the flies encountered in this study—are reportedly kleptoparasites that feed most commonly on the hemolymph of heteropterans and bees caught by spiders or predacious insects (Marshall 1998, Sivinski et al. 1999).

Our objectives were to (1) test the attractiveness of hexyl and (*E*)-2-hexenyl butyrates to these scavenging flies; (2) determine the electrophysiological activity of hexyl butyrate, (*E*)-2-hexenyl butyrate, and related esters to the flies; and (3) determine if sex pheromone components of plant bugs are also attractive to the flies.

This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or a recommendation by the USDA for its use.

[1] Department of Entomology, University of Maryland, College Park, MD 20742.

[2] USDA-ARS Beltsville Agricultural Research Center-West, Chemicals Affecting: Insect Behavior Laboratory, Building 007, Beltsville, MD 20705.

[3] E-mail: aldrichj@ba.ars.usda.gov.

Table 1. Chemicals, sources, release rates, and dispensers used in both field trapping and EAG experiments

Chemical (acronym)	Sources[a]	Release rate (mg/24 h)[b]	Dispenser[c]	Filed experiment no.								EAG
				1	2	3	4	5	6	7	8	
Hexyl butyrate (HB)	1	4.2	100 μl in a closed PE-vial	✓	✓	✓			✓			
		ND	0.1–30 mg loaded onto rubber septa							✓		
		"	1 μg on filter paper									✓
(*E*)-2-Hexenyl butyrate (E2HB)	1	1.4	100 μl in a closed PE-vial	✓	✓	✓			✓			
		ND	0.1–30 mg loaded onto rubber septa							✓		
		"	1 μg on filter paper									✓
HB + E2HB		4.2 + 1.4	100 μl each in 2 separate closed PE vials	✓	✓				✓			
		2.3 + 0.7	50 μl + 50 μl in a closed PE vial			✓						
		ND	100 μl of mixture with different ratios in each closed PE-vial				✓					
		"	0.5 μg + 0.5 μg on filter paper									✓
(*E*)-2-Hexenyl (*E*)-2-hexenoate (E2HE2H)	2	"	20 μl in a closed PE-vial					✓				
γ-Caprolactone (Lactone)	3	"	50 μl in a closed PE-vial					✓				
3GLVs												
1-Hexanol	3	"						✓				
(*Z*)-3-Hexenol	3	"	100 μl of 1:1:1 mix in a close PE-vial					✓				
(*E*)-2-Hexenol	3	"						✓				
Hexyl acetate (HA)	3	"	20 μl loaded onto rubber septa								✓	
(*E*)-2-Hexenyl acetate (E2HA)	2	"	"								✓	
(*E*)-2-Octenyl acetate (E2OA)	1	"	"								✓	
Butyl butyrate (BB)	3	"	1 μg on filter paper									✓
Heptyl butyrate (HB)	3	"	"									✓

[a] 1, Bedoukian Research Inc., Danbury, CT; 2, = Dr. James Oliver, CAIBL, ARS, USDA; 3, Aldrich Chemical Co., Milwaukee, WI.
[b] Measured in a chemical hood at 20–23°C for a week. ND, not determined.
[c] All the field dispensers were replaced by new ones after 1 wk of exposure, except for the rubber septa dispensers used in experiment 7, which were renewed evey third day. PE, polyethylene.

Materials and Methods

Field Trapping. Field experiments were carried out at two sites during the summer of 2002, using Jackson delta traps with removable sticky inserts (Agrisense, Fresno, CA). Site 1 was an alfalfa field (8.5 ha) located at the USDA Beltsville Agricultural Research Center (BARC), Prince George's County, MD, and site 2 was an oak-pine mixed forest (≈2 ha) on the South Farm of BARC, ≈5 km south of site 1. Traps were deployed in lines on either metal posts 30 cm from the edge of an alfalfa field at a height of 50–60 cm (site 1) or on tree trunks ≈1.8 m above ground (site 2), with a spacing of 10 m between traps within each trap line and 15 m between trap lines. For each experiment, one to three sets of traps were deployed, with their initial trap positions randomized and rearranged after each replicate (if >50–100 flies were caught in the best traps) in a Latin-square design (Byers 1991) to minimize positional effects. Sticky inserts with captured flies were removed and replaced by fresh inserts after each replicate. The numbers and species of flies on each sticky insert were counted in the laboratory. Several samples of 5–20 flies recovered from the sticky inserts were soaked in xylene to remove the sticky material and sent for species and gender determinations to the Systematic Entomology Laboratory, USDA-ARS, Beltsville, MD.

Experiments 1–5 were conducted at site 1 (alfalfa). The initial intent of experiment 1 was to test the potential effect of the scent gland compounds, hexyl butyrate (HB) and (*E*)-2-hexenyl butyrate (E2HB), and their combination (1:1 by volume) on the tarnished plant bug, *L. lineolaris*. In addition to butyrates produced in the metathoracic scent glands, each trap was baited with three virgin females of *L. lineolaris* (≈7 d old) kept in a 1-oz plastic cup, with a mesh lid and 30 small holes in the bottom, which was suspended from the trap roof. For each treatment, two pieces of fresh green bean (3 cm long) were provided as food for the females. Both virgin females and green beans were renewed after a 5-d exposure in the field. Experiment 2 was similar to experiment 1 but excluded the virgin females of *Lygus* bugs. Experiment 3 had the same treatments as in experiment 2, except that the release rate for the combination of HB and E2HB was lower than that in the previous tests, i.e., the two compounds were mixed at 1:1 ratio in one polyethylene vial dispenser (BEEM, Bronx, NY) rather than in two separate vials (Table 1). In experiment 4, the attraction of differing blends of HB and E2HB, with the percentages of each ester ranging from 0 to 100%, was tested. In experiment 5, three sets of delta traps baited with (*E*)-2-hexenyl (*E*)-2-hexenoate, γ-caprolactone, or a mixture of three green leaf alcohols [GLVs: 1-hexanol, (*E*)-2-hexenol, and (*Z*)-3-hexenol] or their combinations were deployed at the alfalfa field site. (*E*)-2-Hexenyl (*E*)-2-hexenoate and γ-caprolactone have been detected from tarnished plant bugs (J.R.A., unpublished data) and therefore were tested in the field as possible attractants for *L. lineolaris*, along with GLVs known to synergize pheromone attraction for various insects (Dickens et al. 1990).

Experiments 6–8 were conducted at site 2 (oak-pine). Experiment 6 was carried out with treatments exactly the same as in experiment 2. Dose responses to HB or E2HB by one of the major fly species at site two

were tested in experiment 7 by adding different concentrations of hexane solutions of each compound to rubber septa dispensers (5 mm sleeve-type; The West Co., Lititz, PA). Experiment 8 tested the potential activity of hexyl acetate, (*E*)-2-hexenyl acetate, and (*E*)-2-octenyl acetate (sex pheromone components from the metathoracic scent gland of female *Phytocoris* spp.) in a three-way factorial design; i.e., three individual components, and all possible binary/ternary blends (loaded onto gray rubber septa dispensers).

Electroantennogram Recordings. Electroantennogram (EAG) responses of a female chloropid, *Oscella trigramma* (Loew), to butyl, pentyl, hexyl, and (*E*)-2-hexenyl butyrates were recorded by using a Syntech EAG setup (Hilversum, The Netherlands). A glass capillary indifferent electrode was filled with Beadle-Ephrussi Ringer (Zhang et al. 2000), grounded through a silver wire, and inserted into the open side of the severed fly head. A similar recording electrode connected to a high-impedance DC amplifier with automatic baseline drift compensation was placed in contact with the distal ends of both antennae. The antennal signals were stored and analyzed on a personal computer equipped with a serial Intelligent Data Acquisition Card (IDAC) interface box and the program EAG2000 (Syntech, Hilversum, The Netherlands). Stimuli were prepared by applying the chemicals (1 μg for each compound or blend) in 2 μl of hexane on a piece of filter paper (7 × 15 mm) in a Pasteur pipette. The stimuli were tested in a random order, and "control" stimuli (2 μl of hexane solvent) were applied before and after the five ester treatments were tested. Each stimulation was followed by a minimum of a 60-s purge period of filtered air to ensure recovery of antennal receptors.

Chemicals. All chemicals, sources, release devices, and release rates or loading amounts are listed in Table 1.

Statistical Analysis. Means were compared by one-way analysis of variance (ANOVA) of untransformed (EAG) data and data transformed by log ($X + 1$) (field trapping), followed by the REGW-Q test (SPSS 10.0 for Windows; Day and Quinn 1989). In all cases, $\alpha = 0.05$. Zero values, with no variance, were excluded in analyses to achieve homogeneity of variances for ANOVA.

Results

Insects. The flies caught in sticky traps belong to the following two families, Chloropidae [*O. trigramma* (Loew), *O. cinerea* (Loew), and *Conioscinella* sp.] and Milichiidae (*Lepitometopa latipes* Meigen). The samples submitted for identification were all females. There were a few individuals of another chloropid species identified (*Oscinella* sp.), but they were not separated from their morphologically similar congeners in the analyses.

Field Trapping. In all experiments, flies were only caught in the daytime. In experiment 1, two species of chloropids (*O. trigramma* and *O. cinerea*) and one

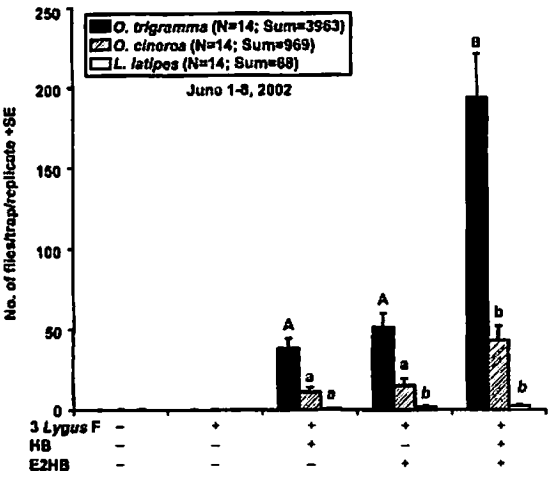

Fig. 1. Captures of female chloropid and milichiid flies in traps baited with three virgin female tarnished plant bugs, *L. lineolaris*, plus hexyl butyrate (HB), (*E*)-2-hexenyl butyrate (E2HB), or the combination of these esters in an alfalfa field in Beltsville, MD. Bars within each species followed by the same letter are not significantly different ($P > 0.05$), one-way ANOVA on log ($X + 1$) transformed data, followed by REGW-Q test.

milichiid species (*L. latipes*) were caught in butyrate-baited traps. No flies were found in blank control traps or traps baited with three *Lygus* virgin females. Traps with added HB or E2HB caught significant numbers of the two *Oscella* species; there were no differences in trap catches between these two butyrates. The combination of the two butyrates captured more than three times as many *Oscella* flies as did traps baited with the individual butyrates (Fig. 1). A few individuals of *L. latipes* were also trapped, but significant attraction was found only to the traps baited with E2HB or its combination with HB (Fig. 1). Similar attraction patterns of *O. trigramma* to HB, E2HB, and their combinations were observed in experiments 2 and 3 (Fig. 2, A and B). Traps baited with the mixture of HB and E2HB (in one dispenser) in experiment 3, with an overall release rate similar to that for individual treatments, caught significantly higher numbers of flies than did the traps baited with the individual butyrates (Fig. 2B). In experiment 4, HB and E2HB individually again showed strong attraction to *O. trigramma* females, whereas mixtures of these esters caught even more flies; optimal ratios ranged from 1:1–9:1 HB to E2HB (Fig. 3). In experiment 5, traps baited with γ-caprolactone (lactone) or a mixture of 3GLVs or their combination did not catch any flies nor did the blank control traps, whereas (*E*)-2-hexenyl (*E*)-2-hexanoate was significantly attractive to both *O. cinerea* and *L. latipes* females (Fig. 4). Addition of the lactone or its combination with the 3GLVs to (*E*)-2-hexenyl (*E*)-2-hexenoate–baited traps significantly reduced the numbers of both fly species caught (Fig. 4).

The pattern of attraction for *O. trigramma* to HB, E2HB, and their combination in experiment 6 (oak-

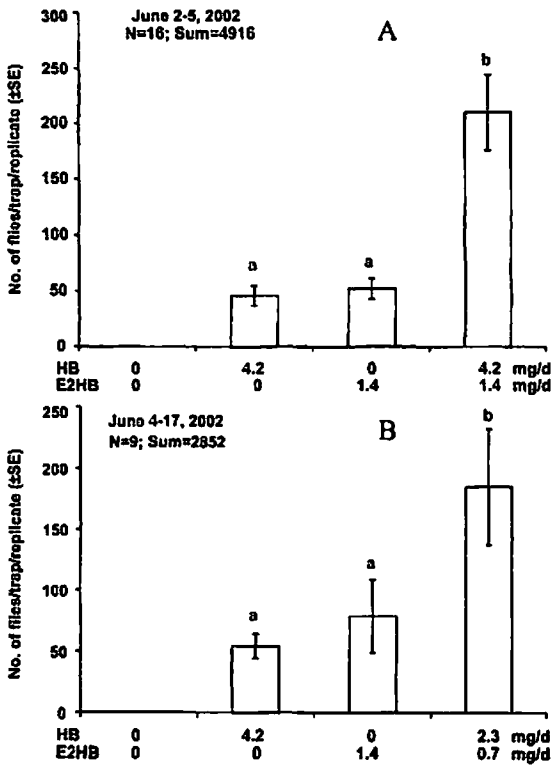

Fig. 2. Captures of female *O. trigramma* in traps baited with hexyl butyrate (HB), (*E*)-2-hexenyl butyrate (E2HB), or their binary blend, in an alfalfa field in Beltsville, MD. (A) Overall release rate of binary blend higher than individual butyrates. (B) Overall release rate of binary blend approximately equal to that for individual butyrates. Bars within each experiment followed by the same letter are not significantly different ($P > 0.05$), one-way ANOVA on log (X + 1) transformed data, followed by REGW-Q test.

pine forest; site 2; Fig. 5) was similar to the results for experiment 2 (alfalfa field; site 1; Fig. 2), except that another chloropid species (*Conioscinella* sp.) was also caught in even larger numbers than that of *O. trigramma* in the traps baited with HB and E2HB (Fig. 5). Traps baited with the blend of these two butyrates caught significantly more *Conioscinella* sp. than traps baited with either butyrate alone (Fig. 5). In experiment 7, traps baited with 0.3 mg or less of HB or E2HB did not catch any *Conioscinella* sp. However, the trap catches significantly increased as the doses of either butyrate increased when loading amounts were >0.3 mg (Fig. 6). The minimum threshold of attraction for *Conioscinella* sp. to these two butyrates was between 0.3 and 1.0 mg per dispenser. At the same loading dosage, HB seemed to be more attractive to *Conioscinella* sp. than did E2HB (Fig. 6).

In experiment 8, three species of chloropids (*O. trigramma*, *O. cinerea*, and *Conioscinella* sp.) and one milichiid species (*L. latipes*) were caught (Table 2). *L. latipes* was the most abundant species (>60% of total catches), followed by *Conioscinella* sp. (20.7%), *O. trigramma* (16.9%), and *O. cinerea* (<2%). Individually, hexyl acetate and (*E*)-2-hexenyl acetate were inactive, as was their binary blend, whereas traps baited with (*E*)-2-octenyl acetate alone caught significant numbers of *L. latipes*, *O. trigramma*, and *Conioscinella* sp. (Table 2). Addition of (*E*)-2-hexenyl acetate to (*E*)-2-octenyl acetate did not influence the trap catches. However, the numbers of all four fly species caught in the traps baited with (*E*)-2-octenyl acetate plus hexyl acetate were significantly higher than that for traps loaded with (*E*)-2-octenyl acetate alone (Table 2). The ternary blend was also significantly more attractive to *L. latipes* than the (*E*)-2-octenyl acetate alone, but it was less active than the binary blend of (*E*)-2-octenyl acetate and hexyl acetate.

EAG Responses to C_4–C_6 Butyrates by *O. trigramma* Females. The life span of our antennal preparations was relatively short (<10 min); nevertheless, successful EAG recordings were made using antennae from three *O. trigramma* females. EAG responses to butyl butyrate and pentyl butyrate were not different from

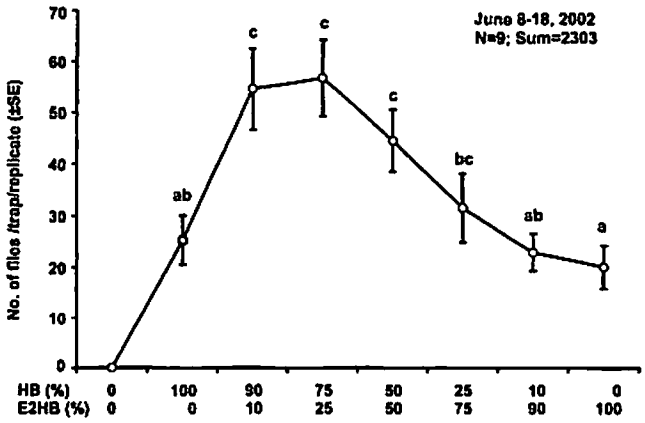

Fig. 3. Captures of female *O. trigramma* in traps baited with different ratios of hexyl butyrate (HB) and (*E*)-2-hexenyl butyrate (E2HB) in an alfalfa field in Beltsville, MD. Means followed by the same letter are not significantly different ($P > 0.05$), one-way ANOVA on log (X + 1) transformed data, followed by REGW-Q test.

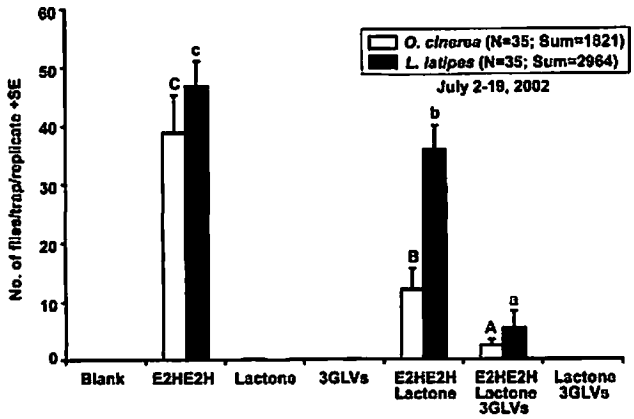

Fig. 4. Captures of female chloropids (*O. cinerea*) and milichiids (*L. latipes*) in traps baited with (*E*)-2-hexenyl (*E*)-2-hexenoate (E2HE2H), γ-caprolactone (Lactone), a mixture of three green leaf alcohols (3GLVs), or their combination in an alfalfa field in Beltsville, MD. Bars within each species followed by the same letter are not significantly different ($P > 0.05$), one-way ANOVA on log ($X + 1$) transformed data, followed by REGW-Q test.

the blank control (hexane only), whereas HB and E2HB elicited significantly higher EAG reactions than the control (Fig. 7). E2HB was more electrophysiologically active than HB, and the blend of these butyrates did not elicit responses significantly different from E2HB alone.

Discussion

Many chloropids and milichiids—including some of the flies encountered in this study—are reportedly kleptoparasites that feed most commonly on the hemolymph of heteropterans and bees caught by spiders or predacious insects (Marshall 1998, Sivinski et al. 1999). Eisner et al. (1991) found that species in the stink bug (Pentatomidae) and squash bug (Coreidae) families that are caught in spider webs are highly attractive to several milichiid, chloropid, and phorid flies because of defensive compounds, including hexanal and (*E*)-2-hexenal, released by the ensnared bugs. Later, Aldrich and Barros (1995) showed that these kinds of chloropid and milichiid flies are attracted to the common $C_{6,8,10}$ α,β-unsaturated aldehydes of true bugs independent of any predators. Previous pheromone studies of stink bugs revealed that, when a bug dies, the volatile chemicals begin leaking from its metathoracic scent gland (e.g., Aldrich et al. 1987). Therefore, it seems that the flies exploit heteropteran allomones as kairomones to find heteropterans on which to feed. Kleptoparasitism may be an opportunistic subset of the more general scavenging behavior of the flies' search for dead bugs.

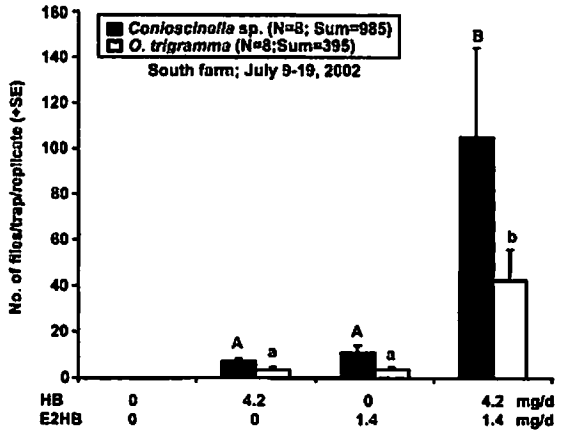

Fig. 5. Captures of female *O. trigramma* and *Conioscinella* sp. in traps baited with hexyl butyrate (HB), (*E*)-2-hexenyl butyrate (E2HB), or their binary blend in an oak-pine mixed forest in Beltsville, MD. Bars within each species followed by the same letter are not significantly different ($P > 0.05$), one-way ANOVA on log ($X + 1$) transformed data, followed by REGW-Q test.

The results of this study clearly show that females (but not males) of *O. trigramma*, *O. cinerea*, and *Conioscinella* sp. (Chloropidae) are strongly attracted to HB and E2HB, major metathoracic scent gland components of *Lygus* spp. (Aldrich et al. 1988, Ho and Millar 2002) and many other mirids (Smith et al. 1991, McBrien and Millar 1999, Groot et al. 2001). These butyrates act synergistically to attract chloropid females, with optimal synergistic ratios for *O. trigramma* similar to natural ratios of these compounds in the scent gland secretions of lygus bugs. Furthermore, our electrophysiological study of *O. trigramma* established that the antennae of females are particularly sensitive to HB and E2HB. For *Conioscinella* females, dose-response curves to HB and E2HB were similar. The threshold of attraction to these esters was about the same amount normally produced by an individual plant bug (J.R.A., unpublished data). Thus, the attraction of these chloropids to blends of E2HB and HB is ecologically relevant and might reflect a natural association of the flies with the butyrate-related mirid bugs. For the milichiid fly, *L. latipes*, only a few individuals were caught in traps baited with E2HB, suggesting that the association of this species with plant

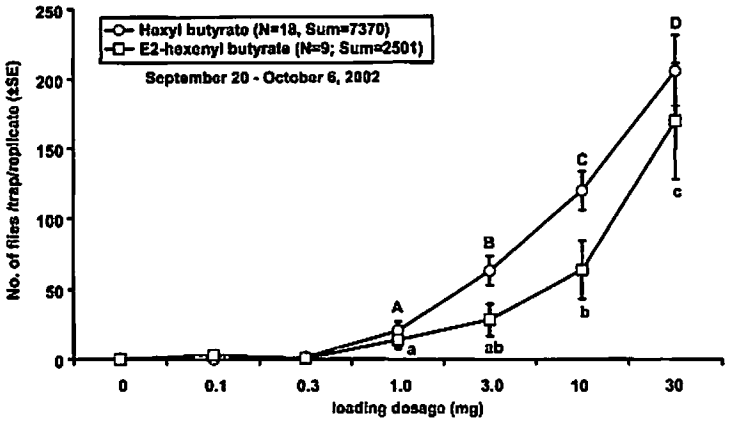

Fig. 6. Captures of female *Conioscinella* sp. in traps baited with different dosages of hexyl butyrate (HB) or (*E*)-2-hexenyl butyrate (E2HB) in an oak-pine mixed forest in Beltsville, MD. Means within each compound followed by the same letter are not significantly different ($P > 0.05$), one-way ANOVA on log ($X + 1$) transformed data, followed by REGW-Q test.

bugs, if any, is unlikely driven by the butyrates (however, see Frost 1913).

Females of *Phytocoris* spp. (Miridae) release sex pheromones consisting of simple esters such as hexyl acetate (females and males), and (*E*)-2-hexenyl acetate and (*E*)-2-octenyl acetate or butyrate (female-specific) (Millar et al. 1997, Millar and Rice 1998, Zhang and Aldrich 2003). We found that (*E*)-2-octenyl acetate, a key pheromone component of eastern U.S. *Phytocoris* spp. (Zhang and Aldrich 2003), was more attractive to the milichiid, *L. latipes*, than to the chloropids, *O. trigramma*, *O. cinerea*, and *Conioscinella* sp., and that hexyl acetate (itself inactive) synergized the attraction of all of these flies to (*E*)-2-octenyl acetate. These data suggest that, in addition to allomones, the flies may use pheromone compounds to locate injured or dead bugs on which to feed.

Although attraction of lygus bugs to (*E*)-2-hexenyl (*E*)-2-hexenoate was negligible, it was highly attractive to *O. cinerea* and *L. latipes*. Surprisingly, the addition of γ-caprolactone and the green leaf volatiles significantly inhibited the attraction of both species to (*E*)-2-hexenyl (*E*)-2-hexenoate. The biological significance of γ-caprolactone for these flies is unclear. However, the inhibitory effect of green leaf volatiles might be ecologically relevant to these scavenging flies if a live bug on a plant or tree that releases scent gland secretion is less attractive to the flies than is a dead bug on the ground, a microhabitat of low green leaf volatile concentration.

In addition to aliphatic esters (see also Sugawara and Muto 1974) and aldehydes (Eisner et al. 1991, Aldrich and Barros 1995), certain Chloropidae and Milichiidae are drawn to pentanoic or hexanoic acid (Jantz and Beroza 1967, Hwang et al. 1976, Hibbard et al. 1997) and to toxic pyrrolizidine alkaloids of various plants (Boppre and Pitkin 1988) (Table 3). Both male and female flies are attracted to plants that produce pyrrolizidine alkaloids (or more likely volatile breakdown products) and feed on these toxins, probably to render themselves unpalatable (Boppre and Pitkin 1988). Pentanoic and hexanoic acids are sex pheromone compounds produced in exceptionally high amounts (100 μg per female) in some common, widespread species of click beetles (Elateridae) (Mayer and McLaughlin 1991), but are also compounds that may be associated with ovipositional sites such as decaying plant or animal tissue. In nearly identical stud-

Table 2. Captures (mean ± SE; #/trap/replicate) of female chloropid and milichiid flies in traps baited with *Phytocoris* pheromone components, 22 July–30 August; 2002, Beltsville, MD

Chemical[a]	*O. trigramma* ($N = 41$)[b]	*O. cinerea* ($N = 22$)	*Conioscinella* sp. ($N = 46$)	*Leptometopa latipes* ($N = 46$)
Blank	0.0 = 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
HA	0.5 ± 0.3[a]	0.1 ± 0.1[a]	0.0 ± 0.0	0.3 ± 0.1[a]
E2HA	0.1 ± 0.1[a]	0.0 ± 0.0[a]	0.1 ± 0.1[a]	0.1 ± 0.1[a]
E2OA	2.8 ± 0.6[b]	0.3 ± 0.2[ab]	2.9 ± 0.5[bc]	4.7 ± 0.8[b]
HA + E2HA	0.3 ± 0.1[a]	0.0 ± 0.0	0.1 ± 0.1[a]	0.2 ± 0.1[a]
HA + E2OA	4.0 ± 0.5[c]	1.2 ± 0.2[c]	4.9 ± 0.8[d]	21.0 ± 3.3[d]
E2HA + E2OA	1.7 ± 0.3[b]	0.2 ± 0.1[ab]	2.3 ± 0.3[b]	4.1 ± 0.5[b]
HA + E2HA + E2OA	3.6 ± 0.9[bc]	0.9 ± 0.4[b]	3.9 ± 0.7[cd]	11.1 ± 2.3[c]
Total	534	59	655	1912

[a] HA, hexyl acetate; E2HA, (*E*)-2-hexenyl acetate; E2OA, (*E*)-2-octenyl acetate.

[b] Means followed by the same letter in a column are not significantly different ($P > 0.05$), ANOVA on Log ($X - 1$), followed by REGW-Q test.

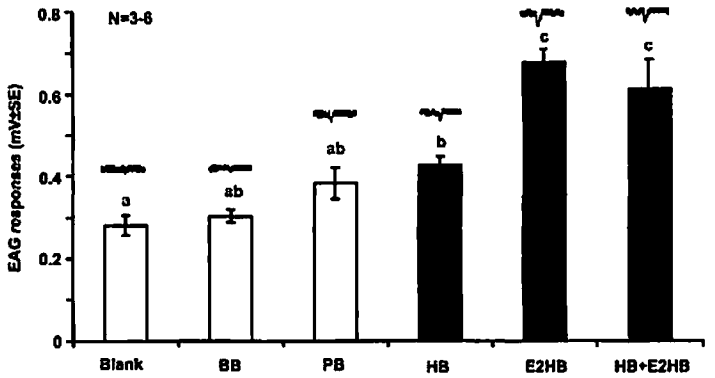

Fig. 7. Mean EAG responses of *O. trigramma* female antennae to C_4–C_6 butyrates. BB, butyl butyrate; PB, pentyl butyrate; HB, hexyl butyrate; E2HB, (*E*)-2-hexenyl butyrate. Bars followed by the same letter are not significantly different ($P > 0.05$), one-way ANOVA on untransformed data, followed by REGW-Q test.

ies, Jantz and Beroza (1967) and Hibbard et al. (1997) found that individuals of *Olcella parva* (Adams) were most attracted to hexanoic acid and pentanoic acid, respectively, but the former authors reported an excess of males, whereas the latter authors recorded a great excess of females in traps. These disparate results may be because of the competing kairomonal attraction to ovipositional sites versus dead or injured insects releasing C_{5-6} acids. Therefore, attraction of chloropids and milichiids to esters, aldehydes, and at least sometimes, acids is fundamentally different than their attraction to alkaloids in that predominantly females are attracted to the volatile compounds associated with insects, whereas both sexes go to and evidently sequester pyrrolizidine alkaloids. Similarly, observations of kleptoparasitism for these and related flies reveal that usually only females are attracted to and feed on prey (Sivinski et al. 1999); occasional records of male attraction are associated with mating, with little or no feeding by the males taking place (Sivinski 1985, Marshall 1998).

The sex-specific attraction to volatiles characteristic of injured or dead insects suggests that the female flies need a protein-rich meal for maximum fecundity, as do anautogenous mosquitoes (Culicidae) (Browne 2001). Anautogeny, the requirement for a meal of blood or proteinaceous food to produce eggs, is known for species in several other dipteran families, including Muscidae, Calliphoridae, Simuliidae, Psychodidae, Ceratopogonidae, Tabanidae, and Tephritidae (Leprince and Lewis 1983, Magnarelli et al. 1984, Vogt and Walker 1987, Adams and Nelson 1990, Smith and Hay-

Table 3. Chemical attraction of chloropid and milichiid flies[a]

Species	Chemicals[b]													Reference
	C_6-ald	C_8-ald	C_{10}-ald	HA	E2OA	HB	E2HB	OB	HH	DH	E2HE2H	C_5-acid	C_6-acid	PA	
Chloropidae															
Chlorops sp.														✓	1
Conioscinella sp (USA)				⊕	✓	✓	✓								2
Conioscinella sp (Japan)						✓									3
Eutropha fulvifrons (Haliday)														✓	1
Melanochaeta spp.														✓	1
Olcella sp.	✓														4
O. cinerea	✓			⊕	✓	✓	✓				✓				2, 5
O. parva	✓											✓	✓		5, 6, 7
O. trigramma	✓	✓	✓	⊕	✓	✓	✓								2, 5
Oscinella sp (USA)						✓	✓							✓	2
Oscinella sp (Kenya)														✓	1
Siphonella sp.								✓							3
Milichiidae															
Leptometopa latipes				⊕	✓		✓				✓				2
Milichiella sp.	✓														4
M. arcuata	✓														5
Neophyllomyza sp.						✓									3
Paramyia nitens	✓														4

[a] ✓, attractive; ⊕, alone inactive, synergistically attractive in binary blend with E2OA.

[b] C_6-ald, (*E*)-2-hexenal; C_8-ald, (*E*)-2-octenal; C_{10}-ald, (*E*)-2-Decenal; HA, hexyl acetate; E2OA, (*E*)-2-octenyl acetate; HB, hexyl butyrate; E2HB, (*E*)-2-hexenyl butyrate; OB, octyl butyrate; HH, hexyl hexanoate; DH, decyl hexanoate; E2HE2H, (*E*)-2-hexenyl, (*E*)-2-hexenoate; C_5-acid, pentanoic acid; C_6-acid, hexanoic acid; PA, pyrrolizidine alkaloids.

[c] 1, Boppre and Pitkin 1988; 2, this paper; 3, Sugawara and Muto 1974; 4, Eisner et al. 1991; 5, Aldrich and Barros 1995; 6, Hibbard et al. 1997; 7, Jantz and Beroza 1967.

ton 1995, Cribb 2000, Browne 2001, Cohen and Voet 2002). However, to our knowledge, anautogeny has not been demonstrated for any milichiid or chloropid species, including *Hippelates* eye gnats, for which only females go to and feed on the mucus of animals (males are attracted to flowers) (Harwood and James 1979). Further research is needed to verify if chloropid and milichiid flies are anautogenous.

If, as we suspect, female chloropids and milichiids are anautogenous and are attracted by semiochemicals to dead or injured insects on which to feed, other chemical cues from insects associated with these flies undoubtedly remain to be discovered. For example, records of kleptoparasitism for these kinds of flies often refer to bees (in addition to true bugs) (e.g., Richards 1953); therefore, it seems likely that 2-heptanone and/or isopentyl acetate will prove attractive to species associated with bees because these are the most abundant alarm pheromone components of honey bees (Collins et al. 1989). Discovery of the semiochemical cues attractive to females for economically important chloropids such as the wheat stem maggot, *Meromyza americana* Fitch, and *Hippelates* eye gnats might provide insight into how to more effectively track and manage these pests.

Acknowledgments

We thank Drs. A. L. Norrbom and F. C. Thompson [Systematic Entomology Laboratory (SEL), USDA-ARS, Beltsville, MD], for identification of flies, and R. Carlson of SEL for facilitating the identification process. Live tarnished plant bugs were kindly provided by G. L. Snodgrass (ARS Southern Insect Management Research Unit, Stoneville, MS). Field assistance by N. Zahm (University of Maryland) was greatly appreciated.

References Cited

Adams, T. S., and D. R. Nelson. 1990. The influence of diet on ovarian maturation, mating, and pheromone production in the housefly, *Musca domestica*. Invertebr. Reprod. Dev. 17: 193–201.

Aldrich, J. R., and T. M. Barros. 1995. Chemical attraction of male crab spiders (Araneae, Thomisidae) and kleptoparasitic flies (Diptera, Milichiidae and Chloropidae). J. Arach. 23: 212–214.

Aldrich, J. R., J. E. Oliver, W. R. Lusby, J. P. Kochansky, and J. A. Lockwood. 1987. Pheromone strains of the cosmopolitan pest, *Nezara viridula* (Heteroptera: Pentatomidae). J. Exp. Zool. 244: 171–176.

Aldrich, J. R., W. R. Lusby, J. Kochansky, M. P. Hoffmann, L. T. Wilson, and F. G. Zalom. 1988. *Lygus* bug pheromones *vis-à-vis* stink bugs., pp. 213–216. *In* Proceeedings of Beltwide Cotton Production Research Conference. National Cotton Council, Memphis, TN.

Armstrong, J. S., S. D. Pilcher, and B. C. Kondratieff. 1995. Two chloropid flies (Diptera: Chloropidae) infecting proso millet (*Panicum miliaceum* L.) in northeastern Colorado. J. Kans. Entomol. Soc. 68: 478–480.

Boppre, M., and B. R. Pitkin. 1988. Attraction of chloropid flies to pyrrolizidine alkaloids (Diptera: Chloropidae). Entomol. Gen. 13: 81–85.

Browne, L. B. 2001. Quantitative aspects of the regulation of ovarian development in selected anautogenous Diptera: integration of endocrinology and nutrition. Entomol. Exp. Appl. 100: 137–149.

Byers, J. A. 1991. BASIC algorithms for random sampling and treatment randomization. Comput. Biol. Med. 21: 69–77.

Cohen, H., and H. Voet. 2002. Effect of physiological state of young *Ceratitis capitata* females, on resource foraging behavior. Entomol. Exp. Appl. 104: 345–351.

Collins, A. M., T. E. Rinderer, H. V. Daly, J. R. Harbo, and D. Pesante. 1989. Alarm pheromone production by two honeybee (*Apis mellifera*) types. J. Chem. Ecol. 15: 1747–1756.

Cribb, B. W. 2000. Oviposition and maintenance of *Forcipomyia* (*Lasiohelea*) *townsvillensis* (Diptera: Ceratopogonidae) in the laboratory. J. Med. Entomol. 37: 316–318.

Day, R. W., and G. P. Quinn. 1989. Comparisons of treatments after an analysis of variance in ecology. Ecol. Monographs. 59: 433–463.

Dickens, J. C., E. B. Jang, D. M. Light, and A. R. Alford. 1990. Enhancement of insect pheromone responses by green leaf volatiles. Naturwissenschaften. 77: 29–31.

Eisner, T., M. Eisner, and M. Deyrup. 1991. Chemical attraction of kleptoparasitic flies to heteropteran insects caught by orb-weaving spiders. Proc. Natl. Acad. Sci. USA. 88: 8194–8197.

Frost, C. A. 1913. Peculiar habits of small Diptera. *Desmometopa latipes* Meig. Psyche. 20: 37.

Groot, A. T., F. P. Drijfhout, A. Heijboer, T. A. van Beek, and J. H. Visser. 2001. Disruption of sexual communication in the mirid bug *Lygocoris pabulinus* by hexyl butanoate. Agric. For. Entomol. 3: 49–55.

Harwood, R. F., and M. E. James. 1979. Entomology in human and animal health. Macmillan Publishing Co., New York.

Hibbard, B. E., D. K. Jewett, and L. B. Bjostad. 1997. Pentanoic acid attracts *Olcella parva* (Adams) (Diptera: Chloropidae) in Colorado corn fields. J. Kans. Entomol. Soc. 70: 67–69.

Ho, H. Y., and J. G. Millar. 2002. Identification, electroantennogram screening, and field bioassays of volatile chemicals from *Lygus hesperus* Knight (Heteroptera: Miridae). Zool. Stud. 41: 311–320.

Hwang, Y. S., M. S. Mulla, and H. Axelrod. 1976. Attractants for synanthropic flies. Identification of attractants and coattractants for *Hippelates* eye gnats (Diptera: Chloropidae). J. Agric. Food Chem. 24: 164–169.

Jantz, O. K., and M. Beroza. 1967. Caproic acid as an attractant for *Olcella parva*. J. Econ. Entomol. 60: 290–291.

Leprince, D. J., and D. J. Lewis. 1983. Aspects of the biology of female *Chrysops univittatus* (Diptera: Tabanidae) in southwestern Quebec. Can. Entomol. 115: 421–425.

Magnarelli, L. A., G. B. Modi, and R. B. Tesh. 1984. Follicular development and parity in phlebotomine sand flies (Diptera: Psychodidae). J. Med. Entomol. 21: 681–689.

Marshall, S. A. 1998. Kleptoparasitic Chloropidae (*Olcella quadrivittata* (Sabrosky)) feeding and mating on staphylinid prey of Asilidae and on hemipteran prey of Mantodea. Studia Dipterologica. 5: 13–18.

Mayer, M. S., and J. R. McLaughlin. 1991. Handbook of insect pheromones and sex attractants. CRC, Boca Raton, FL.

McBrien, H. L., and J. G. Millar. 1999. Phytophagous bugs, pp. 277–304. *In* J. Hardie and K. Minks (eds.), Non-Lepidopteran insects associated with agricultral plants. CABI Publishing, New York.

Millar, J. G., and R. E. Rice. 1998. Sex pheromone of the plant bug *Phytocoris californicus* (Heteroptera: Miridae). J. Econ. Entomol. 91: 132–137.

Millar, J. G., R. E. Rice, and Q. Wang. 1997. Sex pheromone of the mirid bug *Phytocoris relativus*. J. Chem. Ecol. 23: 1743–1754.

Richards, O. W. 1953. Professor O. W. Richards made a communication on commensalism of Desmometopa (Diptera: Milichiidae) with predacious insects and spiders. Proc. R. Entomol. Soc. Lond. C. 18: 55–56.

Sabrosky, C. W. 1987. Chloropidae, pp. 1049–1067. In J. F. McAlpine, B. V. Peterson, G. E. Shewell, J. R. Vockeroth, and D. M. Wood (eds.), Manual of Nearctic Diptera, vol. 2. Research Branch Agriculture Canada, Ontario, Canada.

Sivinski, J. 1985. Mating by kleptoparasitic flies (Diptera: Chloropidae) on a spider host. Fla. Entomol. 68: 215–222.

Sivinski, J., S. Marshall, and E. Petersson. 1999. Kleptoparasitism and phoresy in the Diptera. Fla. Entomol. 82: 179–197.

Smith, R. F., H. D. Pierce, Jr., and J. H. Borden. 1991. Sex pheromone of the mullein bug, *Campylomma verbasci* (Meyer) (Heteroptera: Miridae). J. Chem. Ecol. 17: 1437–1447.

Smith, S. M., and A. Hayton. 1995. The gonotrophic-age structure of a population of the *Simulium venustum* complex (Diptera: Simuliidae) in Algonquin Park, Ontario. Great Lakes Entomol. 28: 185–197.

Sugawara, R., and T. Muto. 1974. Attraction of several dipterous insects to aliphatic esters (Diptera: Milichiidae, Chloropidae and Ceratopogonidae). Appl. Entomol. Zool. 9: 11–18.

Vogt, W. G., and J. M. Walker. 1987. Potential and realised fecundity in the bush fly, *Musca vetustissima*, under favourable and unfavourable protein-feeding regimes. Entomol. Exp. Appl. 44: 115–122.

Zhang, Q.-H., and J. R. Aldrich. 2003. Pheromones of milkweed bugs (Heteroptera: Lygaeidae) attract wayward plant bugs: *Phytocoris* mirid sex pheromone. J. Chem. Ecol. 29: 1835–1851.

Zhang, Q.-H., F. Schlyter, and G. Birgersson. 2000. Bark volatiles from nonhost angiosperm trees of spruce bark beetle, *Ips typographus* L. (Coleoptera: Scolytidae): Chemical and electrophysiological analysis. Chemoecology. 10: 69–80.

Received for publication 15 January 2003; accepted April 28, 2003.