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Synergy Versus Potency in the Defensive Secretions from Nymphs of two Pentatomomorphan Families (Hemiptera: Coreidae and Pentatomidae)

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Abstract

One characteristic of true bugs (Heteroptera) is the presence of dorsal abdominal glands in the immature nymphal stages. These glands usually produce defensive chemicals (allomones) that vary among taxa but are still similar in closely related groups. Knowledge of the chemistry and prevalence of allomones in different taxa may clarify the evolution of these chemical defensive strategies. Within the infraorder Pentatomomorpha, the known secretions of nymphs of Pentatomidae tend to contain the hydrocarbon, *n*-tridecane, a keto-aldehyde, and an (*E*)-2-alkenal as the most abundant components. In the Coreidae, the dorsal abdominal gland secretions of nymphs often contain little or no hydrocarbon, and the most abundant keto-aldehyde and (*E*)-2-alkenal are often of shorter chain length than those of pentatomids. We hypothesized that the long chain compounds would be less potent than their shorter homologs, and that bugs that carry the former would benefit from a synergistic effect of *n*-tridecane. To test this hypothesis we used three different behavioral assays with ants. A predator–prey assay tested the deterrence of allomones toward predators; a vapor experiment tested the effectiveness of allomones in the gaseous phase toward predators; and application of allomones onto predators tested the effect of direct contact. The results substantiate the hypothesis of a synergistic effect between *n*-tridecane and longer chain keto-aldehyde and (*E*)-2-alkenal in deterring predators. The short chain keto-aldehyde 4-oxo- (*E*)-2-hexenal was highly effective on its own. Thus, it seems that different groups of the infraorder diverged in their strategies involving defensive chemicals. Implications of this divergence are discussed.

Keywords

Allomone; Dorsal abdominal glands; Nymphs; Predators; Keto-aldehydes; *n*-tridecane

Introduction

The prevalence and diversity of chemical defenses in insects and other arthropods are staggering, probably more so than in any other group of terrestrial animals (Eisner, 1970). The suborder Heteroptera (Insecta: Hemiptera) is remarkable among insects in its use of defense secretions. In fact, the monophyly of the group is partly based on their possession of scent glands, located in the metathorax of adults and the abdominal dorsum of nymphs, and these glands are considered autapomorphic (i.e., a derived trait) (Schuh and Slater, 1995; Davidova-Vilimova and Podoubsky, 1999). The dorsal abdominal glands (DAGs) of nymphs are responsible for secretion of irritating defensive chemicals (allomones), and may sometimes also be involved in aggregation and/or dispersal (Aldrich, 1988). These glands usually become non-functional following eclosion (but see exceptions in Weirauch, 2006). The major classes of chemicals isolated from defensive glands of heteropteran adults and nymphs are aldehydes, carboxylic acids, esters, and terpenoids (Millar, 2005). Discharge of heteropteran allomones varies with species and life stage (Staddon, 1979). Some species eject their exocrine secretions at an attacker; for examples, in the pentatomids *Bathycorbia thalassina* (Aryeetey and Kumar, 1973) and *Palomena prasina* (Remold, 1962), the coreid *Thasus neocalifornicus* (Prudic et al., 2008), and the lygaeid *Lygaeus saxatilis* (Remold, 1962). Other modes of discharge include volatilization on the surface of widened intersegmental sutures and evaporative cuticle surrounding the gland openings (Staddon, 1979; Xue and Bu, 2008), or through setae (Aryeetey and Kumar, 1973).

Most semiochemical studies of the Heteroptera have thus far focused on the large and highly diverse infraorder Pentatomomorpha, principally the families Pentatomidae, Coreidae, and Lygaeidae. The chemicals identified in nymphs of these families are usually a mixture of hydrocarbons, aldehydes, and keto-aldehydes, where normally one or two compounds occur in much larger amounts than the rest. Many of the individual compounds are shared across taxa, especially at the generic level, but less so at the familial level (Millar, 2005). Although most of DAG compounds are considered to have a defensive role, some may serve as either aggregation or alarm pheromones (Aldrich, 1988), but apart from the few cases where the chemicals have been specifically studied (Borges and Aldrich, 1992; Prudic et al., 2008), the roles of many pentatomomorph allomones are not fully resolved.

In Pentatomidae, DAG secretions of nymphs of many studied species (Table 1) often include the alkane, *n*-tridecane, aldehydes (most commonly (*E*)-2-decenal or (*E*)-2-octenal), and keto-aldehydes. Interestingly, the first instar nymphs of some pentatomid species possess 4-oxo-(*E*)-2-decenal along with *n*-tridecane, while subsequent instars produce 4-oxo-(*E*)-2-hexenal instead of 4-oxo-(*E*)-2-decenal, and (*E*)-2-decenal is most commonly the abundant aldehyde (Borges and Aldrich, 1992). Surprisingly, even though the DAGs are sometimes non-functional in adults, *n*-tridecane and (*E*)-2-decenal remain as the main components of the metathoracic scent gland secretions of a number of adult pentatomids (Pavis, 1987).

Coreidae exhibit a somewhat different pattern of exocrine gland chemistry (Table 1). Most coreid nymphs thus far studied produce mainly the aldehyde, (*E*)-2-hexenal, and the keto-aldehyde, 4-oxo-(*E*)-2-hexenal (Baker and Jones, 1969; Aldrich and Yonke, 1975; Prestwich, 1976; Pavis, 1987; Blatt et al., 1998; Prudic et al., 2008). Adults in this family usually possess the related compounds, hexyl acetate and *n*-hexanal. Most often neither nymphs nor adults contain *n*-tridecane in significant amounts (Aldrich and Yonke, 1975; Pavis, 1987; Millar, 2005).

Interestingly, the most abundant aldehydes and ketoaldehydes of the pentatomid nymphs tend to be longer homologs of those found in the coreid nymphs. It has been suggested that

longer chain aldehydes such as *n*-octanal, *n*-nonanal, and *n*-decanal are not as efficient at penetrating the cuticle of fly larvae as their shorter homolog, *n*-hexanal (Remold, 1963). This author also showed that longer chain aldehydes showed increased penetration rates in the presence of *n*-tridecane, while no such effect of the hydrocarbon was observed when paired with the shorter aldehyde, *n*-hexanal. *n*-tridecane also was shown to be the most effective of the medium-length alkanes tested in synergistically improving the action of (*E*)-2-hexenal (Gunawardena and Herath, 1991). However, there is also the possibility that the defensive compounds act as deterrents. In such cases, the bugs do not necessarily spray the compounds directly onto the attackers, but rely on the deterrence of their noxious fumes. Here, *n*-tridecane might serve as a fixative to delay evaporation of the aldehydes, as suggested by Blum and Traynham (1960).

Based on this information, we hypothesized that 4-oxo-(*E*)-2-decenal and (*E*)-2-decenal, found in pentatomid nymphs, would be less potent against predators compared to their shorter-chain homologs, 4-oxo-(*E*)-2-hexenal and (*E*)-2-hexenal found in coreid nymphs. We further hypothesized that *n*-tridecane would synergistically enhance the activity of 4-oxo-(*E*)-2-decenal and (*E*)-2-decenal. We define potency as the ability to effectively deter or inflict damage on the predator at low doses, and synergy as an increase in the effectiveness of a combination of two compounds such that it is higher than the sum of effectiveness of each compound when applied separately. We tested these hypotheses using synthetic chemicals in three types of assays: behavioral predator assay to assess the deterrent effect of the compounds, and fumigation and spray assays to test the effect of volatiles and droplets, respectively, on predators.

Methods and Materials

Chemicals

An Agilent 6890N gas chromatograph (GC) coupled to an Agilent 5975B mass selective (MS) detector (Agilent Technologies, Palo Alto, CA, USA) was used for analysis of authentic compounds. The GC was operated with splitless injection, and fitted with a 30 m, 0.25 mm ID, 0.25 μ m film thickness HP-5MS column (Agilent). The GC injector temperature was 200 °C, and the oven was programmed from 40 to 230 °C at 8 °C/min after an initial delay of 3 min and held at 230 °C for 10 min. 4-oxo-(*E*)-2-Hexenal and 4-oxo-(*E*)-2-decenal were synthesized according to Moreira and Millar (2005) using purchased precursors (2-ethylfuran >99 %, SAFC, St. Louis, MO, USA; 2-*n*-hexylfuran, 97 %, Alfa Aesar, Ward Hill, MA, USA). 4-oxo-(*E*)-2-Hexenal MS (*m/z*, relative intensity): 112 (M⁺, 17), 97 (1), 84 (16), 83 (100), 57 (15), 55 (65), 53 (6). 4-oxo-(*E*)-2-decenal MS: *m/z* 168 (M⁺, 1), 153 (1), 139 (65), 125 (19), 111 (8), 98 (75), 83 (69), 70 (55), 55 (100), 43 (88). (*E*)-2-Hexenal (98 %, Sigma-Aldrich, St. Louis, MO, USA), (*E*)-2-decenal (97 %, Alfa Aesar) and *n*-tridecane (>99 %, Sigma-Aldrich) were dissolved in hexane (Omnisolve grade, EMD Chemicals, Gibbstown, NJ, USA) to the desired concentrations. The relative abundance of the various compounds in the extracts was quantified by comparing peak areas.

Experimental Procedures

We conducted 3 assays to assess the effectiveness of the defensive compounds.

(1) Predator deterrence assay—For this experiment, we used workers of the jumping ant, *Harpegnathos saltator*, a predatory ant. These ants were used because of their solitary hunting habits. The effect on an individual ant is easier to track with solitary hunters compared to group hunters (many ant species, including fire ants, attack their prey in large groups). Colonies of *H. saltator* initially were excavated at Jog Falls, Karnataka, Southern India in the years 1994, 1995, and 1999, and are currently kept in a USDA-approved

containment room at Arizona State University, Tempe, AZ, USA, in the laboratory of Juergen Liebig. The ant colonies were maintained in plastic containers lined with a layer of dental plaster with a molded chamber covered with a glass sheet to maintain moisture in the nest and separate it from the foraging area. The ants were provided with live crickets twice a week, and kept at 25 °C and 12:12, L:D cycle.

A physiologically-relevant amount of each synthetic chemical (200 µg for the keto-aldehydes; 200, 500, and 1,000 µg dose-range for aldehydes; based on amount of 4-oxo-(*E*)-2-hexanal found in the glands of a single coreid nymph (*Thasus californicus*; Prudic et al., 2008). Synthetic allomone components were dissolved in 1–1.5 µl hexane (99.9 %, EMD chemicals), and topically applied using a microliter syringe to the hard cuticle of the pronotum of a live fourth-instar cricket nymph (*Acheta domestica*) that was chilled on ice before and during the application. An equivalent amount of hexane was applied to crickets that served as controls. The treated and control crickets were presented to jumping ant workers housed individually in a small (10 × 10 × 10 cm) plastic container lined with dental plaster. A ventilated lid was placed on the container during the observation to prevent the insects from escaping, and to minimize external disturbance. The ants' reactions were recorded for 5 min, during which each ant either attacked the cricket by holding it with its mandibles and stinging it, or refrained from doing so. The ants for both control and treatments were taken from the same colony, and each treatment was repeated at least 10 times, with 10 different colonies. At the end of each replicate, ants and crickets were discarded.

(2) Effectiveness of defensive chemicals as paralyzing fumigants—For this test, we used fire ant workers, *Solenopsis xyloni*. The ants were collected as individuals from colonies around the University of Arizona campus, and kept in plastic tubes half-filled with water stopped with a cotton stopper. The tube was covered with aluminum foil and placed in a plastic container with an additional water tube. Ants were provided with live crickets and dry cat food. Physiologically-relevant doses of the synthetic chemicals (50 µg to 1,500 µg), dissolved in hexane (up to 10 µl), were applied to a filter paper disc (Whatman, grade 3, 2.3 cm) and placed in a capped 200 ml glass jar (40 mm OD × 85 mm high). An equivalent amount of hexane was applied to the filter paper disc that was placed in a jar that served as control. Ten individual fire ants of roughly the same size (~4 mm long) were placed in each jar and observed for 30 min. The number of paralyzed ants in each jar at the end of the time period was recorded. Each treatment was repeated at least 9 times. For this experiment, we used the fire ants because they are small enough to be quickly affected by the compounds. Preliminary trials with fire ants of varying sizes showed that the larger ones take longer to be affected by the noxious fumes; therefore, we took care to choose ants of the same, smaller size for all subsequent experiments.

(3) Spray experiment—To simulate a situation in which a heteropteran ejects defensive secretions as a spray, we sprayed physiologically-relevant amounts of the defense chemicals (200 µg in 2 µl hexane) onto individual ants, using a microsyringe held at about 2 cm distance from the ant. An equal volume of hexane spray served as control. For this experiment, we wanted ants that would be more robust, and able to recover the treatment. For this we chose *Pogonomyrmex rogersi* ants collected as individuals from colonies around the University of Arizona campus, and kept as described for fire ants.

For the experiment, *P. rogersi* workers were placed in a glass Petri dish (9 cm OD) with a plastic round wall (made of a plastic cup, 5.5 cm OD × 7 cm high, with the bottom cut off) lined with Fluon®, and the glass dish was washed with water, methanol, and acetone between each trial. This experiment was conducted blindly, such that the sprayer was not informed of the identity of the spray. To avoid olfactory identification of the chemicals by

the sprayer, vials were held at a distance that did not allow for clear recognition of the contents. Ants reacted in a manner that exemplified discomfort, i.e., curled position, walked backwards, walked frantically, etc., and the duration of discomfort (in seconds) after the spray of the contents of each vial was recorded. Each treatment was repeated at least 25 times.

Statistical Analysis

Fisher's exact test was used to determine significant differences between treatments in predator deterrence assay. Oneway ANOVA and Tukey-Kramer *post hoc* tests were used to determine significant differences between doses of the same compound for fumigation and spray against predators, following Levene's tests for homogeneity of variance (Holliday, 2010). The analyses were performed on Rkward (<http://rkward.sourceforge.net>).

Results

Deterrent Effectiveness

The predatory ant *H. saltator* attacked crickets applied with 4-oxo-(*E*)-2-hexenal less frequently than those applied with hexane control (20 % vs. 100 %, Fisher's Exact Test; $P < 0.001$), 4-oxo-(*E*)-2-decenal, or *n*-tridecane (77 % and 76 %, respectively; $P < 0.001$ for both). However, crickets applied with a combination of 4-oxo-(*E*)-2-decenal and *n*-tridecane deterred ants more than crickets treated with 4-oxo-(*E*)-2-decenal alone (45 % vs. 77 %; $P = 0.01$; Fig. 1). These chemicals were all presented at the same dose of 200 μg . (*E*)-2-Hexenal and (*E*)-2-decenal required higher doses to deter the predators from attacking the crickets. Only 1,000 μg reduced attack significantly for both (*E*)-2-alkenals from 84 % to 100 % attack rate of control crickets to 32 % and 50 % attack rate of crickets fortified with (*E*)-2-hexenal and (*E*)-2-decenal, respectively (Fisher's Exact Test; $P < 0.05$). *n*-Tridecane did not increase the deterrence of either of the (*E*)-2-alkenals, with 60 % and 50 % attack for *n*-tridecane combined with (*E*)-2-hexenal and (*E*)-2-decenal, respectively (Fig. 2).

Paralysis Effectiveness

The proportion of fire ants that were paralyzed within 30 min increased in a dose-response manner when the ants were exposed to volatiles of the following defense chemicals: 4-oxo-(*E*)-2-hexenal, 4-oxo-2-(*E*)-decenal, and (*E*)-2-hexenal. *n*-Tridecane was never effective by itself. No synergistic effect of *n*-tridecane with either 4-oxo-(*E*)-2-decenal, (*E*)-2-hexenal, or (*E*)-2-decenal was detected (Fig. 3). 4-oxo-(*E*)-2-Hexenal required lower doses (100 μg) to reach maximum paralysis than 4-oxo-(*E*)-2-decenal (200 μg), (*E*)-2-decenal (300 μg), or (*E*)-2-hexenal (600 μg). 4-oxo-(*E*)-2-Hexenal also achieved maximum paralysis faster than any of the other chemicals (not shown).

Repellent Effectiveness

Sprays of (*E*)-2-hexenal and (*E*)-2-decenal caused more disturbance and agitation than hexane control (29.9 and 32.0 sec compared to 12.9 and 14.9 sec for the two control treatments) although the difference between aldehydes was not significant (one-way ANOVA [not assuming equal variances, Levene's test, $P < 0.05$] followed by Tukey-Kramer, $P > 0.05$) (Fig. 4). Nevertheless, when combined with *n*-tridecane, (*E*)-2-decenal caused disturbance for significantly longer (58.9 sec), both compared to control (Tukey-Kramer; $P < 0.01$) and to (*E*)-2-alkenals without *n*-tridecane ($P < 0.01$). No such effect was found for (*E*)-2-hexenal when combined with *n*-tridecane (29.8 sec).

Discussion

Our results show that members of two families within the Heteroptera differ in their chemical defensive strategies. More specifically, coreid nymphs often produce a potent keto-aldehyde, while pentatomid nymphs predominantly produce (*E*)-2-alkenals that are less potent on their own, but show increased effectiveness when combined with *n*-tridecane. It is interesting to note that most of the pentatomids studied, and some of those we collected and analyzed (data not shown), do not have significant amounts of 4-oxo-(*E*)-2-hexenal. However, some pentatomids, especially those native to tropical regions, do produce large amounts of 4-oxo-(*E*)-2-hexenal (Pareja et al., 2007; Moraes et al., 2008; and see Table 1). Thus, Neotropical species may differ from Nearctic species in semiochemical defense, a hypothesis that may extend to tropical vs. temperate species in general, and one that requires further investigation.

Results of our predator deterrence and volatile paralysis assays suggest that 4-oxo-(*E*)-2-decenal was not as effective as its shorter homolog, 4-oxo-(*E*)-2-hexenal. This could be explained by the difference in penetration rates of these compounds. It has been shown that penetration rate through the cuticle decreases with increase in the length of the residue chain of saturated aldehydes, although toxicity is not affected (Remold, 1963).

Data from our three assays, using three different ant species as predators, support the hypothesis that *n*-tridecane acts as a synergist only with longer chain aldehydes, such as those found in pentatomid nymphs. They also provide support for the suggested mode of action of this synergy, which is facilitation of penetration through the cuticle. *n*-Tridecane has been suggested to act as a synergist with (*E*)-2-hexenal (Gunawardena and Herath, 1991), but we did not find support for this observation in any of our assays.

Few studies have dealt with synergism between chemicals in insect systems. Examining the effect of beetle defensive compounds, individually and in combinations, Dettner and Reissenweber (1991) found that 4-oxo-(*E*)-2-hexenal had a synergistic effect on 2-methylbutyl-2-methyl-butanoate when applied to fly larvae. However, they did not test the toxicity of 4-oxo-(*E*)-2-hexenal on its own, and its effect is quite strong, as evident by the results of our experiments. In the same study, the authors found that esters increase the effect of aldehydes, and suggested it was because they act as solvents that facilitate the penetration of aldehydic toxicants. In the lace bug, *Corythuca cydoniae*, the products of the anterior and posterior DAGs act in synergy to elicit an alarm reaction (Aldrich et al., 1991). The anterior gland products are (*E*)-2-hexenal and nerolidol aldehyde, while the posterior gland products are the terpenoids linalool and geraniol. These compounds are not secreted, rather the glands must be ruptured in order to elicit alarm.

In summary, the dorsal abdominal gland secretions of coreid nymphs often contain little or no hydrocarbon, and the most abundant keto-aldehyde and (*E*)-2-alkenal are often of shorter chain *n*-length than those of pentatomid nymphs. The short chain keto-aldehyde, 4-oxo-(*E*)-2-hexenal, was a highly effective deterrent on its own. In pentatomid nymphs, *n*-tridecane synergizes the longer chain keto-aldehydes and (*E*)-2-alkenals in deterring predators. Thus, it seems that different groups of the infraorder have diverged in their strategies involving defensive chemicals. In this context, it would be interesting to compare the strategies of other families within the Heteroptera to determine the prevalence of these or other semiochemical defenses.

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Fig. 1.

Effect of defensive compounds topically applied onto live crickets on the attack rate of *Harpegnathos saltator* ants. 4OHE and the combination of 4ODE and C13 resulted in reduced attack by ants. 4OHE=4-oxo-(*E*)-2-hexenal; 4ODE=4-oxo-(*E*)-2-decenal; C13= *n*-tridecane. Numbers inside bars indicate sample size, and letters indicate statistical significance: different letters represent significant difference according to Fisher's Exact test ($P < 0.05$). Different color bars represent control (white) or treatment (grey)

Fig. 2.

Dose response curves for (*E*)-2-hexenal (2-hex; $N=19$) and (*E*)-2-decenal (2-dec; $N=10$), and their respective combinations with *n*-tridecane (C13), on the attack rate by individually-caged *Harpegnathos saltator* ants. Asterisks indicate statistically significant difference from control, as tested by Fisher's Exact test ($P<0.05$). No synergistic effect of C13 was observed

Fig. 3.

Dose–response curves for the effect of volatilized defensive compounds on the proportion of paralyzed *Solenopsis xyloni* ants. Ten ants were caged in a glass jar with a filter paper carrying the indicated dose for 30 min. Each point represents the mean + SEM of 9 replicates. Hexane was used as control (dose 0). 4OHE=4-oxo-(*E*)-2-hexenal; 4ODE=4-oxo-(*E*)-2-decenal; 2-hex=(*E*)-2-hexenal; 2-dec=(*E*)-2-decenal; C13=*n*-tridecane

Fig. 4. Effect of defensive compounds when sprayed directly onto *Pogonomyrmex rogersi* ants. Discomfort in seconds (mean + SEM) was determined as any behavior that deviates from normal (see text). Hexane was used as control. Each set of trials was repeated 25 times for 2-hex (light bars) and 37 times for 2-dec (dark bars). 2-hex=(*E*)-2-hexenal; 2-dec=(*E*)-2-decenal; C13=*n*-tridecane. Asterisk indicates statistical significance compared to all other treatments, as tested by one-way ANOVA and Tukey-Kramer ($P<0.05$)

Table 1
The defense compounds of nymphs of Pentatomidae and Coreidae

Family	Species (# of specimens; instar examined)	Most abundant compound (rel. abundance, when available)	Next most abundant compound (rel. abundance, when available)	Other compounds (rel. abundance, when available; instar where compound is found)	Reference
Pentatomidae	<i>Apateticus bracteatus</i> (mid-instar)	<i>n</i> -Tridecane (62)	(<i>E</i>)-2-octenal (30)		Percy et al. (1980)
	<i>Apodiphus amygdali</i>	<i>n</i> -Tridecane	(<i>E</i>)-2-decenal		Pavis (1987)
	<i>Chinavia aseada</i> (<i>Acrosternum aseadum</i>) (1st, 2nd instars)	<i>n</i> -Tridecane	(<i>E</i>)-2-decenal	4-oxo-(<i>E</i>)-2-decenal (1st instar)	Borges and Aldrich (1992); name change: Schwertner and Grazia (2007)
	<i>Chinavia hilaris</i> (<i>Acrosternum hilare</i>) (1st, 2nd instars)	<i>n</i> -Tridecane (29–39)	(<i>E</i>)-2-decenal (23)	4-oxo-(<i>E</i>)-2-decenal (1st instar)	Borges and Aldrich (1992); name change: Schwertner and Grazia (2007)
	<i>Chinavia impicticornis</i> (5th instar)	4-oxo-(<i>E</i>)-2-hexenal (70)	<i>n</i> -Tridecane (18)	(<i>E</i>)-2-decenal (10)	Pareja et al. (2007)
	<i>Chinavia ubica</i> (5th instar)	4-oxo-(<i>E</i>)-2-hexenal (30)	(<i>E</i>)-2-decenal (20)	<i>n</i> -Tridecane (15)	Pareja et al. (2007)
	<i>Chlorochroa ligata</i> (1st, 2nd instars)	<i>n</i> -Tridecane (44–65)	(<i>E</i>)-2-decenal (30)	(<i>E</i>)-2-octenal (16; 1st instar)	Fucarino et al. (2004)
	<i>Chlorochroa sayi</i> (1st, 2nd instars)	<i>n</i> -Tridecane (27–45)	(<i>E</i>)-2-octenal (15–29)		Fucarino et al. (2004)
	<i>Dichelops melacanthus</i> (5th instar)	4-oxo-(<i>E</i>)-2-hexenal (55)	<i>n</i> -Tridecane (23)		Pareja et al. (2007)
	<i>Edessa mediatibunda</i> (1st, 2nd, 5th instars)	<i>n</i> -Undecane	(<i>E</i>)-2-octenal		Borges and Aldrich (1992)
	<i>Eocanthocon furcellata</i> (last instar)	4-oxo-(<i>E</i>)-2-hexenal (78)	<i>n</i> -Tridecane (11)		Ho et al. (2003)
	<i>Euridema rugosa</i> (1st–3rd instars)	<i>n</i> -Tridecane; (<i>E</i>)-2-hexenal			Ishiwatari (1974)
	<i>Euridema pulchra</i> (1st–3rd instars)	(<i>E</i>)-2-hexenal	<i>n</i> -Tridecane		Ishiwatari (1974)
	<i>Euschistus conspersus</i> (1st, 2nd instars)	<i>n</i> -Tridecane (37–49)	<i>n</i> -Tetradecanal (13–28)	4-oxo-(<i>E</i>)-2-decenal (20; 1st instar)	Borges and Aldrich (1992)
	<i>Euschistus heros</i> (1st, 2nd, 5th instars)	<i>n</i> -Tridecane	<i>n</i> -Tetradecanal, 4-oxo-(<i>E</i>)-2-decenal (1st instar), 4-oxo-(<i>E</i>)-2-hexenal		Borges and Aldrich (1992); Pareja et al. (2007)
Family	Species (# of specimens; instar examined)	Most abundant compound (rel. abundance, when available)	Next most abundant compound (rel. abundance, when available)	Other compounds (rel. abundance, when available; instar where compound is found)	Reference
	Pentatomidae (cont'd)				
	<i>Euschistus tristigmus</i> (1st, 2nd, 4th instars)	<i>n</i> -Tridecane	4-oxo-(<i>E</i>)-2-hexenal, (<i>E</i>)-2-octenal	4-oxo-(<i>E</i>)-2-decenal (1st instar)	Borges and Aldrich (1992)
	<i>Lincus spureus</i> (5th instar)	<i>n</i> -Undecane (55–62)	(<i>E</i>)-hexenal (20–27)	4-oxo-(<i>E</i>)-2-hexenal (14)	Cassier et al. (1994)
Pentatomidae (cont'd)	<i>Nezara viridula</i> (1st–5th instars)	<i>n</i> -Tridecane	(<i>E</i>)-2-decenal	4-oxo-(<i>E</i>)-2-decenal (1st instar), 4-oxo-(<i>E</i>)-2-hexenal	Fucarino et al. (2004); Borges and Aldrich

Family	Species (# of specimens; instar examined)	Most abundant compound (rel. abundance, when available)	Next most abundant compound (rel. abundance, when available)	Other compounds (rel. abundance, when available; instar where compound is found)	Reference
Coreidae	<i>Pallantia maculainia</i> (1st–5th instars)	<i>n</i> -Tridecane (30–44)	(<i>E</i>)-2-hexenal (20–34); 4-oxo-(<i>E</i>)-2-hexenal (8–32)	(<i>E</i>)-2-decenal (8–27)	(1992); Pavis et al. (1994)
	<i>Piezodorus guildinii</i> (5th instar)	4-oxo-(<i>E</i>)-2-hexenal (20)	<i>n</i> -Tridecane (9)		Fávaro et al. (2011)
	<i>Podisus maculiventris</i> (5th instar)	(<i>E</i>)-2-hexenal (36)	<i>n</i> -Tridecane (28)	4-oxo-(<i>E</i>)-2-hexenal (13); linalool (15)	Pareja et al. (2007)
	<i>Tessaratoma aetiops</i> (5th instar)	<i>n</i> -Tridecane	(<i>E</i>)-2-decenal	(<i>E</i>)-2-octenal	Aldrich et al. (1984)
	<i>Thyanta pallidovirens</i> (1st, 2nd instars)	<i>n</i> -Tridecane (37)	(<i>E</i>)-2-decenal (10–19)		Calam and Youdeowi (1968)
	<i>Acanthocerus galeator</i> (not indicated)	(<i>E</i>)-2-hexenal	4-oxo-(<i>E</i>)-2-hexenal	4-oxo-(<i>E</i>)-2-decenal (9); 1st instar	Fucarino et al. (2004)
	<i>Amblypelta nitida</i> (1st–5th instars)	<i>n</i> -Hexanal		(<i>E</i>)-2-octenal	Aldrich and Yonke (1975)
	<i>Anoplocnemis dallasiana</i> (not indicated)	(<i>E</i>)-2-hexenal (58)			Pavis (1987)
	<i>Archimerus alternatus</i> (not indicated)	(<i>E</i>)-2-hexenal	4-oxo-(<i>E</i>)-2-hexenal (35)		Prestwich (1976)
	<i>Holopterna allata</i> (not indicated)	(<i>E</i>)-2-hexenal (90)	4-oxo-(<i>E</i>)-2-hexenal (10)	(<i>E</i>)-2-octenal	Aldrich and Yonke (1975)
	<i>Leptoglossus clypealis</i> (not indicated)	(<i>E</i>)-2-hexenal	4-oxo-(<i>E</i>)-2-hexenal		Prestwich (1976)
	<i>Leptoglossus occidentalis</i> (3rd and 4th instars)	(<i>E</i>)-2-hexenal			Aldrich and Yonke (1975)
	Species (# of specimens; instar examined)	Most abundant compound (rel. abundance, when available)	Next most abundant compound (rel. abundance, when available)	Other compounds (rel. abundance, when available; instar where compound is found)	Blatt et al. (1998)
Coreidae (cont'd)	<i>Leptoglossus opposites</i> (not indicated)	(<i>E</i>)-2-hexenal	4-oxo-(<i>E</i>)-2-hexenal		Aldrich and Yonke (1975)
	<i>Piemistria bispina</i> (not indicated)	(<i>E</i>)-2-octenal (44–59)	4-oxo-(<i>E</i>)-2-hexenal (25–50)	(<i>E</i>)-2-hexenal (0–19)	Baker and Jones (1969)
	<i>Thasus neocalifornicus</i> (3rd and 4th instars)	(<i>E</i>)-2-hexenal	4-oxo-(<i>E</i>)-2-hexenal		Prudic et al. (2008)