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Inter- and intraspecific variation in defensive compounds produced by five neotropical stink bug species (Hemiptera: Pentatomidae)

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Abstract

The differences in composition of defensive secretions between nymphs, adult males and adult females of *Chinavia impicticornis* (= *Acrosternum impicticorne*), *Chinavia ubica* (= *Acrosternum ubicum*), *Euschistus heros*, *Dichelops melacanthus* and *Piezodorus guildinii* (Hemiptera, Pentatomidae) were analysed within and between species using compositional log-ratio statistics and canonical variates analysis. Differences in composition between nymphs, males and females were found for all species, as well as when all species were pooled. In particular, tetradecanal appears to be a predominantly nymphal compound in *D. melacanthus*, *E. heros* and *P. guildinii*. In the two *Chinavia* species 4-oxo-(*E*)-2-hexenal and an unknown compound were more dominant in nymphs. The interspecific analysis revealed a good separation of defensive compounds according to their taxonomic relationship. Thus, the two *Chinavia* species grouped together, with (*E*)-2-decenal and (*E*)-2-hexenyl acetate, contributing to this separation. The other three species also differed from each other, with (*E*)-2-octenal associated to *D. melacanthus*, (*E*)-2-hexenal to *P. guildinii* and (*E,E*)-2,4-decadienal and tetradecanal to *E. heros*. The pooled analysis of stage ignoring species revealed tetradecanal and 4-oxo-(*E*)-2-decenal (tentative identification) strongly associated to nymphs. Thus, there are predictable differences between stages, and many of the differences are conserved between species. Consideration of these differences could prove to be important in understanding stink bug–natural enemy interactions, and in optimising biocontrol efforts.
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Keywords: *Euschistus*; *Chinavia*; *Piezodorus*; *Dichelops*; *Acrosternum*; Alarm pheromone; Compositional analysis

1. Introduction

Heteropteran species are well known for producing blends of odoriferous compounds that serve the dual purpose of defence against predation and as alarm pheromones. Despite the vast information on this defensive chemistry of many pentatomid species (Borges and Aldrich, 1992; Aldrich et al., 1993a; Zarbin et al., 2000) studies on the differences in the blends produced by nymphs and adults (Aldrich and Yonke, 1975; Borges and Aldrich, 1992; Farine et al., 1992b; Blatt et al., 1998) and between males and females (Aldrich et al., 1993b; Ho et al., 2003) have carried out mainly qualitative analyses of differences, and there have been few quantitative assessments of variations in volatile blends. Furthermore, little is known about how conserved blends are between nymphs, males and females between species, and whether these blends reflect the taxonomic relationships between pentatomid species. To a large extent this is because few statistical techniques that allow the evaluation of entire blends of compounds have been applied, since individual components of exocrine blends are not independent of each other, and they may share biosynthetic pathways. Thus, statistical techniques that allow for analysis of multicomponent blends need to be used, such as the log-ratio multivariate approach (Aitchison, 1986), which was developed for analysis of compositional data constrained by the total amount of compounds (in this case) produced.

Hemipteran exocrine secretions have important roles in within-species interactions (Lockwood and Story, 1985; Fucarino et al., 2004), but can also play important roles in interspecific interactions, in particular with predators and parasitoids (Aldrich, 1995). Though these compounds

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evolved for defence, there is evidence that they can also serve as kairomones for specific natural enemies. For example, there is evidence that (*E*)-2-hexenal, (*E*)-2-decenal and (*E*)-2-octenal are attractive to parasitoids in the genus *Telenomus* both in the laboratory (Laumann, Moraes and Borges, unpublished data) and in the field (Borges and Aldrich, 1994; Peres, 2004). Thus, quantitative knowledge of similarities and differences within and between species could prove to be important when designing control strategies using natural enemies, including entomopathogenic fungi, since some compounds have fungicidal activity (Borges et al., 1993).

The aims of this study were (a) to quantitatively differentiate the defensive chemistry of adult female, adult male and 5th instar nymphs of five common neotropical stink bug species that are important agricultural pests in Brazil and other South American countries: *Chinavia impicticornis* (= *Acrosternum impicticorne*) (Stål), *Chinavia ubica* (= *Acrosternum ubicum*) (Rolston) (note: the Neotropical and Nearctic species of *Acrosternum* have been placed in the *Chinavia* genus (Orian, 1965) according to Schwertner (2005) and Schwertner and Grazia (2006)), *Euschistus heros* (F.), *Dichelops melacanthus* (Dallas) and *Piezodorus guildinii* (Westwood); and (b) to test whether the differences in blends between different species mirrors the taxonomic differentiation of the species. The understanding of the variation within and between species is important from an evolutionary perspective, in that it can provide information on the evolution of insect defence and chemical communication. From an applied perspective the exploitation of these compounds by natural enemies and their anti-microbial properties could make them important in biological control.

2. Materials and methods

2.1. Insect rearing

Chinavia impicticornis, *C. ubica*, *E. heros*, *D. melacanthus* and *P. guildinii* were reared in 8-l plastic containers in a controlled environment room at 26 ± 1 °C, 65 ± 10% relative humidity and 14:10 light:dark photoperiod. They were fed sunflower seeds (*Helianthus annus* (L.)), soybeans (*Glycine max* (L.) Merrill.), raw peanuts (*Arachis hypogaea* (L.)) and green beans (*Phaseolus vulgaris* (L.)).

2.2. Extraction of defensive compounds

For extracting the defensive compounds of adults, 10–15-day-old insects that had recently died (within the previous 12 h) were pinned through the thorax with the dorsal side up, the abdomen was opened with dissection scissors and the viscera removed, revealing the metathoracic scent gland (MTG) (Zarbin et al., 2000). We used this methodology to avoid stressing live insects, which can cause emptying of the MTG. The gland was then pierced with a flame-stretched glass capillary, causing the contents to rise through the capillary. The contents were then emptied into 200 μl of solvent by immersing the tip of the capillary into the solvent and briefly passing charcoal-filtered air through the capillary. For each species, the contents of glands from 10 males and 10 females were collected. Five of the male samples were dissolved in redistilled *n*-hexane and five in dichloromethane (CH_2Cl_2) and similarly for the 10 female samples were also treated.

The defensive compounds of 5th instar nymphs are produced by the dorsal abdominal gland (DAG), which is shed along with the exuvia when moulting, and defensive compounds can be collected from these exuviae (Borges and Aldrich, 1992). Fresh exuviae (<12 h old) were collected and immersed individually in a glass vial with 500 μl of solvent (Borges and Aldrich, 1992; Aldrich et al., 1993a). The cuticle was macerated with a Teflon rod, and left to stand for 5 min. Two hundred microlitres were transferred to a clean glass vial to separate the sample from the macerated tissue, and the remaining sample was discarded. Ten exuviae of each species were collected, five of which were immersed in *n*-hexane and five in CH_2Cl_2.

2.3. Chemical analyses

The defensive compounds were qualitatively analysed by coupled gas chromatography–mass spectrometry (GC–MS) equipped with a TRB-5 column (30 m, 0.25 mm ID, 0.25 μm film thickness, Teknokroma, Barcelona, Spain) on a temperature program of 50 °C/2 min, 15 °C/min to 250 °C/5 min and helium as the carrier gas. Ionisation was by electron impact (70 eV, source temperature 250 °C) and a quadrupole mass analyser (Shimadzu GCMS-QP2010) was used. Tentative identification was carried out by comparing mass spectra and retention indices of compounds against mass spectral databases or spectra in the literature. Identifications were confirmed by comparing mass spectra and KI retention indices against those from authentic standards injected under the same conditions.

Quantitative analysis was carried out by GC-flame ionisation detector (FID-250 °C). An aliquot of 50 μl of the sample was taken, and 5 μl of internal standard (IS, (*Z*)-3-hexenyl acetate 1.02 μg/ml) was added. This sample was then injected into the GC (Shimadzu 17A) equipped with a DB-5 column (30 m, 0.25 mm ID, 0.25 μm film thickness, J&W Scientific, Folsom, CA, USA) on a temperature ramp of 50 °C/2 min, 15 °C/min to 250 °C/5 min and helium as the carrier gas. For accurate quantification of the major compounds response factors (RF) were calculated by separately coinjecting 100 μg/ml solutions of the IS and the following authentic standards: hexanal (RF = 0.70), (*E*)-2-hexenal (0.79), hexan-1-ol (0.95), 4-oxo-(*E*)-2-hexenal (0.27), (*E*)-2-hexenyl acetate (0.90), (*E*)-2-octenal (1.03), (*E*)-2-octenyl acetate (0.72), nonanal (0.89), (*E*)-2-decenal (1.13), undecane (1.57), dodecane (1.65), tridecane (1.38) and tetradecanal (1.18) . For the other compounds in the blend a response factor = 1 with respect

to the IS was assumed. All authentic standards were purchased from Sigma-Aldrich (St. Louis, MO, USA), Fluka (Buchs., Switzerland), Bedoukian (Danbury, CT, USA) or TCI (Tokyo, Japan). Synthetic 4-oxo-(*E*)-2-hexenal and (*E*)-2-decenyl acetate were provided by J.R. Aldrich (USDA, Beltsville, MD, USA).

2.4. Statistical analyses

To evaluate the variation in the relative contributions of different compounds to the blends of nymphs, females and males within each species a compositional log-ratio approach was taken (Aitchison, 1986), which considers the contribution of each component to the blend as a whole and was recently applied to the analysis of volatile blends (Pareja et al., 2007). This analysis uses a multivariate approach, and corrects for the unit sum constraint inherent in data that form part of a blend using the log-ratio transformation. Not correcting for these characteristics inherent in the data has led to huge confusion in the analysis of proportional data in the past (Aitchison, 1986).

The concentration of each compound was converted to a proportion in the total, and any zero values were corrected according to Aitchison (1986). These values were then expressed as a proportion of a single component (here the IS was used), and this value was converted to logarithms (log ratios). The data were then analysed by multivariate analysis of variance (MANOVA), incorporating each compound as a variate and stage (nymph, male or female) and solvent as factors, giving the significance of differences between nymphs, females and males by means of an *F*-test. Assessment of which compounds contributed most to separating them was done by means of a canonical variate analysis (CVA), which gives a linear combination of the variables, which best discriminates between the levels of a factor. The coefficient estimates for each variable (loadings) the importance of each variate in discriminating between the levels (in our case stages or species), and visualised by means of a CVA biplot.

The interspecific variation in defensive chemistry was analysed using the same approach. The factors included in the model were species, stage and solvent. Two separate descriptive CVAs were carried out: one to discriminate species, independent of stage within species, and the second to discriminate stage independent of species.

3. Results

A total of 38 compounds were detected from the five species (Figs. 1 and 2). Two tentatively identified compounds ((*E,Z*)-2,4-decadienal and tridecanal) were not quantified, the first being present in all species other than *P. guildinii*, but often masked by tridecane. The second was present only in two samples from *C. ubica* females. The identity of four compounds remains unknown, though unknown 1 had the same mass spectrum as the unknown reported by Drijfhout et al. (2002), and is likely to be the same compound. Of the 38 compounds only 4-oxo-(*E*)-2-hexenal, unknown 1 and tridecane were found in all stages of all five species. Undecane, dodecane, 1-tridecene (tentative), tetradecane, pentadecane and unknown 4 were found in all species, but not all stages. In the two *Chinavia* species, 4-oxo-(*E*)-2-hexenal and tridecane were the two most abundant compounds. In both species, these compounds were found in approximately the same concentration in adults, but in nymphs the concentration of 4-oxo-(*E*)-2-hexenal was higher than tridecane for both species. In *D. melacanthus* the most abundant compound was 4-oxo-(*E*)-2-hexenal in all stages, while in *E. heros* 4-oxo-(*E*)-2-hexenal was the most abundant compound in males and nymphs, but tridecane was greater in females. In *P. guildinii* 4-oxo-(*E*)-2-hexenal was the most abundant in all stages, and tridecane and (*E*)-2-hexenal are found produced in large quantities, though the latter, only in males and females. The solvent used affected the detection of compounds for every analysis carried out (see results below), with the more polar compounds being retrieved more efficiently in CH_2Cl_2 (Fig. 3). This effect was most evident with 4-oxo-(*E*)-2-hexenal and the tentative identification of 4-hydroxy-4-methyl-2-pentanone. 4-oxo-(*E*)-2-decenal, also tentative, was present in low amounts, but was only detected with CH_2Cl_2 (Fig. 3).

When log ratios for each species were analysed by MANOVA significant effects of stage and solvent were found for every species (Table 1). The major compounds separating stages varied between species (Table 1), but in *D. melacanthus*, *E. heros* and *P. guildinii* tetradecanal was strongly associated to nymphs.

For the interspecific analysis the five species fitted as groups in the model had a significant effect on blend composition, as did stage and solvent (Table 1). For the descriptive CVA for species independent of stage there are four dimensions that explain 100% of the variation, since there are $t-1$ possible canonical variates (where t is the number of levels in the factor; in this case $t = 5$ species). However, the first two dimensions explained 85% of the variation. So only these are presented. It can be seen clearly that the two *Chinavia* spp. separated from the other three (Fig. 4A). With the large number of compounds interpretation is more difficult, but the major compounds separating each species are listed in Table 1, and shown graphically in Fig. 4A.

When the CVA was carried out for stage independent of species, nymphs separated well from both adult stages (Fig. 4B), with the relative proportion of tetradecanal being very important in determining this separation.

This is the first report of the defensive chemistry of *C. impicticornis*, *C. ubica* and *D. melacanthus*. For *E. heros* and *P. guildinii* several new compounds are reported. In *E. heros* this is, to our knowledge, the first report of (*E*)-2-hexenal, decane, (*E*)-2-hexenyl acetate, limonene, (*E*)-2-octenal, (*E*)-2-octen-1-ol, undecane, linalool, (*E*)-2-hexenyl butyrate, dodecane, (*E*)-2-octenyl acetate, tetradecane, (*E*)-2-decenyl acetate and pentadecane. Of the tentative

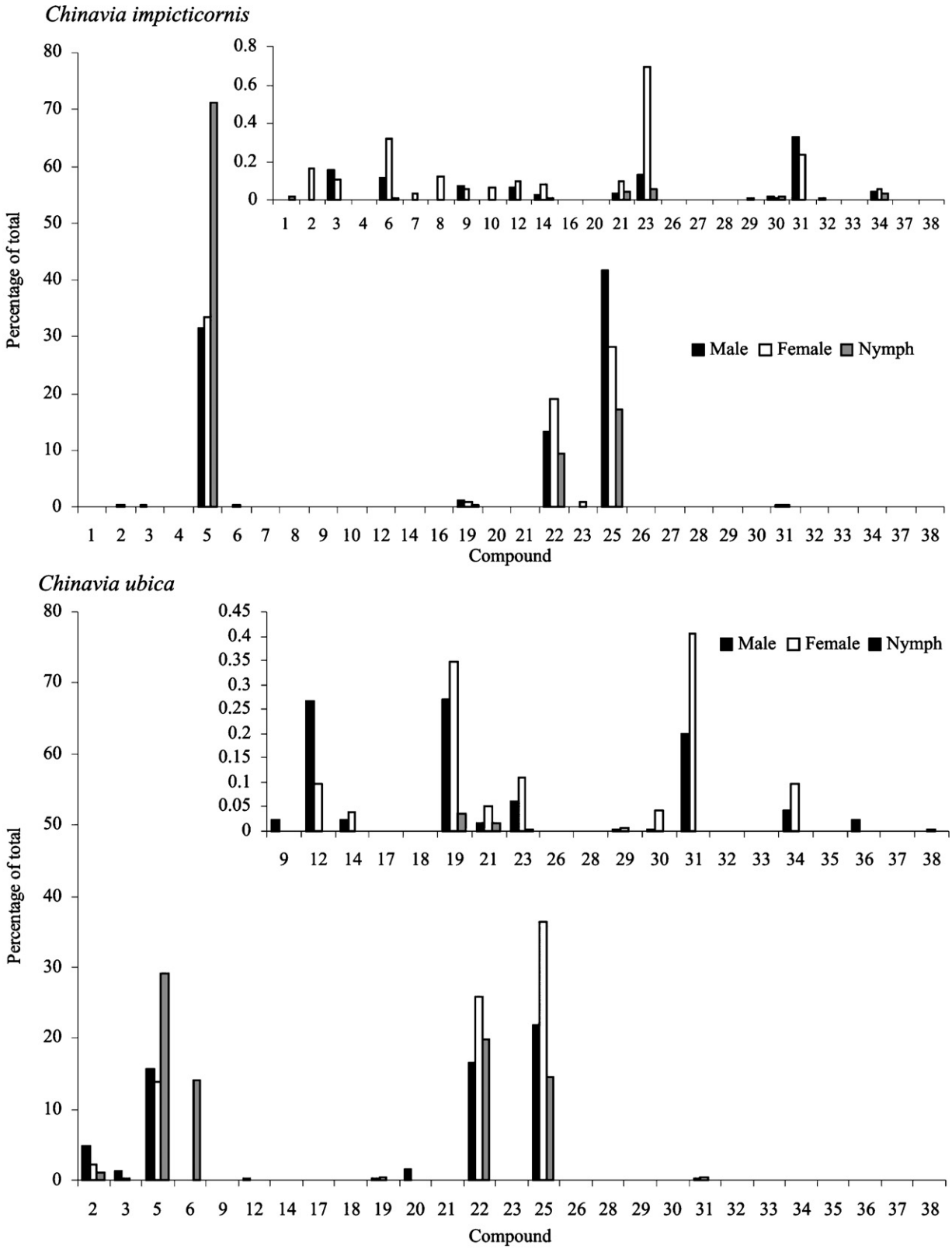

Fig. 1. Mean percentage (with respect to the total) recovered from males, females and nymphs of *Chinavia impicticornis* (top) and *C. ubica* (bottom). The numbers in the graphs indicate compounds that are present in the extracts, including those that are present in trace quantities (bars too small to visualise). The insets show the compounds present in relatively less amounts and asterisks indicate tentative identifications. **1.** hexanal; **2.** 4-hydroxy-4-methyl-pentanone*; **3.** (*E*)-2-hexenal; **4.** hexan-1-ol; **5.** 4-oxo-(*E*)-2-hexenal; **6.** Unknown 1; **7.** decane; **8.** hexyl acetate; **9.** (*E*)-2-hexenyl acetate; **10.** limonene; **12.** (*E*)-2-octenal; **14.** undecane; **16.** nonanal; **17.** (*E*)-2-nonenal; **19.** dodecane; **20.** (*E*)-2-octenyl acetate; **21.** (*Z*)-2-decenal*; **22.** (*E*)-2-decenal; **23.** 1-tridecene*; **25.** tridecane; **26.** (*E*,*E*)-2,4-decadienal; **27.** 4-oxo-(*E*)-2-decenal*; **28.** unknown 2; **29.** 1-tetradecene*; **30.** tetradecane; **31.** (*E*)-2-decenyl acetate; **32.** unknown 3; **33.** 1-pentadecene*; **34.** pentadecane; **37.** unknown 4; **38.** tridecan-1-ol*.

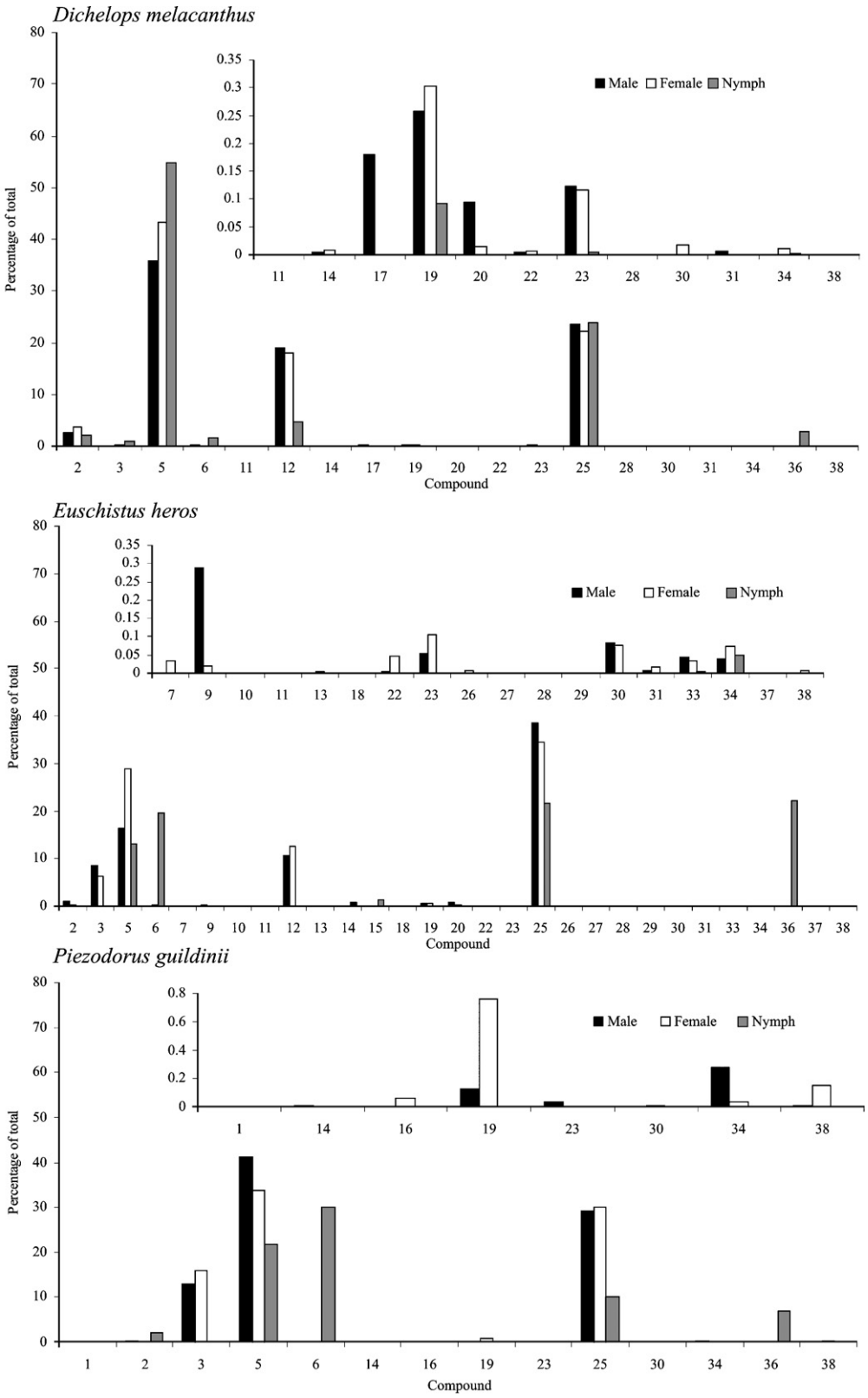

Fig. 2. Mean percentage (with respect to the total) recovered from males, females and nymphs of *Dichelops melacanthus* (top), *Euschistus heros* (middle) and *Piezodorus guildinii* (bottom). The numbers in the graphs indicate compounds that are present in the extracts, including those that are present in trace quantities (bars too small to visualise). The insets show the compounds present in lower relative amounts and asterisks indicate tentative identifications. **1.** hexanal; **2.** 4-hydroxy-4-methyl-pentanone*; **3.** (*E*)-2-hexenal; **5.** 4-oxo-(*E*)-2-hexenal; **6.** unknown 1; **7.** decane; **9.** (*E*)-2-hexenyl acetate; **10.** limonene; **11.** (*Z*)-2-octenal; **12.** (*E*)-2-octenal; **13.** (*E*)-2-octen-1-ol; **14.** undecane; **15.** linalool; **16.** nonanal; **17.** (*E*)-2-nonenal; **18.** (*E*)-2-hexenyl butyrate; **19.** dodecane; **20.** (*E*)-2-octenyl acetate; **22.** (*E*)-2-decenal; **23.** 1-tridecene*; **25.** tridecane; **26.** (*E*,*E*)-2,4-decadienal; **27.** 4-oxo-(*E*)-2-decenal*; **28.** unknown 2; **29.** 1-tetradecene*; **30.** tetradecane; **31.** (*E*)-2-decenyl acetate; **33.** 1-pentadecene*; **34.** pentadecane; **36.** tetradecanal; **37.** unknown 4; **38.** tridecan-1-ol*.

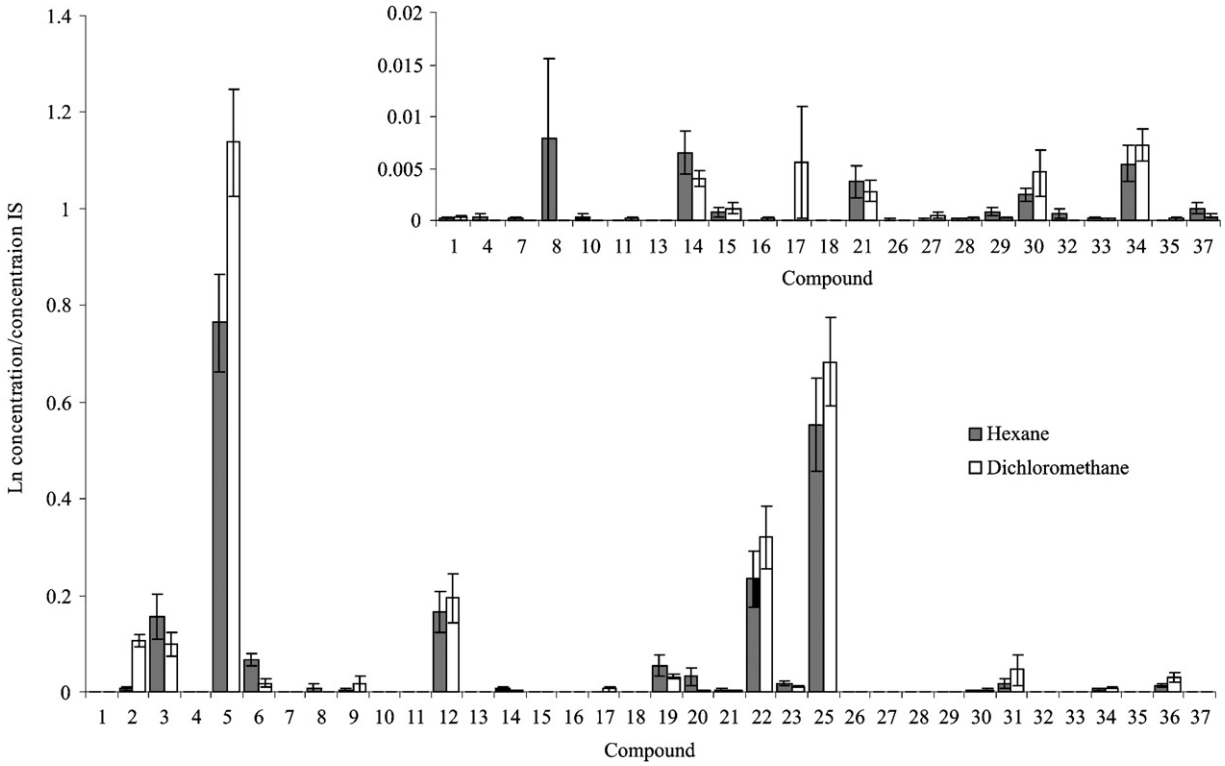

Fig. 3. Mean (±s.e.) of the ratio of each compound with respect to the internal standard (IS) extracted using *n*-hexane and dichloromethane. The insect shows some of the less abundant compounds in detail. Asterisks indicate tentative identifications. **1.** hexanal; **2.** 4-hydroxy-4-methyl-pentanone*; **3.** (*E*)-2-hexenal; **4.** hexan-1-ol; **5.** 4-oxo-(*E*)-2-hexenal; **6.** unknown 1; **7.** decane; **8.** Hexyl acetate; **9.** (*E*)-2-hexenyl acetate; **10.** limonene; **11.** (*Z*)-2-octenal; **12.** (*E*)-2-octenal; **13.** (*E*)-2-octen-1-ol; **14.** undecane; **15.** linalool; **16.** nonanal; **17.** (*E*)-2-nonenal; **18.** (*E*)-2-hexenyl butyrate; **19.** dodecane; **20.** (*E*)-2-octenyl acetate; **21.** (*Z*)-2-decenal*; **22.** (*E*)-2-decenal; **23.** 1-tridecene*; **25.** tridecane; **26.** (*E*,*E*)-2,4-decadienal; **27.** 4-oxo-(*E*)-2-decenal*; **28.** unknown 2; **29.** 1-tetradecene*; **30.** tetradecane; **31.** (*E*)-2-decenyl acetate; **32.** unknown 3; **33.** 1-pentadecene*; **34.** pentadecane; **35.** tridecanal **36.** tetradecanal; **37.** unknown 4.

identifications, the novel compounds for this species are 4-hydroxy-4-methyl-2-pentanone, (*Z*)-2-octenal, 1-tridecene, (*E*, *Z*)-2,4-decadienal, tetradecene, 1-pentadecene. In *P. guildinii* this is the first report of hexanal, tetradecane, pentadecane and tetradecanal, and is also the first tentative identification of 4-hydroxy-4-methyl-2-pentanone.

4. Discussion

Using a quantitative statistical evaluation of differences between entire blends produced by nymphs, adult males and adult females we have shown that there are within-species differences in volatile blends, and also that between species there are certain compounds that are life stage specific. This stage and sex specificity has been previously demonstrated in other Heteroptera (Aldrich and Yonke, 1975; Borges and Aldrich, 1992; Aldrich et al., 1993a; Ho et al., 2003), but often no differences between sexes are found despite different behavioural responses by males and females to different blends (Kou et al., 1989; Farine et al., 1992a, b). From these results, we suggest that the major nymphal compounds are tetradecanal in *D. melacanthus*, *E. heros* and *P. guildinii*, and 4-oxo-(*E*)-2-hexenal and unknown 1 in *C. impicticornis* and *C. ubica*. Unknown 1 appears to be an isomer of 4-oxo-(*E*)-2-hexenal (Table 1). So the production of these compounds is likely to be related. Several other studies have reported a similar compound, or one eluting at the corresponding retention time (Borges and Aldrich, 1992; Drijfhout et al., 2002; Fucarino et al., 2004) suggesting that this compound is a common natural product in Pentatomidae. In *C. impicticornis* the tentatively identified 4-oxo-(*E*)-2-decenal was present in higher amounts in nymphs, and it might serve as an aggregation pheromone in early instars (Borges and Aldrich, 1992; Pavis et al., 1994; Fucarino et al., 2004), and possibly in fifth instars as well.

The blends of the five species grouped closely following the accepted taxonomic relationships of the species, with a high association of the two *Chinavia* species, and a closer grouping of *D. melacanthus* and *E. heros*. Thus alarm pheromones and defensive chemistry could have chemotaxonomic values, as has been highlighted previously (Blum, 1985). The balance between compounds conserved between species and between-species variability could give important evolutionary insights into the evolution of pheromonal communication. The conserved compounds could be due to very strong pheromonal or defensive functions, thus possibly having an adaptive value, moulded by natural selection. On the other hand, the conserved compounds might be a result of trade-offs or evolutionary

Table 1
Results for the within-species analyses for differences between stages, and the between-species analysis for differences between species and between stages

Analysis	Factor	F (df)	P	Compounds with largest loadings associated to each stage/species
Chinavia impicticornis	Stage	6.11 (28,30)	<0.001	*Females:* 4-Hydroxy-4-methyl-2-pentanone*; *Males:*Tridecane; *Nymphs:* 4-Oxo-(*E*)-2-hexenal, pentadecane
	Solvent	3.08 (14,15)	0.02	
Chinavia ubica	Stage	7.94 (34,24)	<0.001	*Females:*, (*E*)-2-Decenyl acetate, pentadecane, (*Z*)-2-decenal*; *Males:* (*E*)-2-Hexenal, (*E*)-2-octenal; *Nymphs:* Unknown 1
	Solvent	7.26 (17,12)	<0.001	
Dichelops melacanthus	Stage	5.39 (30,22)	<0.001	*Females:* (*E*)-2-Octenal, tetradecane; *Males:* Tridecane, pentadecane; *Nymphs:* Undecane, dodecane, tetradecanal
	Solvent	14.63 (15,11)	<0.001	
Euschistus heros	Stage	14.76 (36,18)	<0.001	*Females:* Decane; *Males:* (*E*)-2-Octenal, 1-tridecene*; *Nymphs:* Tetradecanal
	Solvent	3.76 (18,9)	0.02	
Piezodorus guildinii	Stage	15.53 (24,28)	<0.001	*Females:* Undecane, tetradecane; *Males:* 4-Oxo-(*E*)-2-hexenal, tridecane, pentadecane; *Nymphs:* Tetradecanal
	Solvent	4.28 (17,12)	0.006	
Between species	Species	9.54 (108,471)	<0.001	*Chinavia spp:* (*E*)-2-Decenal, (*E*)-2-hexenyl acetate, (*Z*)-2-decenal*, unknown 3, 1-tetradecene*, tridecane; *D. melacanthus:* (*E*)-2-Octenal, 4-oxo-(*E*)-2-hexenal, unknown 2; *E. heros:* (*E,E*)-2,4-Decadienal, tetradecanal, 1-pentadecene*, (*Z*)-2-octenal*, linalool; *P. guildinii:* (*E*)-2-Hexenal, unknown 4, decane
	Stage	5.50 (54,236)	<0.001	*Females:* Undecane, (*E,E*)-2,4-decadienal; *Males:* (*E*)-2-Hexenyl acetate, 1-tridecene*, unknown 3; *Nymphs:* Tetradecanal, tridecane, 4-oxo-(*E*)-2-hexenal
	Solvent	4.81 (27,118)	<0.001	

Results from the multivariate analysis of variance (F values, with degrees of freedom-df, and significance as indicated by the P-value) are presented. The major compounds separating stages or species are given by the loading (coefficients) derived from the canonical variate analysis. Asterisks indicate tentative identifications.

constraints on biosynthetic pathways. Thus, divergence between species might not occur because these pathways might serve other functions that are important, and change has deleterious effects in other areas of the organism's biology.

The variable compounds between species could have equally strong species-specific functions, but could also be redundant elements. Some *E*-isomers have been shown to be important alarm compounds (Pavis et al., 1994; Fucarino et al., 2004), but the function of *Z*-isomers remains unknown, and it is possible that they are present in equilibrium with the *E*-isomers in low amounts. There is strong evidence that males, females and nymphs respond differently to blends from different stages or sexes (Kou et al., 1989; Blatt et al., 1998). Our study shows that there are predictable changes in blends within species that might be responsible for these behavioural effects and compositional differences that are potentially behaviourally relevant. (*E*)-2-Hexenal and (*E*)-2-hexenyl acetate appear to be active components of the alarm pheromone blend of *N. viridula* (Lockwood and Story, 1987) and also serve as repellents (Gunawardena and Herath, 1991). Tridecane has been shown to contribute to the toxicity of defensive blends (Gunawardena and Herath, 1991) and potentially acts as an aggregation pheromone and an alarm pheromone in a dose-dependent manner in *Nezara viridula* (Lockwood and Story, 1985), though in a recent study no repellence or aggregation of *N. viridula* was observed (Fucarino et al., 2004). Stressed *E. heros* and *P. guildinii* often do not release one or all sex pheromone components, while releasing large amounts of tridecane and other MTG secretions (Borges et al., in press; Moraes, Laumann and Borges, submitted manuscript), suggesting that the release of tridecane can play an important role in both intra and interspecific interactions.

Besides the action of these compounds as alarm pheromones, it is possible that the balance between defensive and aggregation needs of nymphs and adults is different, and this might have contributed to differences in defensive chemistry detected in this study. In the Pentatomidae the male releases the sex pheromone (Moraes et al., 2005b; Borges et al., 2006, in press). Furthermore, both males and females produce sexual vibratory signals (Moraes et al., 2005a), which are exploited by parasitoids for host location (Laumann et al., 2007), so are more detectable than nymphs. This greater exposure could have led to selection on the adults, particularly the male, for more potent defences against natural enemies (Aldrich, 1996). Aldrich and Yonkes (1975) argued that the compositions of Heteropteran defensive chemistry in nymphs is highly conserved between species, and our results support this claim.

Natural enemies are known to extensively exploit volatile components of exocrine secretions (Aldrich, 1995). For example, the egg parasitoid *Trissolcus basalis* prefers female odours over male odours from a distance (Colazza

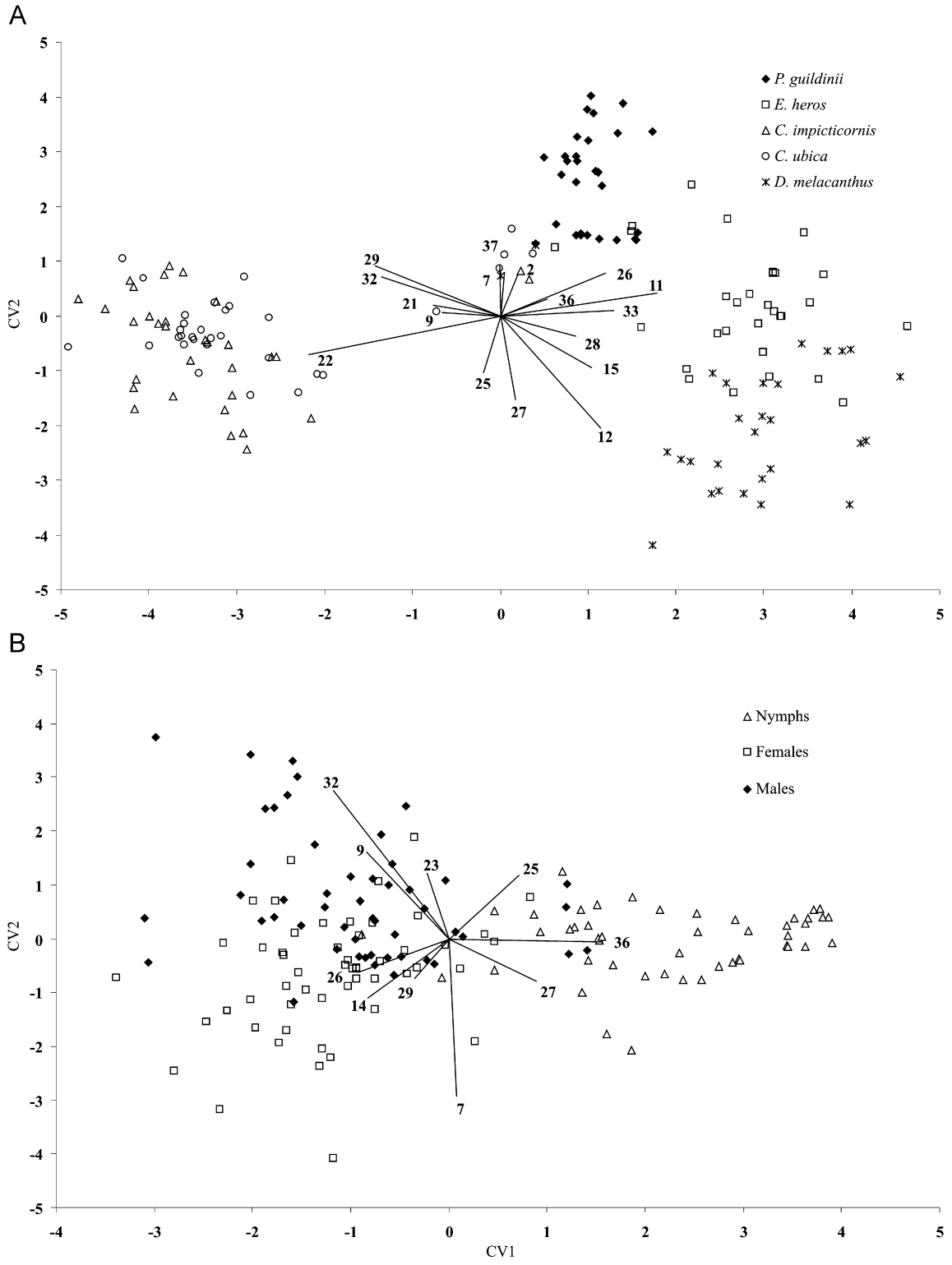

Fig. 4. Canonical variates analysis (CVA) biplot for (A) the analysis differentiating between species, and (B) the stage analysis independent of species. The points are the individual scores for each replicate, calculated from the CVA equation that maximises differences between treatments along the two dimensions defined by the two canonical variates (CV1 and CV2). The lines are the loadings for each of the variates (compounds), and the length of the line represents the relative magnitude of the importance of each compound in differentiating between treatments in the two dimensions (multiplied by a constant for better visualisation). For (A) only the first two canonical variates (representing 85% of the variability) are presented out of the four possible canonical variates. Asterisks indicate tentative identification. **2.** 4-hydroxy-4-methyl-pentanone*; **7.** decane; **9.** (*E*)-2-hexenyl acetate; **11.** (*Z*)-2-octenal*; **12.** (*E*)-2-octenal; **14.** undecane; **15.** linalool; **21.** (*Z*)-2-decenal*; **22.** (*E*)-2-decenal; **23.** 1-tridecene*; **25.** tridecane; **26.** (*E*,*E*)-2,4-decadienal; **27.** 4-oxo-(*E*)-2-decenal*; **28.** unknown 2; **29.** 1-tetradecene*; **32.** unknown 3; **33.** 1-pentadecene*; **36.** tetradecanal; **37.** unknown 4.

et al., 1999), and this could be due to volatile exocrine secretions, since this parasitoid was attracted to (*E*)-2-decenal from a distance (Mattiacci et al., 1993). Another egg parasitoid, *Telenomus podisi* uses adult kairomones to find its host, and parasitoid strains from different geographical origins have different responses to host kairomones (Borges et al., 2003). Also, *T. podisi* is attracted to both (*E*)-2-hexenal and 4-oxo-(*E*)-2-hexenal (Laumann, Moraes and Borges, unpublished data), the two major components found here in female *E. heros*, the preferred host for this parasitoid (Sujii et al., 2002). These forms of attraction could be cases of infochemical detour (Vet and Dicke, 1992), where the egg parasitoid uses a more detectable cue from a host stage closely associated to the egg stage, which is the target for parasitism. In the field overall parasitism was increased on *N. viridula* with the presence of septa containing (*E*)-2-hexenal, while for *E. heros* it was greater with septa containing both (*E*)-2-hexenal and tridecane (Peres, 2004). Further work is being carried out to understand whether the attraction to chemical compounds by different parasitoid species corresponds to the major components in the defensive blends of the preferred hosts, since it has been shown for other systems that the specificity of the parasitoid–pentatomid association is reflected in the use of host semiochemicals by parasitoids (Conti et al., 2004).

These semiochemically mediated interactions with natural enemies show that understanding the variability in the production of these compounds, and their effects on predators and parasitoids could be important in determining the potential success of biocontrol strategies. Pentatomid species differ in their susceptibility to entomopathogenic fungi, with *E. heros* being less susceptible than *P. guildinii* or *N. viridula* (Sosa-Gómez and Moscardi, 1998), and it has been shown that two components of the exocrine secretions of *N. viridula*, (*E*)-2-hexenal and (*E*)-2-decenal, inhibit spore germination and mycelial development of this fungus (Borges et al., 1993). It is possible that components of the exocrine blend contribute to these differences in susceptibility. Infection of the pentatomid rice pest *Tibraca limbativentris* by this fungus is higher than the species mentioned above (da Silva Martins et al., 2004), and neither (*E*)-2-hexenal nor (*E*)-2-decenal is present in aeration extracts from this species (Borges et al., 2006), nor were they found in preliminary MTG extracts (Moraes, Laumann and Borges, unpublished data).

In conclusion, this study has provided evidence for differences in volatile blends between nymphs and adults, and also between males and females. Furthermore, the differences between nymphs and adults are relatively conserved, as evidenced by the separation of nymphs from adults in the pooled analysis. Understanding the differences between blends and the variability in production could prove very important in understanding the evolution of pentatomid defensive chemistry, and also how these affect their interactions with natural enemies and their potential use in pest management.

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