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Metasternal Gland Volatiles and Sexual Communication in the Triatomine Bug, *Rhodnius prolixus*

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Abstract Twelve compounds produced by the metasternal glands (MGs) of the triatomine bug *Rhodnius prolixus* were identified by solid phase microextraction (SPME) combined with coupled gas chromatography-mass spectrometry (GC-MS) using achiral and chiral columns. All substances were ketones or alcohols, and the same compound profile was found in the secretions produced by either sex. The most abundant compounds were 2-methyl-3-buten-2-ol, (2*S*)-pentanol, (3*E*)-2-methyl-3-penten-2-ol, and (2*R*/2*S*)-4-methyl-3-penten-2-ol. Emission of these compounds was detected more frequently from females than males, and females released them more frequently during the early hours of the scotophase, the period when sexual activity in this species is at its peak. These compounds were also detected in the headspace above mating pairs. Finally, the occlusion of the MG orifices of male or female bugs with paraffin resulted in a significant decrease in copulation frequency compared to sham-operated insects. Together, these data suggest that the MG secretions of *R. prolixus* may be involved in sexual communication.

Keywords *Rhodnius prolixus* · Sexual behavior · Metasternal glands · Pheromone · Volatiles · Identification

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Introduction

Rhodnius prolixus Stål 1859 (Heteroptera: Reduviidae) is the main vector of Chagas disease in northern South America and in parts of Central America (Schofield 1994). This species is well adapted to live in rural houses and is considered to be of major epidemiological importance (Monteiro et al. 2003). Approximately 16–18 million people in Latin America are infected with the Chagas disease parasite, *Trypanosoma cruzi*, and another 120 million are at risk (WHO 2005).

Baldwin et al. (1971) reported that copulating pairs of *R. prolixus* emit a pheromone that is attractive to males. This phenomenon has also been observed for another vector of Chagas disease, the bug *Triatoma infestans* (Manrique and Lazzari 1995). Relatively little is known about the mechanisms of long- or short-distance orientation that mediate sexual encounters between adults of species in the subfamily Triatominae, although Baldwin et al. (1971) suggested that feeding triggers the development of sexual attraction in *R. prolixus* and that unfed males of this species do not respond to the apparent odor emitted by mating pairs.

R. prolixus adults have a pair of metasternal glands (MGs) that open to the ventral metathorax (Brindley 1930). Their function and the chemical identity of any secretions are unknown. Another set of glands of this insect, the Brindley's glands, which secrete isobutyric acid as the most abundant compound, are likely associated with alarm and defense functions (Ward 1981; Cruz López et al. 1995; Rojas et al. 2002; Manrique et al. 2006). It has been suggested that compounds produced by Brindley's glands are involved in the sexual chemical communication of triatomines (Cruz López et al. 2001; Rojas et al. 2002; Guerenstein and Guerin 2004).

In a recent study, Manrique et al. (2006) suggested that the secretions of the MGs of *T. infestans* are involved both in sexual and alarm communication, and that the secretions of Brindley's glands are restricted to alarm and defensive roles. These authors identified several highly volatile ketones and alcohols produced by the MGs and showed that the contents of these glands are emitted by adults of this species during copulation.

Our primary objective in this study was to identify volatile secretions produced by the MGs of *R. prolixus*. We further tested whether the MG compounds are emitted at different phases of the light/dark cycle by virgin males and females and examined if mating pairs of this species emit these substances. Finally, we tested whether the compounds emitted from these glands influence copulation.

Methods and Materials

Insects Insects were reared at 26±2°C and 60±10% r.h. Groups of fifth instars were sorted by sex and placed in separate flasks to keep them unmated until use in experiments. All insects used were kept under the 12:12 L:D photoperiod for at least 3 d before any experiment. Virgin insects were used for all experiments, and their nutritional status was varied according to the experiment performed. For odor-identification studies, unfed insects were dissected at d 20 after ecdysis. For the detection of emission of MG compounds, insects were fed at d 10, and used 20 d after ecdysis. For the remaining two experiments (i.e., detection of emission of MG odors during mating and evaluation of the effect of gland occlusion on mating success), insects were used at d 20 after being fed at d 10. All assays were performed at 26±2°C and 60±10% r.h.

Identification of Compounds Produced by Metasternal Glands Samples of 12 glands were obtained from six insects and stored in 2-ml vials sealed with Teflon®/silicone-lined caps. Control samples were prepared with pieces of tissue and cuticle from hind leg coxae. Female and male tissue was stored at −8°C for not more than 10 d before analysis. No change of the chemical profile was observed after storage when compared with freshly prepared samples.

Gland samples were sonicated (Thornton T14, Inpec Eletrônica, Brazil, 40 kHz) for 5 min and then heated at 50°C for 30 min. A solid phase microextraction (SPME) fiber (2 cm, DVB/CAR/PDMS-50/30 μm, Supelco, Bellefonte, PA, USA) was exposed in the headspace of the samples (in vials) for 10 min at 50°C immediately before analysis by gas chromatography-mass spectrometry (GC-MS). GC-MS analysis was performed by using coupled Shimadzu 17A-5050A machines. Desorption time in the splitless injection port of the GC was 1 min. Helium at 30 cm s^{-1} was used as carrier gas. Transfer line and GC injector temperatures were 250 and 230°C, respectively. Analyses were performed by using a SupelcoWax-10 column (30 m×0.25 mm i.d.×0.25 μm film; Supelco), with an oven program of 40°C for 5 min, 3°C min^{-1} to 120°C, then 15°C min^{-1} to 200°C.

Tentative identification of volatile compounds was based on the comparison of retention indices (Kováts 1965) and mass spectra with data from the literature and spectral library (NIST-02). All tentative identifications were confirmed by peak enhancement in co-injections with authentic synthetic samples (Birkett et al. 2004). A typical procedure for co-injection/peak enhancement is given for 2-pentanol: A gland sample was prepared and analyzed as described. After a chromatogram was obtained, the gland sample was treated again according to the same protocol (i.e., sonicated, heated, and sampled by SPME). In parallel, a sample of synthetic standard was prepared: 1 μl of the compound was absorbed on a small piece of filter paper (1×1 cm) in a 10-ml open vial. The vial was heated at 50°C for 3 min and cooled to ambient temperature for 1 min. The same SPME fiber was exposed to the standard sample for 2 sec, and the odors were desorbed from the fiber into the GC injector. The results of both injections were compared and the identity of the compound confirmed when three criteria were fulfilled. First, the peak from the MG compound and the synthetic compound overlapped fully. Second, the peak area increased in the second injection. Third, no difference between the mass spectral profiles was observed after a scan-by-scan analysis.

The stereochemistry of chiral compounds was determined by GC with flame-ionization detection (FID; Shimadzu 17A) and GC-MS analysis. Gland samples were heated at 50°C for 30 min, and the SPME fiber was exposed in the headspace for a given time depending on the relative abundance of a compound. The method used for analysis was the same as for the GC-MS analysis, except the carrier gas velocity was 31 cm sec^{-1}, the injector and detector temperatures were both 225°C, and a CYCLOSILB column (30 m×0.25 mm i.d.×0.25 μm film, J & W Scientific) at either 80°C, for 4-methyl-3-penten-2-ol, or 30°C, for the other compounds, was used. Because a number of peaks overlapped with this column and conditions, chiral GC-MS analysis was carried out by using a GammaDex 225 column (30 m×0.25 mm i.d.×0.25 μm film) at 30°C. The retention times of compounds were compared with synthetic standards, and co-injection (peak enhancement) was carried out to confirm the identities of the enantiomers of all compounds. The configuration of 2-methyl-3-penten-2-ol was confirmed by co-injection with the synthetic (*E*)-isomer, derived from *trans*-methyl crotonate (for synthesis details, see below).

Emission of MG Compounds by Virgin Adults Groups of three virgin adults of the same sex were separated 7 d after ecdysis and transferred into 10-ml vials covered with gauze with a piece of filter paper inside as a substrate for the bugs. These vials were enclosed separately in 150-ml closed plastic containers so as to isolate each group of bugs. We worked with groups of insects to increase both the likelihood of emission and the amount of MG odors (preliminary assays with individual insects failed probably because of low levels of compounds). Each treatment included three groups of three insects. The different series of assays monitored odor emission by: (1) unfed females during the dark phase; (2) unfed females during the light phase; (3) females fed at d 9 after ecdysis, during the dark phase; (4) females fed at d 9 after ecdysis, during the light phase; (5) unfed males during the dark phase; (6) unfed males during the light phase; (7) males fed at d 9 after ecdysis, during the dark phase; and (8) males fed at d 9 after ecdysis, during the light phase. Odor sampling with a SPME fiber was carried out for 1 hr for each treatment. Volatile compounds on the fiber were desorbed immediately after sampling the headspace. This procedure was repeated every second day over a period of 12 d with all groups, i.e., giving a total of six samples per group of three insects and 18 samples for each of the eight treatments. Control samples were obtained by SPME analyses of vials containing a piece of filter paper.

The data from studies of emission of MG compounds by *R. prolixus* adults were analyzed both for individual substances and for pooled samples. This allowed the comparison of emission activity between series (treatments). Every time a MG odor was detected over the samples, this was recorded as a "detection event".

Emission of MG Compounds During Copulation One *R. prolixus* female and one male were gently transferred onto a piece of filter paper inside a 10-ml vial, so as to avoid disturbance and the consequent emission of Brindley's glands' products (Manrique et al. 2006). The vial was closed with a Teflon®/silicone-lined cap. After copulation had begun, volatiles present in the headspace were sampled for 60 min with a SPME fiber. Volatile compounds were analyzed immediately after sampling the headspace. Twenty assays were performed. Each pair of insects was used only once and then discarded.

Odors Emitted by MGs and Possible Effect on Mating in R. prolixus A pair of bugs was gently introduced into a Petri dish (10×2 cm) lined with a piece of filter paper and covered with glass to prevent escape. Whether the pair copulated or not was observed for 60 min; if the pair did not commence copulation within this time, it was considered that no copulation occurred. To evaluate the relevance of MG odors for the success of copulation, the proportion of mating pairs under different treatments was compared: (1) pairs in which males had the MG orifices occluded with paraffin (N=20), (2) pairs in which females had the MG orifices occluded with paraffin (N=20), and (3) pairs in which both males and females had the MG orifices occluded with paraffin (N=20). To test whether this treatment affected the behavior of the insects, two series of control assays were performed: (4) a group in which sham males had paraffin applied on a different area of the cuticle without covering the MG orifices (N=20), and (5) a group in which sham females had paraffin applied on a different area of the cuticle without covering the MG orifices (N=20). An additional control series (6) evaluated the mating frequency in intact pairs (N=20). All experiments were performed at 26±2°C and 60±10% r.h. The behavior of insects was studied during the first half of the dark phase of their activity cycle.

Chemicals 2-Butanone, 2-pentanone, (2*R*)-2-butanol, (2*S*)-2-butanol, 2-methyl-3-buten-2-ol, 3-pentanol, 2-pentanol, 4-methyl-2-pentanol, 3-hexanol, and 2-methyl-1-butanol were purchased from Sigma-Aldrich (Brazil). (2*S*)-3-Methyl-2-butanol, (2*S*)-2-pentanol, and (2*S*)-4-methyl-2-pentanol were purchased from Lancaster Synthesis (UK). (3*E*)-2-Methyl-3-penten-2-ol was synthesized from methyl crotonate according to Stavinoha et al. (1981), and 4-methyl-3-penten-2-ol was synthesized according to Johnson and Rickborn (1970).

(3E)-2-Methyl-3-penten-2-ol Methyl crotonate (0.96 g, 9.6 mmol) was added dropwise at <−10°C under nitrogen to an ether solution of 1.6 M methyllithium (20-ml, 32 mmol) over 30 min. The mixture was stirred for an additional 3 hr at 0°C before Baeckströms reagent (celite/Na_2SO_4, 1:1 *w*/*w*) was added. The mixture was filtered and concentrated *in vacuo*, giving (3*E*)-2-methyl-3-penten-2-ol as a colorless liquid (0.57 g, 60%). ^{1}HNMR: δ: 5.63 (m, 2H), 1.68 (d, 4.5 Hz, 3H), and 1.30 (s, 6H). ^{13}CNMR: δ: 139.37, 122.16, 70.89, 29.97, and 17.87 ppm.

4-Methyl-3-penten-2-ol Sodium borohydride (0.19 g, 5.0 mmol) was dissolved in ethanol (50%, 10-ml). 4-Methyl-3-penten-2-one (mesityl oxide, 1.0 g, 10.0 mmol) was added dropwise, while stirring at 0°C. The reaction mixture was stirred at ambient temperature overnight. K_2CO_3 was added until the solution was saturated, after which the product was extracted with diethyl ether (2× 20-ml). The ether phase was washed with brine (20-ml) and dried over $MgSO_4$. Evaporation of the solvent gave 4-methyl-3-penten-2-ol as a colorless liquid (0.88 g, 87%). ^{1}HNMR: δ: 5.20 (d, 8.6 Hz, 1H), 4.55 (dq, 8.4, 6.3 Hz, 1H), 1.71 (s, 3H), 1.68 (s, 3H), and 1.22 (d, 6.3 Hz, 3H).

^{13}CNMR: δ: 134.43, 129.57, 65.03, 25.87, 23.84, and 18.23 ppm.

A mixture enriched in (2*S*)-4-methyl-3-penten-2-ol was obtained from the racemate by a lipase-catalyzed reaction (Amano PS immobilized on diatomite, Sigma-Aldrich, Sweden; Brenna et al. 1998). Racemic 4-methyl-3-penten-2-ol (20 mg, 0.20 mmol) and vinyl acetate (100 mg, 0.86 mmol) were dissolved in dichloromethane (1-ml). Amano PS-DI (20 mg) was added to the mixture, which was left for 5 hr, with occasional shaking. Chiral GC-MS analysis showed a product enriched in the *S*-enantiomer. The assignment of the stereochemistry was based on the well-known stereochemical preference of the Amano-PS lipase (Kazlauskas et al. 1991). A mixture enriched in (3*S*)-hexanol was obtained according to the same protocol.

For all synthesized compounds, ^{1}H-NMR and ^{13}C-NMR spectra of $CDCl_3$ solutions were recorded at 500 and 125 MHz by using a Varian Unity spectrometer. Chemical shifts were expressed in ppm in relation to tetramethylsilane. The starting materials were obtained from commercial suppliers and used without further purification. NMR data corresponded well with literature data (Ando et al. 1982; Gau et al. 1999).

Statistical Analyses The results, expressed as the numbers of mating pairs per group of bugs, with or without their glands occluded, were analyzed by means of a Chi-square test followed by multiple comparisons using the Bonferroni correction. Therefore, only comparisons having $P<0.003$ were considered to show a significant difference.

Results

Identification of Compounds Produced by MGs Our results showed that the MGs of *R. prolixus* are the sources of a complex mixture of volatile substances. Twelve ketones and alcohols were identified in the MGs of *R. prolixus*, with the most abundant compound being 2-methyl-3-buten-2-ol, followed by 2-pentanol, (3*E*)-2-methyl-3-penten-2-ol, and 4-methyl-3-penten-2-ol (Table 1). The same substances were detected in the MGs of both sexes. The chiral alcohols 2-butanol, 2-pentanol, 4-methyl-2-pentanol, and 3-hexanol were found as *S*-enantiomers only, while 4-methyl-3-penten-2-ol (mesityl alcohol) was found as a mixture of the two enantiomers (Table 1). Because 3-methyl-2-butanol and 2-methyl-1-butanol were present at very low concentrations and/or co-eluted with other major compounds on both of the columns used, the chiral analysis was not unequivocal.

Emission of MG Compounds by Virgin Adults MG compounds were consistently detected in the headspace of adult bugs (Fig. 1, Table 2). Detection events (i.e., each time any MG compound was detected) were recorded more frequently in both females and males during the scotophase than the

Table 1 Compounds identified in metasternal glands of *R. prolixus*

Compound	RT[a]	Retention Index[b]	Relative Amount[c] (%) ♀	Relative Amount[d] (%) ♂	Relative Amount[e] (%) ♀ + ♂
2-Butanone	2.53	909	–[f]	–[f]	–[f]
2-Pentanone	3.68	979	–[f]	–[f]	–[f]
(2*S*)-Butanol	4.92	1,030	5.7±1.4	2.2±0.7	4.0±2.1
2-Methyl-3-buten-2-ol	5.48	1,048	61±10	62.2±7.2	61±8.6
3-Methyl-2-butanol	7.31	1,108	1.1±0.5	0.9±0.3	1.0±0.4
3-Pentanol	7.99	1,120	1.4±0.4	1.1±0.2	1.2±0.4
(2*S*)-Pentanol	8.50	1,131	20±4.9	21±5.3	20±5.0
(3*E*)-2-Methyl-3-penten-2-ol	10.07	1,166	6.1±3.0	5.3±1.5	5.7±2.3
(2*S*)-4-Methyl-2-pentanol	10.80	1,181	–[f]	–[f]	–[f]
(3*S*)-Hexanol	12.00	1,207	–[f]	–[f]	–[f]
2-Methyl-1-butanol	12.49	1,217	3.5±5.1[g]	3.3±4.1[g]	3.4±4.5[g]
(2*S*/2*R*)-4-Methyl-3-penten-2-ol	14.99	1,267	2.1±1.3	2.5±0.8	2.3±1.0

[a] Retention time (SupelcoWax-10 column)
[b] Retention indices calculated according to Kováts (1965)
[c] Relative amount (mean and SD) from eight female samples
[d] Relative amount (mean and SD) from eight male samples
[e] Relative amount (mean and standard deviation) from all 16 samples
[f] Average amount ≤0.5% of total amount of compounds in sample
[g] Three (one ♂ and two ♀) out of 16 samples contained 10.5–13.1%, and the remaining 13 samples 0.0–3.4%

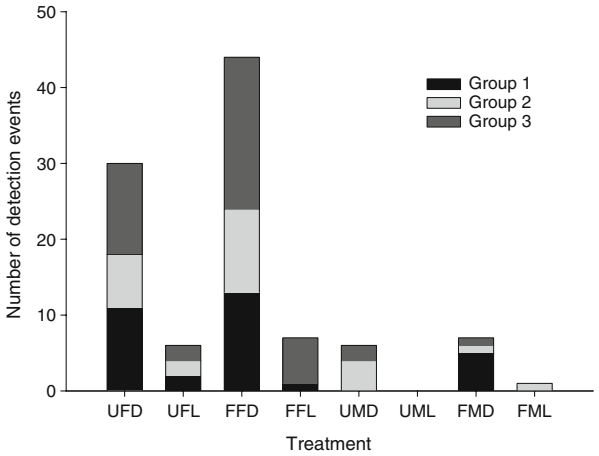

Fig. 1 Number of detection events of various *R. prolixus* metasternal gland compounds in different groups (each of three individuals) of fed/unfed males and females during the light and dark phases. *UFD* Unfed females during dark phase, *UFL* unfed females during light phase, *FFD* fed females during dark phase, *FFL* fed females during light phase, *UMD* unfed males during dark phase, *UML* unfed males during light phase, *FMD* fed males during dark phase, *FML* fed males during light phase

photophase (Fig. 1, Table 2). In general, more detection events were recorded from females than males under all experimental conditions (Fig. 1, Table 2). There was no apparent difference between unfed and fed insects.

Nine out of the 12 compounds found in the MGs were detected in the headspace over females over all the different treatments (Table 2), whereas only three of them were found in the headspace over males (Table 2). 2-Methyl-3-buten-2-ol was the most frequently detected compound in these analyses (Table 2).

Emission of MG Compounds During Copulation In 19 out of 20 assays with pairs, a successful copulation resulted. The average duration of copulation was 49.7±3.6 min. At least one of the compounds identified in the MGs was detected during 70% of the copulations. The most abundant compound produced by the MGs (2-methyl-3-buten-2-ol) was detected in 40% of the copulations. The compound most frequently found during copulation (in 60% of the samples) was 2-methyl-1-butanol. 2-Pentanone was detected in 10% of the assays.

Relevance of the Odors Emitted by MGs for the Success of Mating The percentage of copulation of untreated control pairs was 95% (Fig. 2, N=20). This was not significantly different from the percentages of copulation observed in sham-operated male and female treatments (Fig. 2). However, occlusion of female MG orifices or male MG orifices resulted in significant (P<0.003) decreases in copulation frequencies (30%, N=20 and 15%, N=20, respectively). Occlusion of both male and female orifices also resulted in a significant (P<0.003) decrease (relative to the controls) of mating percentage (15%, N=20).

Discussion

The results show that the metasternal glands of *R. prolixus* are a rich source of volatile compounds. GC-MS analysis

Table 2 The detection of *Rhodnius prolixus* metasternal gland compounds in various treatments, related to sex, feeding status, and time of day

Compound	UFD	UFL	FFD	FFL	UMD	UML	FMD	FML
2-Butanone	1	0	3	0	0	0	0	0
2-Pentanone	2	0	2	0	0	0	0	0
(2*S*)-Butanol	3	1	8	1	0	0	0	0
2-Methyl-3-buten-2-ol	7	2	11	3	4	0	6	1
3-Methyl-2-butanol	5	0	4	0	0	0	0	0
3-Pentanol	0	0	0	0	0	0	0	0
(2*S*)-Pentanol	4	1	5	1	1	0	0	0
(3*E*)-2-Methyl-3-penten-2-ol	0	1	0	1	0	0	0	0
(2*S*)-4-Methyl-2-pentanol	0	0	0	0	0	0	0	0
(3*S*)-Hexanol	0	0	0	0	0	0	0	0
2-Methyl-1-butanol	7	1	11	1	1	0	1	0
(2*S*/*R*)-4-Methyl-3-penten-2-ol	0	1	0	1	0	0	0	0
Total	29	7	44	8	6	0	7	1

Numbers indicate the detection frequency for each compound (18 SPME samples per treatment).
UFD Unfed female sampled during dark phase, *UFL* unfed female sampled during light phase, *FFD* fed female sampled during dark phase, *FFL* fed female sampled during light phase, *UMD* unfed male sampled during dark phase, *UML* unfed male sampled during light phase, *FMD* fed male sampled during dark phase, *FML* fed male sampled during light phase

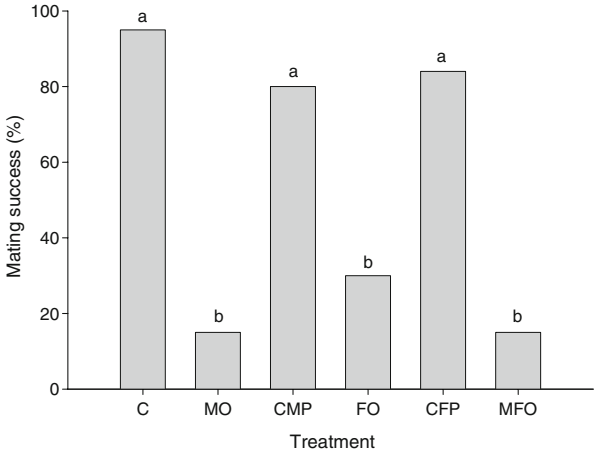

Fig. 2 Copulation (%) of *R. prolixus* pairs: *C* Control (intact) pairs, *MO* males with occluded MG orifices, *CMP* control males treated with paraffin on a different part of their body surface, *FO* females with occluded MG orifices, CFP control females treated with paraffin on a different part of their body surface, *MFO* males and females with occluded MG orifices. *Different letters atop treatments* represent significant differences (chi-square test followed by Bonferroni multiple comparisons, $P<0.003$)

revealed a mixture of 12 volatile ketones and alcohols, with the most abundant compounds being 2-methyl-3-buten-2-ol, (2*S*)-pentanol, (3*E*)-2-methyl-3-penten-2-ol, and the enantiomers of 4-methyl-3-penten-2-ol. None of these compounds had previously been reported in a triatomine species. However, 2-methyl-3-buten-2-ol has been reported as part of the aggregation pheromones of several species of bark beetles (Giesen et al. 1984; Klimetzek et al. 1989; Schlyter et al. 1992) and also as part of the alarm pheromone of the hornet wasp, *Vespa crabro* (Veith et al. 1984). 2-Pentanol has been found in the alarm pheromone of hornet wasps (Ono et al. 2003; Ono 2005), as an attractant to fruits for the coleopterans, *Carpophilus hemipterus* and *Conotrachelus nenuphar* (Phelan and Lin 1991; Prokopy et al. 2001), and as part of the defensive secretions of *Polyzosteria* and related cockroaches (Wallbank and Waterhouse 1970). To our knowledge, 2-methyl-3-penten-2-ol and 4-methyl-3-penten-2-ol have not been reported as semiochemicals for any insect species. Minor components of the secretions, 2-butanone, 2-methyl-1-butanol, and 3-hexanol have been found previously in MG secretions of *T. infestans* by Manrique et al. (2006).

Interestingly, the saturated alcohols we identified in *R. prolixus* all had an (*S*)-configuration, suggesting a common enzymatic system in their biosynthesis. In accord with what is known about the biosynthesis of 2-methyl-3-buten-2-ol in bark beetles (Lanne et al. 1989; Martin et al. 2003; Seybold et al. 2006), it is conceivable that a common allylic carbocation in the biosynthetic pathway gives rise to both (3*E*)-2-methyl-3-penten-2-ol and 4-methyl-3-penten-2-ol. Hydration at the allylic positions of the carbocation would form 2-methyl-3-penten-2-ol and 4-methyl-3-penten-2-ol. The latter addition appears not to be stereoselective as both enantiomers of 4-methyl-3-penten-2-ol are formed.

We demonstrated that the volatile compounds found in the MGs of *R. prolixus* are emitted by virgin adult bugs of both sexes. Emission of these compounds was detected more frequently from females than males. Females also released these chemicals more frequently during the early hours of the scotophase, the period when sexual activity in this species is at its peak (Manrique, personal communication). That these compounds may be involved in sexual communication is suggested by their detection, albeit in low amounts (e.g., 10–100 pg for 2-pentanol) over copulating pairs of *R. prolixus*. That only three of the MG compounds were detected over copulating pairs could have been due to the very low concentration of compounds emitted by bugs. It is worth noting that the SPME collections for analysis of MG content were of headspace above 12 glands heated to 50°C, whereas, at most, the headspace above a pair of bugs emitting volatiles consisted of the contents of four glands at 26°C. For most of the compounds identified in the glands (i.e., from two MGs), the amount was close to the detection limit of our instrument. Manrique et al. (2006) detected 3-pentanone, the main component of the MG secretions of *T. infestans*, over copulating pairs, and suggested that this species may use MG odors for communication during mating. A role for the MG odors in the sexual behavior of *R. prolixus* was further suggested by our occlusion experiments in which occlusion of the MG orifices of either males or females resulted in a significant decrease in copulation, relative to the various controls. A similar result was obtained for *T. infestans* (Crespo and Manrique 2007).

It is worth noting that we did not detect any Brindley's gland secretions during our sampling of copulating pairs. These secretions have previously been detected (Ríos Candelaria 1999; Guerenstein and Guerin 2004) over *R. prolixus* mating pairs, and it has been suggested that they may be involved in sexual communication. However, it cannot be excluded that the detection of Brindley's gland compounds in those studies may have been the result of an alarm response (Manrique et al. 2006) rather than a sexual signal. Further work is needed to clarify the role of Brindley's glands secretions in the sexual behavior of *R. prolixus*.

Overall, our data show that the release of compounds found in the MGs of adult *R. prolixus* corresponds with sexual activity of this species, and that furthermore, females

appear to release greater quantities of these compounds than males. However, whether these chemicals actually mediate sexual behavior in this species is unknown. Further work is required to determine whether the compounds are directly involved in mediating sexual behavior of adults and, if so, what is their precise role. If these chemicals are attractive to adult *R. prolixus*, they could prove useful as chemical baits in traps for monitoring or controlling *R. prolixus* populations, thereby limiting the transmission of Chagas disease to humans. The development of new methods for controlling *R. prolixus* is critical as certain populations have already developed resistance to the pyrethroid insecticides used in control programs (Zerba 1999).

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