Exocrine Secretions of Wheel Bugs (Heteroptera: Reduviidae: *Arilus* spp.): Clarification and Chemistry

Jeffrey R. Aldrich[a,b,*], Kamlesh R. Chauhan[c], Aijun Zhang[c], and Paulo H. G. Zarbin[d]

[a] Affiliate Department of Entomology, University of California, Davis, CA, USA
[b] J. R. Aldrich consulting LLC, 519 Washington Street, Santa Cruz, CA 95060, USA. E-mail: drjeffaldrich@gmail.com
[c] USDA-ARS Invasive Insect Biocontrol & Behavior Laboratory, 10300 Baltimore Avenue, Bldg. 007, rm301, BARC-West, Beltsville, MD 20705, USA
[d] Universidade Federal do Paraná, Departamento de Química, Laboratório de Semioquímicos, CP 19081, 81531–980, Curitiba – PR, Brazil

* Author for correspondence and reprint requests

Z. Naturforsch. **68 c**, 522–526 (2013); received August 21/November 6, 2013

Wheel bugs (Heteroptera: Reduviidae: Harpactorinae: *Arilus*) are general predators, the females of which have reddish-orange subrectal glands (SGs) that are eversible like the osmeteria in some caterpillars. The rancid odor of *Arilus* and other reduviids actually comes from Brindley's glands, which in the North (*A. cristatus*) and South (*A. carinatus*) American wheel bugs studied emit similar blends of 2-methylpropanoic, butanoic, 3-methylbutanoic, and 2-methylbutanoic acids. The *Arilus* SG secretions studied here are absolutely species-specific. The volatile SG components of *A. carinatus* include (*E*)-2-octenal, (*E*)-2-nonenal, (*E*)-2-decenal, (*E*,*E*)-2,4-nonadienal, (*E*)-2-undecenal, hexanoic acid, 4-oxo-nonanal, (*E*,*E*)-2,4-decadienal, (*E*,*Z*)-2,4- or (*Z*,*E*)-2,4-decadienal, and 4-oxo-(*E*)-2-nonenal; whereas in *A. cristatus* the SG secretion contains *β*-pinene, limonene, terpinolene, terpinen-4-ol, thymol methyl ether, *α*-terpineol, bornyl acetate, methyl eugenol, *β*-caryophyllene, caryophyllene oxide, and farnesol. *Arilus* spp. SG secretions may be sex pheromones, but verification of this hypothesis requires further testing.

Key words: Arilus, *Halyomorpha*, Pheromone

Introduction

Assassin bugs in the genus *Arilus* (Heteroptera: Reduviidae: Harpactorinae) are called "wheel bugs" because of the toothed median ridge on the pronotum (Fig. 1a); they are among the largest of the New World reduviids, and are usually rarely encountered (Mead, 2008). Barth (1961) studied the exocrine glands of *A. carinatus* (Forster) from Brazil; however, this manuscript (in Portuguese) has been largely overlooked. Barth (1961) determined that only females have eversible subrectal glands (SGs) (Fig. 1b) (Weirauch, 2006a) and that the pungent odor sometimes detected when *A. carinatus* females are captured comes from Brindley's glands not from the SGs. [The metathoracic scent glands characteristic of most Heteroptera (Aldrich, 1988), are absent in Harpactorinae (Weirauch, 2006b), whereas Brindley's glands occur laterally below the first abdominal tergite in harpactorines and most other reduviid subfamilies (Staddon, 1979).] Contrary to the findings of Barth (1961), Mead (2008) reported that "…when captured, [*A. cristatus* (L.)] extrude (with little provocation) a pair of bright, orange-red scent sacs near the apex of the venter [that] give off a pungent scent." Garman (1916) realized much earlier that the osmeteria-like SGs of *A. cristatus* are only present in females, and that they are not always everted when females are disturbed, but he incorrectly (as we will show) assumed that the "pungent scent" emanated from the SGs.

Chemical data presented here demonstrates that Brindley's gland secretions are qualitatively alike for North *(A. cristatus)* and South *(A. carinatus)* American wheel bugs, whereas the SG secretions are highly species-specific. The volatile SG components are herein reported for *A. carinatus* and *A. cristatus* for the first time.

Methods and Materials

Insects and gland extracts

Single female *A. carinatus* and *A. cristatus* were collected in Brazil and the USA, respectively, and frozen prior to dissection (Table I). Once thawed,

© 2013 Verlag der Zeitschrift für Naturforschung, Tübingen · http://znaturforsch.com

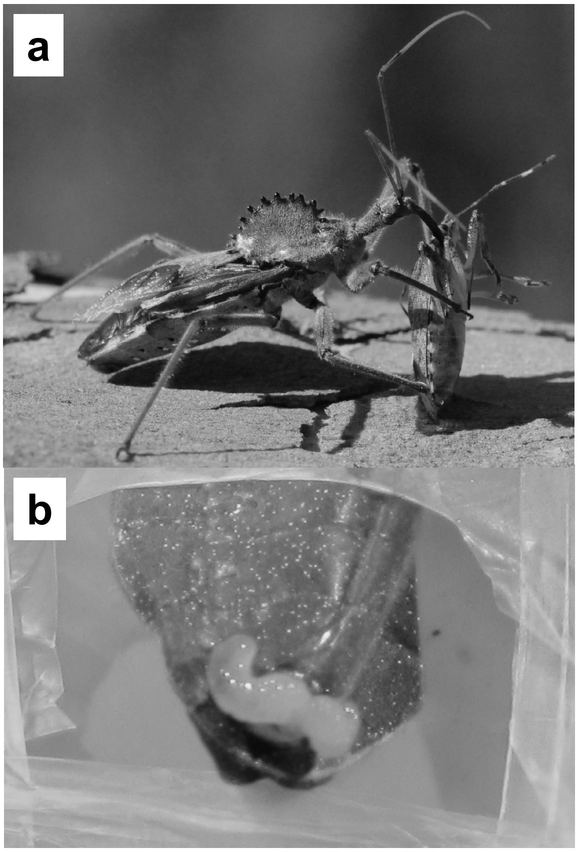

Fig. 1. (a) Male *Arilus cristatus* (L.) feeding on an adult brown marmorated stink bug, *Halyomorpha halys* (Stål) (photo by Michael Raupp). (b) Everted subrectal gland of an *Arilus carinatus* female collected April 17, 2010, Universidade Federal do Paraná, Curitiba, Brazil.

the insects were wrapped with Parafilm leaving the posterior end of the abdomen exposed, the abdominal sternum was compressed causing the SGs to evert (Fig. 1b), and each gland was cut off at its base into 20 μl of redistilled *n*-hexane in a Waters Alliance Total Recovery Vial® (deactivated, 12 mm x 32 mm; Waters, Taunton, MA, USA). Samples of the paired Brindley's glands were dissected from the bugs under tap water, and extracted in 50 μl of *n*-hexane.

Gas chromatography-mass spectrometry (GC-MS)

Samples were analyzed (70 eV) using Hewlett-Packard 6890 gas chromatographs coupled to HP 5973 mass spectrometers (Hewlett-Packard Company, Wilmington, DE, USA), one with an HP-5MS column (Agilent Technologies, Santa Clara, CA, USA; 30 m x 0.25 mm ID, 0.25 μm film thickness, 50 °C for 2 min, to 280 °C at 15 °C/min, hold for 10 min), and the other with a DB-WAXetr column (J & W Scientific, Folsom, CA, USA; 60 m x 0.25 mm ID, 0.25 μm film thickness, 50 °C for 2 min, to 230 °C at 15 °C/min, hold for 10 min). The major acids present in Brindley's gland extracts were unresolved on the above columns, but were resolved using an HP 5890 gas chromatograph with a DB-1701 column (J & W Scientific; 30 m x 0.25 mm ID, 0.25 μm film thickness, 40 °C for 2 min, to 200 °C at 5 °C/min, hold for 10 min). Analyses were done in the splitless mode.

Chemical standards

2-Methylpropanoic acid, butanoic acid, 3-methylbutanoic acid, 2-methylbutanoic acid, octanoic acid, (*E*,*E*)-2,4-octadienal, (*E*,*E*)-2,4-nonadienal, (*E*,*E*)-2,4-decadienal, β-pinene, limonene, terpinolene, terpinen-4-ol, α-terpineol, methyl eugenol, β-caryophyllene, caryophyllene oxide, thymol, and farnesol were from Aldrich Chemical (Milwaukee, WI, USA); bornyl acetate, hexanoic acid, (*E*)-2-octenal, (*E*)-2-nonenal, (*E*)-2-decenal, and (*E*)-2-undecenal were from Bedoukian Research, Inc. (Danbury, CT, USA). 4-Oxo-(*E*)-2-nonenal was prepared according to Moreira and Millar (2005), and 4-oxo-nonanal was obtained by hydrogenation of oxo-nonenal. Thymol methyl ether was synthesized from thymol following standard procedures, treating thymol with methyl iodide in alkaline solution.

Results

The *A. carinatus* female collected in Brazil did not evert its subrectal glands (SGs), yet there was a distinct rancid odor from the bug (J. R. A., personal observation). During past collections of *A. cristatus*, the SGs were sometimes, but not always, everted (J. R. A., personal observation).

The rancid odor of the *Arilus* spp. is from Brindley's glands whose secretions in *A. carinatus* and *A. cristatus* are similar blends of 2-methylpropanoic, butanoic, 3-methylbutanoic, and 2-methylbutanoic acids (Table I). The SG secretions of *Arilus* females are more complex and dilute than Brindley's gland secretions, and their aromas are pleasant but distinctly different from each other (J. R. A., personal observation). In fact, SG volatiles of *A. carinatus* and *A. cristatus* are absolutely species-specific (Table I). The predominant *A. carinatus* SG volatiles are (*E*)-2-octenal, (*E*)-2-nonenal, (*E*)-2-decenal, (*E,E*)-2,4-nonadienal, (*E*)-2-undecenal, hexanoic acid, 4-oxo-nonanal, (*E,E*)-2,4-decadienal (and an unidentified isomer), and 4-oxo-(*E*)-2-nonenal. The *A. cristatus* SG secretion has a pine-like odor (J. R. A., personal observation) whose composition includes monoterpenoids (β-pinene, limonene, terpinolene, terpinen-4-ol, α-terpineol, thymol methyl ether, and bornyl acetate), sesquiterpenoids (β-caryophyllene, caryophyllene oxide, and farnesol), plus the phenylpropene methyl eugenol.

Discussion

Adult *Arilus* females have eversible SGs, like the osmeteria of various caterpillars (Chow and Tsai, 1989), but their secretions are not responsible for the rancid odor emitted when the insects are molested. English literature on *A. cristatus* incorrectly attributes this odor to their SGs. Actually, the rancid odor is due to simultaneous emission of Brindley's gland secretion, which in the North (*A. cristatus*) and South (*A. carinatus*) American wheel bugs studied are similar blends of 2-methylpropanoic, butanoic, 3-methylbutanoic, and 2-methylbutanoic acids. Brindley's glands are thought to be defensive (Staddon, 1979). Releasing Brindley's gland acids may be repulsive to predators, as well as serve to advertise the danger of attacking insects with such a fierce bite (Smith *et al.*, 1958). In addition, *Arilus* adults audibly stridulate when disturbed (Mead, 2008).

Since *Arilus* SGs are female-specific, they may be associated with oviposition and/or sex. To our knowledge, there are no observations of these

Table I. *Arilus* species exocrine gland components[a].

KI [b]	Brindley's gland (%)[c]		Compound
	A. cristatus	*A. carinatus*	
673	27.9	32.5	2-Methylpropanoic acid
685	1.1	0.9	Butanoic acid
698	28.3	37.0	3-Methylbutanoic acid
700	42.7	29.6	2-Methylbutanoic acid[d]
	Subrectal gland (%)[e]		
985	22.1	0	β-Pinene[d]
1033	22.3	0	Limonene[d]
1091	8.7	0	Terpinolene
1179	3.7	0	Terpinen-4-ol[d]
1198	6.6	0	α-Terpineol[d]
1235	9.6	0	Thymol methyl ether
1290	8.3	0	Bornyl acetate[d]
1382	0	8.4	(*E*)-2-Octenal
1405	3.7	0	Methyl eugenol
1458	0	7.3	(*E*)-2-Nonenal
1466	11.1	0	β-Caryophyllene[d]
1573	0.7	0	Caryophyllene oxide[d]
1602	0	5.2	(*E*)-2-Decenal
1661	0	5.8	(*E*,*E*)-2,4-Nonadienal
1707	0	6.8	(*E*)-2-Undecenal
1711	0	12.1	(*E*,*E*)-2,4-Decadienal
1715	1.0	0	Farnesol
1729	0	6.0	Hexanoic acid
1756	0	12.2	4-Oxo-nonanal
1765	0	23.6	(*E*,Z)- or (*Z*,*E*)-2,4-Decadienal
1804	0	12.6	4-Oxo-(*E*)-2-nonenal

[a] Samples from *A. carinatus* females collected April 17, 2010, Curitiba, Brazil, and *A. cristatus* females collected October 25, 2010, Mitchellville, MA, USA.
[b] Kovats index; 30 m DB-5 column.
[c] GC conditions: 30 m DB-1701 column; 40 °C (isothermal for 2 min) to 200 °C at 5 °C/min, hold for 10 min.
[d] Chiral compound; chirality not determined.
[e] GC conditions: 60 m DB-WAXetr column; 50 °C (isothermal for 2 min) to 230 °C at 10 °C/min, hold for 10 min.

glands being everted in the context of egg laying or guarding. By the same token, we know of no reports of these glands being observed in sexual contexts. The high species specificity of SG secretions in *A. carinatus* and *A. cristatus* is consistent with a sex pheromonal role. Many reduviid adults possess active dorsal abdominal exocrine glands (DAGs) (Weirauch, 2006c), and in at least one harpactorine species males produce an attractant pheromone from sexually dimorphic DAGs (James *et al.*, 1994). However, while adult *Arilus* spp. possess DAGs, they are apparently not enlarged or sexually dimorphic (Weirauch, 2006c). *Zelus luridus*, a species quite closely aligned to *Arilus* (Christiane Weirauch, personal communication), possesses non-eversible SGs plus glandular exocrine cells associated with the genital segments of females (Weirauch, 2006a), and there is strong circumstantial evidence that *Zelus* females produce attractant sex pheromones (Edde and Phillips, 2006).

Perhaps the most enticing observation that the SGs of wheel bugs may be the source of sex pheromones comes from Metzger's (1928) discovery 85 years ago that *A. cristatus* adults (sex not determined) were attracted to turpentine oil, which is chemically (Sadeghi *et al.*, 2013) like the SG secretion reported herein for this predator. Moreover, Todd's (1937) field observations showed that the sex ratio in *A. cristatus* was skewed toward females; thus, these widely dispersed predators may have experienced added selection pressure to evolve sex pheromones. Further research is needed to determine whether or not *Arilus* SG secretions constitute sex pheromones. Clarifying the function of *Arilus* SG secretions is all the more timely now since the North American wheel bug, *A. cristatus*, is one of the few predators attacking the invasive brown marmorated stink bug, *Halyomorpha halys* (Pentatomidae) in the USA (Fig. 1a) (Aldrich *et al.*, 2009; J. R. A., unpublished data; Paula Shrewsbury, personal communication; http://www.stopbmsb.org).

Acknowledgements

This study was made possible by CNPq funding for the visit of J. R. A. to the Federal University of Paraná (visiting researcher; proc. 401604/2009–8). We thank Drs. Paula M. Shrewsbury and Michael Raupp, Department of Entomology, University of Maryland at College Park, respectively, for bringing to our attention instances of *A. cristatus* preying upon the brown marmorated stink bug, and providing the photograph of *A. cristatus* preying upon *H. halys*. We are also grateful to Mr. and Mrs. Scales of Bowie, Maryland, for collecting the female *A. cristatus* as it fed on an adult *H. halys*. Finally, we thank Dr. Christiane Weirauch, Department of Entomology, University of California, Riverside, for sharing information and helpful discussions. Mention of commercial products does not constitute an endorsement by the U. S. Department of Agriculture.

Aldrich J. R. (1988), Chemical ecology of the Heteroptera. Annu. Rev. Entomol. **33**, 211–238.

Aldrich J. R., Khrimian A., Chen X., and Camp M. J. (2009), Semiochemically based monitoring of the invasion of the brown marmorated stink bug and unexpected attraction of the native green stink bug (Heteroptera: Pentatomidae) in Maryland. Fla. Entomol. **92**, 483–491.

Barth R. (1961), Sobre o orgae abdominal glandular de *Arilus carinatus* (Forster, 1771) (Heteroptera, Reduviidae). Mem. Inst. Oswaldo Cruz **59**, 37–43.

Chow Y. S. and Tsai R. S. (1989), Protective chemicals in caterpillar survival. Experientia **45**, 390–392.

Edde P. A. and Phillips T. W. (2006), Field responses of nontarget species to semiochemicals of stored-product Bostrichidae. Ann. Entomol. Soc. Am. **99**, 175–183.

Garman H. (1916), The locust borer (*Cyllene robinae*) and other insect enemies of the black locust. Kt. Agric. Exp. Stn. Bull. **200**, 99–135.

James D. G., Moore C. J., and Aldrich J. R. (1994), Identification, synthesis, and bioactivity of a male-produced aggregation pheromone in assassin bug, *Pristhesancus plagipennis* (Hemiptera: Reduviidae). J. Chem. Ecol. **20**, 3281–3295.

Mead F. W. (2008), Wheel bug, *Arilus cristatus* (Linnaeus) (Insecta: Hemiptera: Reduviidae). http://edis.ifas.ufl.edu/pdffiles/IN/IN24300.pdf.

Metzger F. W. (1928), Turpentine oil as an attractant of the wheel bug (*Arilus cristatus* L.). J. Econ. Entomol. **21**, 431–432.

Moreira J. A. and Millar J. G. (2005), Short and simple syntheses of 4-oxo-(*E*)-2-hexenal and homologs: Pheromone components and defensive compounds of Hemiptera. J. Chem. Ecol. **31**, 965–968.

Sadeghi H., Tahery Y., and Moradi S. (2013), Intra- and inter-specific variation of turpentine composition in Eldar pine (*Pinus eldarica* Medw.) and black pine (*Pinus nigra* Arnold). Biochem. Syst. Ecol. **48**, 189–193.

Smith F. D., Miller N. G., Carnazzo S. J., and Eaton W. B. (1958), Insect bite by *Arilus cristatus*, a North American reduviid. A. M. A. Arch. Dermatol. **77**, 324–330.

Staddon B. W. (1979), The scent glands of Heteroptera. Adv. Insect Physiol. **14**, 351–418.

Todd J. N. (1937), Life history of the wheel-bug, *Arilus cristatus* (Linn.) (Hemiptera: Reduviidae). Entomol. News **48**, 226–228.

Weirauch C. (2006a), Observations on the sticky trap predator *Zelus luridus* Stal (Heteroptera, Reduviidae, Harpactorinae), with the description of a novel gland associated with the female genitalia. Denisia **19**, 1169–1180.

Weirauch C. (2006b), Metathoracic glands and associated evaporatory structures in Reduvioidea (Heteroptera: Cimicomorpha), with observation on the mode of function of the metacoxal comb. Eur. J. Entomol. **103**, 97–108.

Weirauch C. (2006c), Dorsal abdominal glands in adult Reduviidae (Heteroptera, Cimicomorpha). Dtsch. Entomol. Z. **53**, 91–102.