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Identification of the Compounds Released by *Megacopta cribraria* (Heteroptera: Plataspidae)[1]

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Megacopta cribraria (F.) (Hemiptera: Plataspidae) was first discovered in the New World in northeast Georgia in October 2009. It was first reported as a nuisance pest aggregating on the exterior of homes (Eger et al. 2010, Insecta Mundi 0121: 1–11; Suiter et al. 2010, J. Integr. Pest Manag. 1: F1–F4). The source of these aggregating adults was found to be kudzu, *Pueraria montana* var. *lobata* (Loureiro) Ohwi (F.), which was later determined to be the preferred host of the insect in its expanded North American range (Zhang et al. 2012, Environ. Entomol. 41: 40–50). The insect spread rapidly throughout the southern United States (Gardner et al. 2012, J. Entomol. Sci. 48: 118–127) and is recognized as an agriculturally important pest of soybean, *Glycine max* (L.) Merrill, in its U.S. range (Greene et al. 2012, U.S. Soybean Board Technol. Transfer Publ., Clemson, SC).

Adult and immature *M. cribraria* produce a mildly offensive odor when disturbed (Ruberson et al. 2013, Appl. Entomol. Zool. 48: 3–13). Such volatile organic compounds (VOCs) are used for both aggregation and deterrence (Adrich 1988, Annu. Rev. Entomol. 33: 211–238). Kitamura et al. (1984, Appl. Entomol. Zool. 19: 33–41) observed that *Megacopta punctatissimum* (Montandon) (as *Coptosoma punctatissimum* Montandon) (Hemiptera: Plataspidae) produced VOCs similar to those of the family Pentatomidae. These commonly consisted of hydrocarbons (undecane, dodecane, tridecane, and pentadecane) and aldehydes (decenyl acetate, octenal, decenal, and oxo-hexenal). The authors suggested that aldehydes played a key role as attractants. More recently, Onnink et al. (2017. J. Entomol. Sci. 52: 39–51) reported that gas chromatography–mass spectrometry (GC-MS) revealed the presence of tridecane and (E)-2-decenal in the VOCs of disturbed and undisturbed *M. cribraria*, but only (E)-2-hexenal was in the VOCs of disturbed *M. cribraria*. Their olfactometer and electroantennographic assays showed that

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Table 1. Mean percentage of chemical compounds found in *Megacopta cribraria* exudate identified using gas chromatography–mass spectrometry and authenticated using pure samples.

	M. cribraria Life Stages			
Chemical Compound	**Immatures**	**Female**	**Male**	**Mixed-Gender Adults**
(E)-2-hexenal	1.23	2.93	2.905	0.165
2-Hexen-1-ol acetate	0.4825	1.345	1.45	0
(E)-2-decenyl acetate	2.975	9.07	10.595	0.25
(E)-4-oxohex-2-enal	11.8025	6.485	6.045	10.835
(E)-2-decenal	19.595	13.38	12.715	16.515
(E)-2-octenal	0.2175	0	0	0
Dodecane	1.5525	1.88	1.855	1.66
1-Tridecene	0.1625	0	0	0.295
Pentadecane	0.1125	0	0	33.165
Tridecane	42.3025	62.81	61.645	35.885
1-Octadecene	0.05	0	0	0.1

adult females responded to the (E)-2-hexenal but not to the tridecane or (E)-2-decenal. Males did not respond to any of the three VOC components. The authors concluded that (E)-2-hexenal could be used as a female attractant. Characterization of these VOCs in the Plataspidae family is relatively incomplete; therefore, the objective of this study was to expand efforts to identify chemicals produced by *M. cribraria* that could be used as attractants or deterrents.

During the summer and fall of 2015, *M. cribraria* adult males, adult females, and immatures (first through fifth instars) were collected in Union County, NC. Specimens within these groups were separated and stored over dichloromethane (Fisher Scientific, Waltham, MA) in a capped 15-mL centrifuge tube (BD Falcon, Tewksbury, MA). VOCs were analyzed using a gas chromatograph–mass spectrometer (Shimadzu GCMS-QP2010 SE, Shimadzu Scientific Instruments, Columbia, MD) equipped with an autosampler/autoinjector (AOC-20s, AOC-20i) and fitted with a SH-Rxi-5Sil-MS column (30 m $\times$ 0.25 mm inner diameter $\times$ 0.25 μm film). A 1-μL sample was injected, and the GC temperature program was 60°C, ramped at 10°C per minute to 200°C with a 15-min hold. For each VOC, the top seven to nine compounds, by percentage of the total, were reported (Table 1). Each was identified by a similarity search based on known compounds in the National Institute of Standards and Technology and the Wiley Databases and Spectral Libraries.

Even if a VOC compound had a very high similarity to a known chemical, its identity was verified by analysis of a standard sample for each known compound.

Table 2. Retention time (RT) of each compound in *Megacopta cribraria* exudate and retention times of authentic samples.

Chemical Compound	RT in Bug (min)	RT of Authentic Sample (min)
(E)-2-hexenal	3.693	3.696
(E)-4-oxohex-2-enal	5.094	5.106
Dodecane	8.771	8.779
(E)-2-decenal	9.681	9.721
1-Tridecene	10.23	10.094
Pentadecane	12.86	12.869
2-Hexen-1-ol acetate	5.904	6.001
(E)-2-decenyl acetate	11.62	11.707
Tridecane	10.21	10.386
(E)-2-octenal	6.612	6.651
1-Octadecene	16.44	16.527

Each analysis was repeated twice. To authenticate VOCs, (E)-2-hexenal, (E)-2-decenal, 1-tridecene, pentadecane, and 2-hexen-1-ol acetate were purchased from Sigma Aldrich (St. Louis, MO), tridecane, (E)-2-octenal, and 1-octadecene were purchased from Fluka (St. Louis, MO), and docecane was purchased from Reagents, Inc. (Charlotte, NC) as standards. (E)-4-oxohex-2-enal was synthesized as per methods of Moreira et al. (2005, J. Chem Ecol. 31: 965–968) without purification.

(E)-2-decenyl acetate was synthesized by a two-step reaction. The first step involved the synthesis of 2-decen-1-ol from 2-decenal. 2-Decenal (Fluka) (5.017 g, 33 mmol) and methanol (20 mL) were mixed in a round-bottom flask using a stirrer bar. The mixture was continuously stirred and cooled to 0°C. Sodium borohydride (0.793 g, 20 mmol) was added in small aliquots. The reaction was stirred for 15 min and then brought to ambient room temperature. The reaction was acidified with 3M HCl and washed with aqueous sodium bicarbonate (5 mL). Sodium chloride (5 g) was then added and mixed. The mixture was extracted with ether (3×30 mL), dried over sodium sulfate, and filtered; the solvent removed *in vacuo*. GC-MS showed 100% conversion to product: 2-decen-1-ol (a white solid).

The second step involved converting 2-decen-1-ol to (E)-2-decenyl acetate. 2-Decen-1-ol (4.667 g, 30 mmol), acetic acid (5.483 g, 91 mmol), and four drops of concentrated sulfuric acid were placed in a round-bottom flask. The reaction was refluxed for 1 h, and then cooled to ambient room temperature after which 40 mL of water was added. The reaction was extracted with ether (50 mL) and washed with water (25 mL), aqueous sodium bicarbonate (25 mL), and aqueous sodium chloride (25 mL). The ether layer was dried over magnesium sulfate and gravity filtered; the solvent removed *in vacuo*. GC-MS confirmed the desired product, (E)-2-decenyl

acetate, was synthesized to give a 67% conversion of 2-decen-1-ol to desired product. The product was not further purified.

Hydrocarbons were identified in both immature and adult samples in large quantities (Table 1). Tridecane was the most prominent hydrocarbon found in immatures and adults; it was likely released as the insects were disturbed during handling. In low quantities, tridecane is an aggregation pheromone; however, it is considered an alarm pheromone when released in large quantities by insects (Ishiwartari 1976, Appl. Entomol. Zool. 9: 153–158; Lockwood and Story 1985, Ann. Entomol. Soc. Am. 78: 474–479; Lockwood and Story 1986, Environ. Entomol. 15: 739–749). Kitamura et al. (1984), however, observed tridecane in larger quantities in *M. punctatissimum* (as *C. punctatissimum*) and concluded that once the hydrocarbons had evaporated in air samples, aldehydes were present. They suggested that the hydrocarbons acted as the solvent and aldehydes were the attractant. Recently, Onnick et al. (2017. J. Entomol. Sci. 52: 39–51) identified large quantities of tridecane, (E)-2-decenal, and (E)-2-hexenal in kudzu bug exudate of disturbed adults; however, electroantennographic assays demonstrated that neither sex was attracted to tridecane or (E)-2-decenal, therefore suggesting that these compounds are not the attractant, but could be involved in emitting the volatile as fumigant, as reported by Kitamura et al. (1984) and Gunawardena and Herath (1991, J. Chem. Ecol. 17: 2449–2258). Interestingly, pentadecane was found in high quantities in mixed-gender adult samples, while in very low quantities, if at all, in samples where adult females, adult males, immatures remained separated (Table 1). This result might be attributed to a sampling error that detected cuticular hydrocarbons or pentadecane may possibly be a defensive compound, as Hayashi et al. (1976, Experientia 32: 418–419) reported with *Aethus nigritus* (F.) (Hemiptera: Cynididae).

(E)-4-oxohex-2-enal was found in larger quantities in the immatures (first through fifth instars) than in the adults. (E)-4-oxohex-2-enal has been reported in exudates from *Graphosoma lineatum* (L.) (Hemiptera: Pentatomidae) and *Thasus neocalifornicus* Brailovsky and Barrera (Hemiptera: Coreidae) as a defensive secretion (Prudic et al. 2008, J. Chem. Ecol. 34: 734–741; Sanda et al. 2012, J. Chromatogr. B. 881–882: 69–75). Additionally, (E)-2-decenal was found in relatively higher quantities in first through fifth instar immatures and adult exudate of disturbed than undisturbed insects. (E)-2-hexenal was found in both disturbed male and female samples. Onnick et al. (2016, J. Entomol. Sci. 52: 39–51) identified (E)-2-hexenal in large quantities of disturbed adult *M. cribraria* and found that females were significantly more attracted to the compound than males, suggesting an aggregation pheromone. Also, Aldrich et al. (1984, Environ. Entomol. 13: 1031–1036) observed (E)-2-hexenal as part of a mixture of long-range aggregation pheromones produced by male spined soldier bugs, *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae). (E)-2-octenal was found only in *M. cribraria* first through fourth instars. (E)-2-hexenal and (E)-2-octenal were found to be defensive secretions emitted by the common bed bug, *Cimex lectularius* (L.) (Hemiptera: Cimicidae), to attract adults (Ulrich et al. 2016, Physio. Entomol. 41: 103–110). Because immatures of hemipteran species are not sexually mature, this suggests that *M. cribraria* instars secrete this as a defensive compound by aggregating more insects in the general area or kudzu patch.

The identification of such compounds can help to determine specific aggregation behavior and may have a role in the potential for insect management. Borges et al. (2011, J. Appl. Entomol. 135: 68–80) tested the sex pheromone methyl 2,6,10-trimethyltridecanoate in field trials by baiting traps to attract Neotropical brown stink bugs, *Euchistus heros* (F.) (Hemiptera: Pentatomidae), for scouting efforts in soybean fields. The pheromone attracted female insects for more than a 30-d period and at low bug populations, which provided a better, more effective alternative to the usual monitoring technique. Additional experiments must be conducted for each compound reported herein to determine specific aggregation behavior of the insect.

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