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Identification of semiochemicals attractive to *Simulium vittatum* (IS-7)

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Abstract. Many blackfly species (Diptera: Simuliidae) are economically important insect pests, both as nuisance biters and as vectors of pathogens of medical and veterinary relevance. Among the important blackfly pest species in North America is *Simulium vittatum* Zetterstedt *sensu lato*. The objective of this study was to identify compounds excreted by mammalian hosts that are attractive to host-seeking *S. vittatum* females. The attractiveness of putative compounds to colonized *S. vittatum* was tested through electrophysiological (electroantennography; $n = 58$ compounds) and behavioural (Y-tube assays; $n = 7$ compounds in three concentrations) bioassays. Five compounds were significantly attractive to host-seeking *S. vittatum* females: 1-octen-3-ol; 2-heptanone; acetophenone; 1-octanol, and naphthalene. These candidate compounds might be useful as attractants in traps that could be developed for use in alternative or complementary management tactics in programmes to suppress nuisance blackfly populations, or for the collection of samples in which to study the transmission ecology of pathogens transmitted by blackflies of the *S. vittatum* complex.

Key words. Simuliidae, attractants, electroantennography, volatile organic compounds, Y-tube assay.

Introduction

Blackflies (Diptera: Simuliidae) are globally important pest insects for humans and livestock (Adler *et al.*, 2004). The annual economic impact of nuisance blackfly populations ranges from thousands to millions of dollars and reflects impacts on both agricultural and recreational activities (Gray *et al.*, 1996, 1999). This has led to the establishment of government and private sector programmes dating from the 1940s to the present that aim to suppress nuisance populations in many states of the U.S.A. and in the Canadian provinces (Gray *et al.*, 1996; O'Connor *et al.*, 2001; Adler *et al.*, 2004). Suppression programmes rely primarily on the application of products containing insecticidal crystalline proteins (ICPs) produced by *Bacillus thuringiensis* var. *israelensis* (Bti ICPs) to the larval habitat, which comprises fast-flowing bodies of water (Barjac & Sutherland, 1990).

Blackflies are also vectors of a variety of pathogens of medical and veterinary importance and thus have further economic impact. For example, vesicular stomatitis, a viral disease that affects various ungulate species, may be vectored by *Simulium vittatum* Zetterstedt (Cupp *et al.*, 1992; Smith *et al.*, 2011). The 1995 vesicular stomatitis outbreak caused losses amounting to US$50–100 m to the cattle industry of the U.S.A. (Bridges *et al.*, 1997), and outbreaks continue to occur in many states at intervals of 2–10 years (Rodríguez, 2002). Various blackfly species are also biological vectors of filarial parasites, including some species of *Onchocerca* (Nematoda: Onchocercidae). Most significantly in terms of human health, this genus includes

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the causative agent of river blindness (*Onchocerca volvulus* Leuckart, 1894). Infections with *O. volvulus* occur in Africa and formerly in six countries in Latin America. In Africa, the vectors of *O. volvulus* are primarily members of the *Simulium damnosum* Theobold species complex, whereas in Latin America, a number of *Simulium* species serve as vectors of *O. volvulus* (World Health Organization, 1995). More recently, it has been reported that sequences identical to *Onchocerca lupi* Rodonaja, 1967 have been detected in *Similium tribulatum* Lugger collected in southern California, implicating *S. tribulatum* as a potential vector for *O. lupi* in this region (Hassan *et al.*, 2015). *Simulium tribulatum* is a member of the *S. vittatum* species complex (Adler *et al.*, 2004). *Onchocerca lupi* infects companion animals (i.e. dogs and cats) and occasionally humans (Labelle *et al.*, 2011; Otranto *et al.*, 2015; Alho *et al.*, 2016; Verocai *et al.*, 2016); it can cause ocular, dermatological or neurological disease (Otranto *et al.*, 2011; Cantey *et al.*, 2016).

The objective of this study was to identify compounds produced by selected mammal hosts that were attractive to *S. vittatum* and its sibling forms. These compounds may eventually be used as attractants in traps developed to collect large numbers of *S. vittatum* in suppression programmes and as tools in research programmes aimed at elucidating the transmission ecology of blackfly-transmitted pathogens in North America.

Materials and methods

Source of blackflies

Experiments were conducted with *S. vittatum* (cytospecies IS-7 Rothfels & Featherston, 1981) females reared in a colony at the Department of Entomology of the University of Georgia (UGA), as described in the literature (Gray & Noblet, 2014).

Chemicals

The 58 test compounds (Table 1) were purchased from Sigma-Aldrich Corp. (St Louis, MO, U.S.A.) or Fisher Scientific, Inc. (Waltham, MA, U.S.A.), and had purity of 97% or higher [with the exception of lactic acid (90%)]. Unless specified, all chiral molecules were racemic mixtures of enantiomers. The choice of compounds was based on published evidence that: (a) the compound elicits a response via electroantennography (EAG) or is attractive to haematophagous dipterans as indicated in behavioural and/or field studies, or (b) the compound can be isolated as a volatile compound excreted by cattle (common hosts for species within the *S. vittatum* complex), humans or dogs (hosts for *O. lupi*) (Table 1).

Electroantennography

All test compounds (including the positive control) were dissolved in hexane [high-performance liquid chromatography (HPLC) grade $\geq 95\%$], tetrahydrofuran (THF) (HPLC grade $\geq 95\%$), or distilled water to yield 1 : 100 solutions (roughly 80 mM). Post-oviposition female blackflies (i.e. host-seeking flies) were used in EAG studies. *Simulium vittatum* is an autogenous species, and therefore females in the post-oviposition phase after completion of the first oviposition cycle, with no previous blood intake, were used in this study. Briefly, inseminated females were kept at 21–25 °C and 70–90% relative humidity (RH) for 5 days in the colony at UGA. These were transported by express courier service to the laboratory at the University of South Florida (USF) in boxes cooled with ice packs and kept at a low temperature to minimize fly loss. Groups of three or four flies were allowed to oviposit in Petri dishes measuring 5.5 cm in diameter containing moist cotton in the bottom and covered by Whatman grade 1 filter paper (Whatman, Inc., Piscataway, NJ, U.S.A.). Dishes were assessed for the presence of egg masses and flies visually examined for evidence of oviposition (i.e. a deflated abdomen as an indicator of recent oviposition and/or close proximity to egg mass). Flies were then collected and placed into paper cages with a water and sucrose solution and maintained at a low temperature (6 °C) until they were used in the EAG procedure. All females were 6–10 days old.

Electroantennography assays followed the procedures previously described (McGaha *et al.*, 2015; Young *et al.*, 2015). Briefly, blackfly samples were prepared in glass capillary tubes containing silver chloride-coated electrodes (0.2 mm in diameter), of which one was connected to the tip of one antenna and the other was exposed to the internal tissues of the metathorax. Electrodes used to monitor the stimulus were submerged into freshly prepared saline solution (750 mg NaCl, 35 mg KCl and 29 mg $CaCl_2$ per 100 mL of distilled water).

Test solutions prepared as described above (10 µL) were applied to a strip of Whatman filter paper grade 1, the solvent allowed to evaporate and the paper strip inserted into a glass Pasteur pipette. An air stimulus controller (CS-55; Syntech International BV, Hilversum, the Netherlands) generated a pulse (0.2 s duration) of filtered air that introduced headspace volatiles from the pipette into a continuous stream of humidified air (1000 mL/min) that was directed to the blackfly's antenna. Individual compounds were randomly assigned to 11 groups of five to seven compounds each and tested on a total of 66 blackflies. Electroantennography assays using solvent alone (negative controls), blanks (air puffs) and a positive control of 1-octen-3-ol (McGaha, 2013) were recorded before and after each group of compounds. All compounds were evaluated in six replicates using EAG.

Electroantennography responses were normalized to the preceding response of the positive control stimulus (1-octen-3-ol). One-way analysis of variance (ANOVA) with multiple comparisons using Dunnett's test was performed in GraphPad Prism Version 6 (GraphPad Software, Inc., San Diego, CA, U.S.A.) to determine if normalized EAG responses to compounds significantly differed from that to the solvent control ($\alpha = 0.005$). Compounds selected for behavioural assays were those that produced a significantly greater response (at least five-fold higher) than that produced by the solvent.

Behavioural assays

The compounds demonstrating activity in EAG were next tested for their attraction to host-seeking *S. vittatum* females

Table 1. Compounds selected for electroantennography studies in host-seeking *Simulium vittatum* females.

Class of compound	Compound name	Mammal species (body part) (References)	Attractive to (References)
Carboxylic acids	Heptanoic acid	Human (groin) (Young *et al.*, 2015) Dog (hair) (Magalhães-Junior *et al.*, 2014)	*Simulium damnosum s.l.* (Young *et al.*, 2015)
	Lactic acid	Human (arms, armpit) (Robinson & Robinson, 1954; Young *et al.*, 2015)	*Anopheles gambiae* (Robinson & Robinson, 1954)
	Octanoic acid	Human (sweat, feet) (Healy & Copland, 2000; Curran *et al.*, 2005; Ara *et al.*, 2006)	*S. damnosum s.l.* (Young *et al.*, 2015)
	Hexanoic acid	Human (sweat, feet) (Cork & Park, 1996; Curran *et al.*, 2005; Ara *et al.*, 2006; Jeanbourquin & Guerin, 2007)	*S. damnosum s.l.* (Young *et al.*, 2015)
		Cattle (rumen) (Healy & Copland, 2000; Curran *et al.*, 2005)	*Stomoxys calcitrans* (Jeanbourquin & Guerin, 2007) *An. gambiae* (Cork & Park, 1996)
	Nonanoic acid	Human (sweat, skin, groin) (Healy & Copland, 2000; Curran *et al.*, 2005; Young *et al.*, 2015)	*S. damnosum s.l.* (Young *et al.*, 2015)
	Isobutyric acid	Human (sweat, feet), Cattle (rumen) (Healy & Copland, 2000; Curran *et al.*, 2005; Ara *et al.*, 2006)	*St. calcitrans* (Healy & Copland, 2000; Jeanbourquin & Guerin, 2007)
	4-Methoxybenzoic acid	Human (armpit) (Young *et al.*, 2015)	—
	DL-Serine	Human (armpit and forehead) (Steullet & Guerin, 1994)	—
	Pentadecanoic acid	Human (sweat) (Healy & Copland, 2000; Curran *et al.*, 2005)	—
	Octadecanoic acid	Human (sweat) (Healy & Copland, 2000)	—
	Hexadecanoic acid	Human (sweat, skin) (Healy & Copland, 2000; Curran *et al.*, 2005)	—
	Adipic acid	Human (skin) (Curran *et al.*, 2005)	—
	Isophthalic acid	Human (armpit) (Young *et al.*, 2015)	—
	Isovaleric acid	Human (feet) (Ara *et al.*, 2006) Cattle (rumen) (Jeanbourquin & Guerin, 2007)	*St. calcitrans* (Jeanbourquin & Guerin, 2007)
	Propionic acid	Human (feet) (Cork & Park, 1996; Ara *et al.*, 2006) Cattle (rumen) (Jeanbourquin & Guerin, 2007)	*An. gambiae* (Cork & Park, 1996) *St. calcitrans* (Jeanbourquin & Guerin, 2007)
	Butyric acid	Human (sweat, feet) (Cork & Park, 1996; Ara *et al.*, 2006) Cattle (rumen) (Jeanbourquin & Guerin, 2007)	*An. gambiae* (Cork & Park, 1996) *St. calcitrans* (Jeanbourquin & Guerin, 2007)
	Decanoic acid	Human (sweat, feet) (Healy & Copland, 2000; Curran *et al.*, 2005; Ara *et al.*, 2006)	—
	Undecanoic acid	Human (armpit) (Young *et al.*, 2015)	—
	Tridecanoic acid	Human (sweat, skin) (Healy & Copland, 2000; Curran *et al.*, 2005)	—
	Linoleic acid	Human (armpit, groin) (Young *et al.*, 2015)	—
	2-Methylhexanoic acid	Human (armpit) (Young *et al.*, 2015)	—
	Oleic acid	Human (sweat) (Healy & Copland, 2000)	—
	Palmitic acid (hexadecanoic acid)	Human (sweat, skin) (Healy & Copland, 2000; Curran *et al.*, 2005)	—
Alcohols	1-Octen-3-ol	Human (feet) (Ara *et al.*, 2006) Cattle (rumen) (Jeanbourquin & Guerin, 2007)	*Simulium ochraceum s.l.* (Young *et al.*, 2015) *Simulium ornatum* (Opoku, 2008) – Y-tube *Cnephia pecuarum* (Atwood & Meisch, 1993) – field Tabanidae (Gibson & Torr, 1999), *Glossina* sp. (Hall *et al.*, 1984) *Lutzomyia longipalpis* (Sant'Anna *et al.*, 2002) *Culicoides* spp. (Bhasin *et al.*, 2001; Harrup *et al.*, 2012) *St. calcitrans*, *Haematobia irritans* (Birkett *et al.*, 2004)

Table 1. Continued.

Class of compound	Compound name	Mammal species (body part) (References)	Attractive to (References)
	Tetrahydrofurfuryl alcohol	Human (skin) (Curran *et al.*, 2005)	—
	3-Octanol	Cattle (rumen) (Jeanbourquin & Guerin, 2007)	*S. ochraceum s.l.* (Young *et al.*, 2015) *St. calcitrans*, *H. irritans* (Birkett *et al.*, 2004; Jeanbourquin & Guerin, 2007)
	1-Octanol	Cattle (rumen) (Jeanbourquin & Guerin, 2007)	*S. ochraceum s.l.* (Young *et al.*, 2015) *St. calcitrans* (Jeanbourquin & Guerin, 2007)
	2-Ethyl 1-hexanol	Cattle (rumen) (Jeanbourquin & Guerin, 2007)	*S. ochraceum s.l.* (Young *et al.*, 2015) *St. calcitrans* (Jeanbourquin & Guerin, 2007)
	cis-3-Hexen-1-ol	Cattle (rumen) (Jeanbourquin & Guerin, 2007)	*S. damnosum s.l.*, *S. ochraceum s.l.* (Young *et al.*, 2015) *St. calcitrans* (Jeanbourquin & Guerin, 2007)
	1-Decanol	Human (groin) (Young *et al.*, 2015)	—
	2-Decanol	Cattle (Birkett *et al.*, 2004)	*St. calcitrans*, *H. irritans* (Birkett *et al.*, 2004)
	1-Heptadecanol	Human (armpit) (Young *et al.*, 2015)	—
	1-Pentadecanol	Human (armpit, groin) (Young *et al.*, 2015)	—
	1-Tetradecanol	Human (groin) (Young *et al.*, 2015)	—
	1-Octadecanol	Human (armpit) (Young *et al.*, 2015)	—
	1-Nonanol	Cattle (Birkett *et al.*, 2004)	*St. calcitrans*, *H. irritans* (Birkett *et al.*, 2004)
Aldehydes	Nonanal	Human (armpit) (Curran *et al.*, 2005; Young *et al.*, 2015) Cattle (Steullet & Guerin, 1994) Dog (hair) (Oliveira *et al.*, 2008; Magalhães-Junior *et al.*, 2014)	*S. ochraceum s.l.* (Young *et al.*, 2015) *Culex quinquefasciatus* (Syed & Leal, 2009)
	Hexanal	Human (armpit) (Curran *et al.*, 2005; Young *et al.*, 2015) Cattle, rabbit (Steullet & Guerin, 1994) Dog (hair) (Oliveira *et al.*, 2008)	*S. damnosum s.l.*, *S. ochraceum s.l.* (Young *et al.*, 2015)
	Tetrahydro-2-furancarboxaldehyde	Human (armpit) (Curran *et al.*, 2005; Young *et al.*, 2015) Cattle, rabbit (Steullet & Guerin, 1994)	—
	1-Decanal	Human (armpit, forearm) (Cork & Park, 1996; Curran *et al.*, 2005; Young *et al.*, 2015) Cattle (rumen) (Jeanbourquin & Guerin, 2007) Dog (hair) (Oliveira *et al.*, 2008; Magalhães-Junior *et al.*, 2014)	*S. damnosum s.l.* (Young *et al.*, 2015) *An. gambiae* (Cork & Park, 1996) *Cx. quinquefasciatus* (Syed & Leal, 2009) *St. calcitrans* (Jeanbourquin & Guerin, 2007)
Alkanes	Pentadecane	Human (groin) (Young *et al.*, 2015) Dog (hair) (Oliveira *et al.*, 2008; Magalhães-Junior *et al.*, 2014)	—
	Undecane	Human (breath) (Sanchez & Sacks, 2006) Cattle (Birkett *et al.*, 2004) Dog (hair) (Oliveira *et al.*, 2008; Magalhães-Junior *et al.*, 2014)	—
	Heptadecane	Human (skin) (Curran *et al.*, 2005) Dog (hair) (Oliveira *et al.*, 2008; Magalhães-Junior *et al.*, 2014)	—
	Hexadecane	Human (armpit, groin) (Young *et al.*, 2015) Dog (hair) (Oliveira *et al.*, 2008)	—
Ketones	(+/−)-Dihydrocarvone	Cattle (rumen) (Jeanbourquin & Guerin, 2007)	*St. calcitrans* (Jeanbourquin & Guerin, 2007)
	Methyl acetoacetate	Human (armpit) (Young *et al.*, 2015)	—

Table 1. Continued.

Class of compound	Compound name	Mammal species (body part) (References)	Attractive to (References)
	6,10-Dimethy-5,9-undecadien-2-one	Human (skin) (Curran *et al.*, 2005)	*S. ochraceum s.l.* (Young *et al.*, 2015)
	6-Methyl-5-hepten-2-one	Human (sweat) (Cork & Park, 1996; Young *et al.*, 2015)	*An. gambiae* (Cork & Park, 1996)
		Cattle (Birkett *et al.*, 2004)	*St. calcitrans*, *H. irritans* (Birkett *et al.*, 2004; Jeanbourquin & Guerin, 2007)
	Sodium pyruvate	Human (armpit, forehead) (Steullet & Guerin, 1994)	—
	4-Methoxy-2H-chromen-2-one	Human (armpit) (Young *et al.*, 2015)	—
	Acetophenone	Human (breath) (Sanchez & Sacks, 2006)	*S. damnosum s.l.*, *S. ochraceum s.l.* (Young *et al.*, 2015)
		Cattle (rumen) (Jeanbourquin & Guerin, 2007)	*St. calcitrans* (Jeanbourquin & Guerin, 2007)
		Dog (hair) (Magalhães-Junior *et al.*, 2014)	
	Acetone	Human (breath) (Sanchez & Sacks, 2006)	*Simulium arcticum* (Sutcliffe *et al.*, 1994)
		Cattle (Birkett *et al.*, 2004)	*S. ornatum* (Opoku, 2008)
			Culicoides obsoletus (Bhasin *et al.*, 2001) – field
	2-Heptanone	Cattle (Birkett *et al.*, 2004)	*St. calcitrans*, *H. irritans* (Birkett *et al.*, 2004)
Others	3-Methyl indole	Human (groin) (Young *et al.*, 2015)	*S. ochraceum s.l.* (Young *et al.*, 2015)
	R-(+)-Limonene	Human (breath) (Sanchez & Sacks, 2006)	*St. calcitrans* (Jeanbourquin & Guerin, 2007)
		Cattle (rumen) (Jeanbourquin & Guerin, 2007)	
	Cedryl acetate	Human (armpit) (Young *et al.*, 2015)	—
	Urea (ammonia)	Human (armpit, forehead) (Kutyshenko *et al.*, 2011)	*Stegomyia aegypti* (=*Aedes aegypti*) (Geier *et al.*, 1999)
		Cattle (manure, urine) (Laubach *et al.*, 2013)	*Hybomitra lasiophthalma* (Hribar *et al.*, 1992)
	Naphthalene	Human (breath) (Sanchez & Sacks, 2006)	*St. calcitrans*, *H. irritans* (Birkett *et al.*, 2004)
		Cattle (Birkett *et al.*, 2004)	
		Dog (hair) (Magalhães-Junior *et al.*, 2014)	

in a behavioural assay using a Y-tube olfactometer model as previously described (Young *et al.*, 2015). Post-oviposition flies were obtained from the colony and kept in an incubator at 25 °C and 70–90% RH until experiments were undertaken (i.e. for 1–3 days). All females were 6–10 days old.

Initially, each group of flies ($n = 20$) was acclimated to the filtered air pumped through the Y-tube while confined in the release chamber for 10 min while no control or test compounds were present in either arm. After acclimation, the flies were allowed to exit and the numbers of flies in each arm of the apparatus, in the base and in the release chamber were recorded after 20 min. The Y-tube was thoroughly cleaned between runs, and control runs were carried out between experimental trials to ensure that no residual attractant remained in the apparatus. In total, six replicates per dilution of each compound were performed, assaying 120 flies per test, including an air (blank) vs. hexane (negative control) assay as an additional control. The positions of the arms containing the control and test stimuli were alternated between replicate runs.

Solutions of the test and control stimuli/compounds were introduced into the Y-tube olfactometer by impregnating Whatman filter papers (grade 1, 2.5 cm in diameter) with 20 µL of each solution. Hexane was allowed to evaporate before an impregnated filter paper was placed in the Y-tube's stimulus chamber. Filter papers were held in place by a copper clamp. All filter papers were pre-washed in hexane before the experiment. The attraction responses to each compound were assessed for each test run, and all compounds were tested using 10-fold dilutions (1 : 10, 1 : 100, 1 : 1000 v/v; all of the compounds used in this phase were dissolved in hexane). The proportions of flies in the test and control arms were compared using a likelihood ratio test based on a multinomial probability model using a custom program written in FORTRAN 95 (Young *et al.*, 2015). This program is available upon request.

Results

Of the 58 compounds tested in the EAG assay, 14 elicited an electrophysiological response that was significantly greater than that seen with the solvent negative control (Fig. 1). The compounds eliciting responses that were both statistically significant and at least five-fold greater than the response elicited by the solvent control were evaluated in the Y-tube olfactometer.

Five of the seven compounds tested in the Y-tube olfactometer were found to be significantly attractive to host-seeking *S. vittatum* females (Fig. 2). These included hexane solutions of 1-octen-3-ol (1 : 1000), 2-heptanone (1 : 1000, 1 : 100), acetophenone (1 : 1000), 1-octanol (1 : 1000, 1 : 100), and naphthalene (1 : 1000, 1 : 100).

Discussion

Studies evaluating the attraction of blackflies have largely been based on field evaluations of traps using a limited number of compounds that are known to attract a phylogenetically wide range of haematophagous dipterans (see Table 1), often in combination with carbon dioxide (CO_2). Only recently has more focused experimentation assessed electrophysiological and behavioural responses in two major blackfly vectors of *O. volvulus* [*Simulium ochraceum* Walker, 1861 (complex) and *S. damnosum* Theobald, 1903 (complex)] to volatile organic compounds that may be involved in host-seeking behaviour (Young *et al.*, 2015). That study used an array of organic compounds and demonstrated that only acetophenone, hexanal and cis-3-hexen-1-ol elicit an electrophysiological response in the two species complexes (Young *et al.*, 2015). Of these, only acetophenone elicited a strong response in host-seeking *S. vittatum* females. Two other compounds (1-octanol and 1-octen-3-ol) were stimulatory to *S. vittatum* and *S. ochraceum s.l.*, and two compounds not previously tested for the *O. volvulus* vectors elicited a strong response in *S. vittatum* (2-heptanone, and naphthalene).

Acetophenone was the only compound that elicited attraction in *S. vittatum*, *S. damnosum* and *S. ochraceum*. This compound has been reported as electro-stimulatory or as an attractant and is presumed to be associated with mosquito species seeking plant sugarmeals (Kwon *et al.*, 2006; Jhumur *et al.*, 2008). As do many blackfly species, female *S. vittatum* feed on plant sources as nutrients for flight and ovarian development (Cupp & Collins, 1979; Adler *et al.*, 2004). Acetophenone is also found in the breath of humans and cattle as a result of food digestion (Spinhirne *et al.*, 2004; Sanchez & Sacks, 2006; Jeanbourquin & Guerin, 2007), as well as in dog hair (Magalhães-Junior *et al.*, 2014). The attraction of *S. vittatum* to acetophenone reinforces the sensory parsimony concept first described in simuliids by Young *et al.* (2015), initially reported for higher dipterans [e.g. the stable fly, *Stomoxys calcitrans* (L.) (Diptera: Muscidae) and tsetse fly, *Glossina* spp. (Diptera: Glossinidae)], that stimuli for locating hosts or shelter are often shared across genera (Jeanbourquin & Guerin, 2007).

As in *S. ochraceum*, but not *S. damnosum* (Young *et al.*, 2015), 1-octen-3-ol was found to be attractive to host-seeking *S. vittatum* females. A related study also using a Y-tube system demonstrated that low concentrations of 1-octen-3-ol are attractive to *Simulium ornatum* Meigen (Opoku, 2008), corroborating the findings in *S. vittatum* reported here. Although 1-octen-3-ol is attractive to numerous families of nematoceran or brachyceran dipterans (Hall *et al.*, 1984; Gibson & Torr, 1999; Bhasin *et al.*, 2001; Sant'Anna *et al.*, 2002; Birkett *et al.*, 2004; Harrup *et al.*, 2012), this compound was not attractive to some Nearctic simuliids [*Simulium meridionale* Riley and *Cnephia pecuarum* (Riley)] in comparison with CO_2 alone under field conditions (Atwood & Meisch, 1993). In fact, these authors suggested that its use in traps may impair sampling of certain blackfly species. A second study conducted in North America showed that 1-octen-3-ol in combination with CO_2 had no apparent attractant effect on *Simulium arcticum* Malloch (Sutcliffe *et al.*, 1994).

Another compound that was attractive to both *S. vittatum* and *S. ochraceum*, but not *S. damnosum s.l.*, was 1-octanol. This compound is associated with cattle and is thought to be a result of digestion (Jeanbourquin & Guerin, 2007); it may also be related to host-oriented behaviour. However, unlike species within the *S. vittatum* complex that feed on cattle and several other domestic and wild ungulates (Knowlton

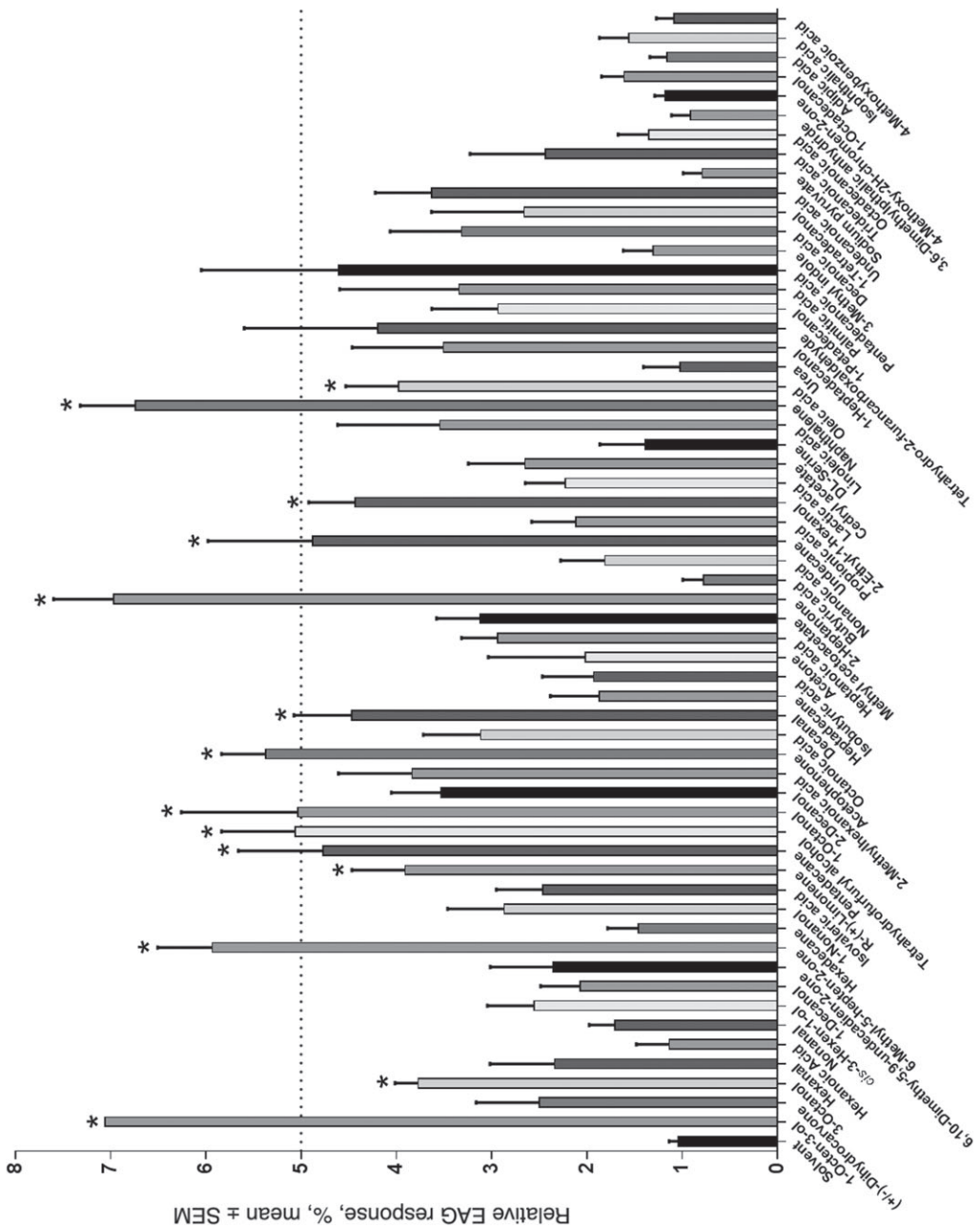

Fig. 1. Electroantennogram (EAG) responses of host-seeking *Simulium vittatum* females to tested compounds. Bars represent mean ± standard error of the mean (SEM) percentage EAG response, relative to the solvent. $^*P < 0.005$; $n = 6$. The mean ± SEM response for the negative control (air) = 0.

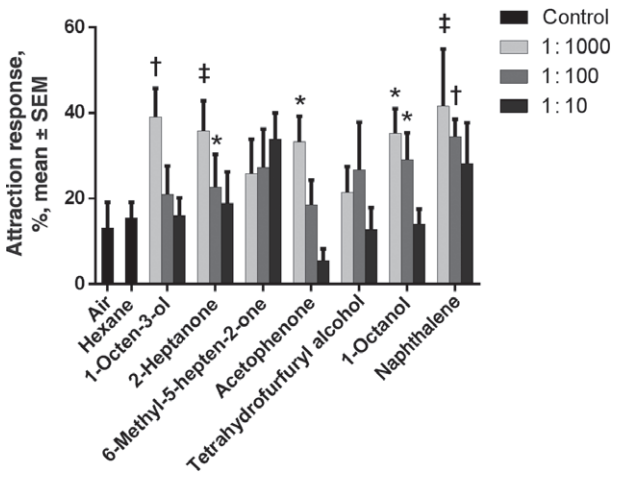

Fig. 2. Attraction responses of host-seeking *Simulium vittatum* females to different concentrations of the tested compounds (1 : 1000, 1 : 100, 1 : 10) using the Y-tube olfactometer assay. Bars indicate the mean ± standard error of the mean (SEM) percentage of flies present in the stimulus arm of the olfactometer at the end of the experimental runs. $^*P < 0.05$; †$P < 0.005$; ‡$P < 0.0005$; $n = 6$.

& Rowe, 1934; Fredeen, 1973; Pledger, 1978; Mullens & Dada, 1992), *S. ochraceum* (cytotype A) feeds preferentially on humans (Dalmat, 1955).

The two last compounds that significantly attracted host-seeking *S. vittatum* females (2-heptanone and naphthalene) have not been tested experimentally in other simuliid species (i.e. electrophysiologically, behaviourally in a Y-tube assay, or in the field), which prevents the drawing of direct comparisons. However, both compounds have elicited responses in both haematophagous and non-haematophagous dipterans that are vectors of pathogens or are nuisance biting pests of cattle (Birkett *et al.*, 2004). Whereas 2-heptanone has been isolated only from cattle (Birkett *et al.*, 2004), naphthalene occurs in humans (Sanchez & Sacks, 2006), cattle (Birkett *et al.*, 2004) and dogs (Magalhães-Junior *et al.*, 2014). Both may be involved in the location of hosts for bloodmeals in the case of *S. vittatum* and related sibling species.

The finding that naphthalene was attractive in the Y-tube olfactometer at low concentrations was surprising as it is a well-recognized repellent with insecticidal activity. Indeed, naphthalene was previously tested against *Musca autumnalis* De Geer (Diptera: Muscidae), a non-haematophagous dipteran that transmits *Thelazia* spp. to ungulates, using a wind tunnel bioassay (Birkett *et al.*, 2004). By contrast with the present study, low concentrations of naphthalene were found to be strongly repellent to flies, whereas responses to higher concentrations did not differ from that to the control. More research will be necessary to determine if this observation actually reflects fly behaviour under natural conditions.

The collection of host-seeking female blackflies has often relied on the use of traps with CO_2 emission (combined or not with volatile compounds/crude extract of host odour), actual host landings or using tents baited with a human subject or cow in the interior (Ham & Sachs, 1986; Rodríguez-Pérez *et al.*, 2013, 2014; Lamberton *et al.*, 2014; Toé *et al.*, 2014; Hassan *et al.*, 2015). The former may be more practical but requires several optimization steps in both the laboratory and the field, whereas the latter often involves significant logistical and ethical issues such as the risk for exposure of humans to *O. volvulus*. The studies described here may provide the information necessary to design an efficient bait formulation that could be used to attract *S. vittatum s.l.* to artificial traps. The development of such a bait will first require studies that evaluate the abilities of the identified compounds to attract flies under field conditions. Furthermore, any compounds used in a final formulation will need to be safe to handle, non-toxic and relatively inexpensive. Finally, they will be required to have sufficient volatility to allow dissemination over a relatively long distance at ambient temperatures, and sufficient non-volatility to permit a constant release over a period of days when incorporated into an appropriate matrix capable of supporting the slow release of a volatile compound, such as aroma beads (Young *et al.*, 2015). However, once optimized, such baits might be used in traps as an alternative or complementary population control measure in programmes for the suppression of nuisance *S. vittatum* populations. In addition, such traps may be useful tools in studies of the transmission ecology of blackfly-transmitted pathogens that occur in North America.

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Conflict of interest

The authors declare no conflicts of interest.

References

Adler, P.H., Currie, D.C. & Wood, M. (2004) *The Black Flies (Simuliidae) of North America*. Comstock Books, New York, NY.

Alho, A.M., Cruz, L., Coelho, A. *et al.* (2016) Aberrant laryngeal location of *Onchocerca lupi* in a dog. *Parasitology International*, **65**, 218–220.

Ara, K., Hama, M., Akiba, S. *et al.* (2006) Foot odour due to microbial metabolism and its control. *Canadian Journal of Microbiology*, **52**, 357–364.

Atwood, D.W. & Meisch, M.V. (1993) Evaluation of 1-octen-3-ol and carbon dioxide as black fly (Diptera: Simuliidae) attractants in Arkansas. *Journal of the American Mosquito Control Association*, **9**, 143–146.

Barjac, H. & Sutherland, D. (1990) *Bacterial Control of Mosquitoes and Black Flies*. Rutgers University Press, New Brunswick, NJ.

Bhasin, A., Mordue, A.J. & Mordue, W. (2001) Field studies on efficacy of host odour baits for the biting midge *Culicoides impunctatus* in Scotland. *Medical and Veterinary Entomology*, **15**, 147–156.

Birkett, M.A., Agelopoulos, N., Jensen, K.-M.V. *et al.* (2004) The role of volatile semiochemicals mediating host location and selection by nuisance and disease-transmitting cattle flies. *Medical and Veterinary Entomology*, **18**, 313–322.

Bridges, V.E., McCluskey, B.J., Salman, M.D., Hurd, H.S. & Dick, J. (1997) Review of the 1995 vesicular stomatitis outbreak in the western United States. *Journal of the American Veterinary Medical Association*, **211**, 556–560.

Cantey, P.T., Weeks, J., Edwards, M. *et al.* (2016) The emergence of zoonotic *Onchocerca lupi* infection in the United States – a case-series. *Clinical Infectious Diseases*, **62**, 778–783.

Cork, A. & Park, K.C. (1996) Identification of electrophysiologically active compounds for the malaria mosquito, *Anopheles gambiae*, in human sweat. *Medical and Veterinary Entomology*, **10**, 269–276.

Cupp, E.W. & Collins, R.C. (1979) The gonotrophic cycle in *Simulium ochraceum*. *American Journal of Tropical Medicine and Hygiene*, **28**, 422–426.

Cupp, E.W., Maré, C.J., Cupp, M.S. & Ramberg, F.B. (1992) Biological transmission of vesicular stomatitis virus (New Jersey) by *Simulium vittatum* (Diptera: Simuliidae). *Journal of Medical Entomology*, **29**, 137–140.

Curran, A.M., Rabin, S.I., Prada, P.A. & Furton, K.G. (2005) Comparison of the volatile organic compounds present in human odor using SPME-GC/MS. *Journal of Chemical Ecology*, **31**, 1607–1619.

Dalmat, H.T. (1955) *The Black Flies (Diptera: Simuliidae) of Guatemala and their Role as Vectors of Onchocerciasis*. Smithsonian Institution, Washington, DC.

Fredeen, F.J.H. (1973) Black flies. *Agriculture Canada Publication*, **1499**, 1–19.

Geier, M., Bosch, O.J. & Boeckh, J. (1999) Ammonia as an attractive component of host odour for the yellow fever mosquito, *Aedes aegypti*. *Chemical Senses*, **24**, 647–653.

Gibson, G. & Torr, S.J. (1999) Visual and olfactory responses of haematophagous Diptera to host stimuli. *Medical and Veterinary Entomology*, **13**, 2–23.

Gray, E.W. & Noblet, R. (2014) Large scale laboratory rearing of black flies. *Rearing Animal and Plant Pathogen Vectors* (ed. by K.M. Maramorosch & F. Mahamood), pp. 42–72. CRC Press, London.

Gray, E.W., Adler, P.H. & Noblet, R. (1996) Economic impact of black flies (Diptera: Simuliidae) in South Carolina and development of a localized suppression program. *Journal of the American Mosquito Control Association*, **12**, 676–678.

Gray, E.W., Adler, P.H., Coscarón-Arias, C., Coscarón, S. & Noblet, R. (1999) Development of the first black fly (Diptera: Simuliidae) management program in Argentina and comparison with other programs. *Journal of the American Mosquito Control Association*, **15**, 400–406.

Hall, D.R., Beevor, P.S., Cork, A., Nesbitt, B.F. & Vale, G.A. (1984) 1-octen-3-ol: a potent olfactory stimulant and attractant for tsetse isolated from cattle odours. *Insect Science and its Application*, **5**, 335–339.

Ham, P.J. & Sachs, C. (1986) The use of modified Challier–Lavéissière tsetse traps to replace human vector collectors in *Simulium damnosum* surveys. *Tropical Medicine and Parasitology*, **37**, 80.

Harrup, L.E., Logan, J.G., Cook, J.I. *et al.* (2012) Collection of *Culicoides* (Diptera: Ceratopogonidae) using CO_2 and enantiomers of 1-octen-3-ol in the United Kingdom. *Journal of Medical Entomology*, **49**, 112–121.

Hassan, H.K., Bolcen, S., Kubofcik, J. *et al.* (2015) Isolation of *Onchocerca lupi* in dogs and black flies, California, U.S.A. *Emerging Infectious Disease*, **21**, 789–796.

Healy, T.P. & Copland, M.J. (2000) Human sweat and 2-oxopentanoic acid elicit a landing response from *Anopheles gambiae*. *Medical and Veterinary Entomology*, **14**, 195–200.

Hribar, L.J.D., Leprince, D.J. & Foil, L.D. (1992) Ammonia as attractant for *Hybomitra lasiophthalma* (Macquart) (Diptera: Tabanidae). *Journal of Medical Entomology*, **29**, 346–348.

Jeanbourquin, P. & Guerin, P.M. (2007) Sensory and behavioural responses of the stable fly *Stomoxys calcitrans* to rumen volatiles. *Medical and Veterinary Entomology*, **21**, 217–224.

Jhumur, U.S., Dotterl, S. & Jurgens, A. (2008) Floral odors of *Silene otites*: their variability and attractiveness to mosquitoes. *Journal of Chemical Ecology*, **34**, 14–25.

Knowlton, G.F. & Rowe, J.A. (1934) New blood-sucking flies from Utah (Simuliidae, Diptera). *Annals of the Entomological Society of America*, **27**, 580–584.

Kutyshenko, V.P., Molchanov, M., Beskaravayny, P., Uversky, V.N. & Timchenko, M.A. (2011) Analyzing and mapping sweat metabolomics by high-resolution NMR spectroscopy. *PLoS One*, **6**, e28824.

Kwon, H.W., Lu, T., Rutzler, M. & Zwiebel, L.J. (2006) Olfactory responses in a gustatory organ of the malaria vector mosquito *Anopheles gambiae*. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 13526–13531.

Labelle, A.L., Daniels, J.B., Dix, M. & Labelle, P. (2011) *Onchocerca lupi* causing ocular disease in two cats. *Veterinary Ophthalmology*, **14**, 105–110.

Lamberton, P.H.L., Cheke, R.A., Walker, M.L. *et al.* (2014) Onchocerciasis transmission in Ghana: biting and parous rates of host-seeking sibling species of *Simulium damnosum* complex. *Parasites & Vectors*, **7**, 511.

Laubach, J., Taghizadeh-Toosi, A., Gibbs, S.J., Sherlock, R.R., Kelliher, F.M. & Grover, S.P.P. (2013) Ammonia emissions from cattle urine and dung excreted on pasture. *Biogeosciences*, **10**, 327–338.

Magalhães-Junior, J.T., Mesquita, P.R.R., Oliveira, W.F.S. *et al.* (2014) Identification of biomarkers in the hair of dogs: new diagnostic possibilities in the study and control of visceral leishmaniasis. *Analytical and Bioanalytical Chemistry*, **406**, 6691–6700.

McGaha, T.W. Jr. (2013) *Oviposition and Host-seeking Behavioral Responses of* Simulium vittatum *(Diptera: Simuliidae) to Potential Attractants in Laboratory Bioassays*. University of Georgia, Athens, GA.

McGaha, T.W. Jr., Young, R.M., Burkett-Cadena, N.D. *et al.* (2015) Identification of communal oviposition pheromones from the black fly *Simulium vittatum*. *PLoS One*, **10**, e0118904.

Mullens, B.A. & Dada, C.E. (1992) Insects feeding on desert bighorn sheep, domestic rabbits and Japanese quail in the Santa Rosa Mountains southern California. *Journal of Wildlife Diseases*, **28**, 476–480.

O'Connor, P.F., Hazelrigg, J.E., Shaw, M.W. & Madon, M.B. (2001) Black fly control program at Greater Los Angeles County Vector Control District. *Proceedings and Papers of the Annual Conference of the Mosquito and Vector Control Association of California*, 21–24 January 2001, Napa, CA. pp. 26–31.

Oliveira, L.S., Rodrigues, F.M., Oliveira, F.S. *et al.* (2008) Headspace solid phase microextraction/gas chromatography–mass spectrometry combined to chemometric analysis for volatile organic compounds determination in canine hair: a new tool to detect dog contamination by visceral leishmaniasis. *Journal of Chromatography B*, **875**, 392–398.

Opoku, A.A. (2008) The effect of CO_2, sweat, chemical vapours and air on *Simulium ornatum*: implications for control. *West African Journal of Applied Ecology*, **13**, 39–44.

Otranto, D., Sakru, N., Testini, G. *et al.* (2011) Case report: first evidence of human zoonotic infection by *Onchocerca lupi* (Spirurida, Onchocercidae). *American Journal of Tropical Medicine and Hygiene*, **84**, 55–58.

Otranto, D., Giannelli, A., Latrofa, M.S. *et al.* (2015) Canine infections with *Onchocerca lupi* nematodes, United States, 2011–2014. *Emerging Infectious Diseases*, **21**, 868–871.

Pledger, D.J. (1978) *Black Flies (Diptera, Simuliidae) of the Swan Hills, Alberta as Possible Vectors of* Onchocerca cervipedis *Wehr & Dikmans 1935 (Nematoda: Onchocercidae) in Moose (*Alces alces *Linnaeus)*. University of Alberta, Edmonton, AB.

Robinson, S. & Robinson, A.H. (1954) Chemical composition of sweat. *Physiological Reviews*, **34**, 202–220.

Rodríguez, L.L. (2002) Emergence and re-emergence of vesicular stomatitis in the United States. *Virus Research*, **85**, 211–219.

Rodríguez-Pérez, M.A., Adeleke, M., Burkett-Cadena, N.D. *et al.* (2013) Development of a novel trap for the collection of black flies of the *Simulium ochraceum* complex. *PLoS One*, **8**, e76814.

Rodríguez-Pérez, M.A., Adeleke, M., Rodríguez-Luna, I.C., Cupp, E.W. & Unnasch, T.R. (2014) Evaluation of a community-based trapping program to collect *Simulium ochraceum sensu lato* for verification of onchocerciasis elimination. *PLoS Neglected Tropical Diseases*, **8**, e3249.

Sanchez, J.M. & Sacks, R.D. (2006) Development of a multibed sorption trap, comprehensive two-dimensional gas chromatography, and time-of-flight mass spectrometry system for the analysis of volatile organic compounds in human breath. *Analytical Chemistry*, **78**, 3046–3054.

Sant'Anna, A.L., Eiras, A.E. & Cavalcante, R.R. (2002) Electroantennographic responses of the *Lutzomyia* (*Lutzomyia*) *longipalpis* (Lutz & Neiva) (Diptera: Psychodidae) to 1-octen-3-ol. *Neotropical Entomology*, **31**, 13–17.

Smith, P.F., Howerth, E.W., Carter, D. *et al.* (2011) Domestic cattle as a non-conventional amplifying host of vesicular stomatitis New Jersey virus. *Medical and Veterinary Entomology*, **25**, 184–191.

Spinhirne, J.P., Koziel, J.A. & Chirase, N.K. (2004) Sampling and analysis of volatile organic compounds in bovine breath by solid-phase microextraction and gas chromatography–mass spectrometry. *Journal of Chromatography A*, **1025**, 63–69.

Steullet, P. & Guerin, P.M. (1994) Identification of vertebrate volatiles stimulating olfactory receptors on tarsus I of the tick *Amblyomma variegatum* Fabricius (Ixodidae). I. Receptors within the Haller's organ capsule. *Journal of Comparative Physiology A, Sensory Neural and Behavioral Physiology*, **174**, 27–38.

Sutcliffe, J.F., Shemanchuk, J.A. & McKeown, D.B. (1994) Preliminary survey of odours that attract the black fly, *Simulium arcticum* (Malloch) (IIS-10.11) (Diptera: Simuliidae) to its cattle hosts in the Athabasca region of Alberta. *International Journal of Tropical Insect Science*, **15**, 487–494.

Syed, Z. & Leal, W.S. (2009) Acute olfactory response of *Culex* mosquitoes to a human- and bird-derived attractant. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 18803–18808.

Toé, L.D., Koala, L., Burkett-Cadena, N.D. *et al.* (2014) Optimization of the Esperanza window trap for the collection of the African onchocerciasis vector *Simulium damnosum sensu lato*. *Acta Tropica*, **137**, 39–43.

Verocai, G.G., Conboy, G., Lejeune, M. *et al.* (2016) *Onchocerca lupi* nematodes in dogs exported from the United States into Canada. *Emerging Infectious Diseases*, **22**, 1477–1479.

World Health Organization (1995) Expert committee report: onchocerciasis and its control. Report No. 852. WHO, Geneva.

Young, R.M., Burkett-Cadena, N.D., McGaha, T.W. Jr. *et al.* (2015) Identification of human semiochemicals attractive to the major vectors of onchocerciasis. *PLoS Neglected Tropical Diseases*, **9**, e3450.

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