

# Identification of Host Volatiles and their Role in the Behavioural Modulation of Host-Seeking *Culicoides* Biting Midges

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## Identification of Host Volatiles and their Role in the Behavioural Modulation of Host-Seeking *Culicoides* Biting Midges

### Abstract

*Culicoides* biting midges are important vectors of Bluetongue and Schmallenberg viruses in Europe. The introduction of these viruses highlighted the need to develop novel surveillance and control tools to monitor and manage biting midges.

Biting midges, as most insects, primarily use olfactory cues to recognize and discriminate resources for their survival and reproduction. Blood feeding insects rely on host-derived volatiles to locate their vertebrate hosts, and these odours can be exploited to manipulate insect-host interaction. The primary goal of this thesis was to identify host volatiles that modulate the host-seeking behaviour of biting midges. Through coupled gas chromatography and electroantennographic detection (GC-EAD) analysis of odour collections from cattle hair and urine, I identified several compounds that, in combination with carbon dioxide, elicited behavioural attraction or inhibition in *Culicoides nubeculosus*, in a dose dependent manner. Some of these host odours were evaluated in the field showing similar behavioural responses of *C. impunctatus*. Both laboratory and field assays emphasized the importance of release rate to obtain optimal behavioural responses.

The impact of using host volatiles to monitor host-seeking biting midges was shown in another study. Here, I showed that incandescent light traps baited with two cattle kairomones, 1-octen-3-ol and carbon dioxide, caught significantly more insects than UV light traps. UV light traps are to date the most commonly used method for monitoring biting midges. That said, differences were observed between trap types, both concerning species composition and the physiological state of the female biting midges caught. Incorporating the novel bioactive compounds, formulated in blends and released in proper ratio and rate, could further increase the efficacy of this trapping method. By incorporating these attractants together with repellent compounds identified in this project, a “push-pull”- system for biting midges could also be envisioned.

The host volatiles identified are detected by the peripheral olfactory system of biting midges. A comparative analysis across biting species with different host preferences revealed a correlation between host preference and the number and types of antennal and maxillary palp sensilla. Functional characterisation of these sensillum types will be the next step to increase our understanding of how host odours are detected by biting midges

With this thesis I have increased our understanding of the chemical ecology of host-seeking female biting midges and their peripheral olfactory system. Future research projects will need to develop viable commercial products that could be incorporated into management strategies for *Culicoides* biting midges.

*Keywords:* Ceratopognidae, host preference, host-derived volatiles, trapping and taxonomy

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## Dedication

To Mikael, I will always remember the late nights in the field with cheese sandwiches and a movie. Those were the days!

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## List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

- I Elin Isberg, Ylva Hillbur, Rickard Ignell (2013). Numbers of antennal and maxillary palp sensilla in the context of phylogeny and host preference of biting midges (*Culicoides: Ceratopogonidae*). *Journal of Medical Entomology* 50(3), 486-492.
- II Elin Isberg, Ylva Hillbur, Rickard Ignell. Evaluation of light- and odor-baited traps for capturing *Culicoides* biting midges. *Manuscript*.
- III Elin Isberg, Ylva Hillbur, Göran Birgersson, Rickard Ignell. Identification of cattle-derived volatiles that modulate the behavioural response of the biting midge *Culicoides nubeculosus* (Diptera: Ceratopogonidae). *Manuscript*
- IV Elin Isberg, Ylva Hillbur, Rickard Ignell. Evaluation of host-derived volatiles for trapping *Culicoides* biting midges. *Manuscript*

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The contribution of Elin Isberg to the manuscripts included in this thesis was as follows:

- I Planned the study together with the co-authors, analysed the results and wrote the article together with the co-authors
- II Planned the study together with the co-authors, performed the field study and analysed the results. Wrote the article together with the co-authors
- III Planned the study together with the co-authors, performed air entrainments, GC-EAD, GC-MS and behavioural analyses. Analysed the results. Wrote the article together with the co-authors.
- IV Planned the study together with the co-authors, performed and analysed the data from the field study. Wrote the article together with the co-authors.

## Abbreviations

GC-EAD	Gas chromatography-electroantennographic detection
GC-MS	Gas chromatography coupled mass spectrometry
BTV	Bluetongue virus
SEM	Scanning electron microscopy
CO <sub>2</sub>	Carbon dioxide

# 1 Introduction

The Bluetongue virus (BTV) is a serious, midge-transmitted, pathogen affecting ruminants. The outbreak of BTV in Europe, in 2006, (Box 1), highlighted our lack of understanding pertaining to the *Culicoides* midges which transmit the disease (Carpenter *et al.*, 2009; Carpenter *et al.*, 2008c). The emergence of the Schmallenberg virus in 2011 (Beer *et al.*, 2013) further emphasised the vulnerability which exists within Europe to midge-transmitted pathogens. A greater understanding of biting midge biology is essential if novel monitor and control strategies effective against *Culicoides* disease vectors are to be developed.

Female biting midges use volatile animal odours to recognize and discriminate between potential hosts on which to blood feed (Logan *et al.*, 2009; Mands *et al.*, 2004; Bhasin *et al.*, 2000b; Bhasin *et al.*, 2000a; Blackwell *et al.*, 1996). Identifying and exploiting these chemicals to attract or repel biting midges is recognised as an important step in developing new strategies effective against *Culicoides* disease vectors (Carpenter *et al.*, 2008a).

The first part of this thesis provides a brief overview of the genus *Culicoides*. Emphasis is placed on the basic biology, ecology and taxonomy of biting midges, and the behaviours associated with blood feeding and consequent virus transmission. The next section outlines the morphology of the peripheral olfactory system. There then follows a summary of results from previous studies on olfactory cues modulating the host-seeking behaviour of female biting midges. A summary of the results obtained during my PhD studies is then provided. The concluding section discusses the implication of my research for future monitoring and control strategies.

### **Box 1. The epidemiology of bluetongue**

Twenty six serotypes of the Blue Tongue Virus (BTV) (Maclachlan & Mayo, 2013) have been recognised worldwide, including nine in Europe. BTV, a non-zoonotic arbovirus, is primarily transmitted by *Culicoides* biting midges, where approximately 30 vector species are known to date (Dijkstra *et al.*, 2008; Meiswinkel *et al.*, 2007; Meiswinkel *et al.*, 2004). The disease affects ruminants, where certain breeds of sheep are particularly sensitive to infections, resulting in high morbidity and mortality (Mellor, 2000; Wittmann & Baylis, 2000). The clinical signs of the disease include fever, nasal discharge, facial oedema, depression and muscle weakness (Mellor, 2000).

#### **BTV emergence in Europe and potential vectors**

The recent arrival of BTV, in northern and western Europe, has not occurred in isolation. The disease has been expanding into southern and eastern Europe for the last decade. This expansion is thought to be caused by climate change (Wilson & Mellor, 2008), as well as an increase in travel and commerce (Maclachlan, 2011). Until 2006, the virus was confined to the Mediterranean basin (Mellor *et al.*, 2008). However, by 2008 BTV was detected as far north as Scandinavia (Carpenter *et al.*, 2009). The primary vector of BTV in southern Europe is not present in the north, and at the outbreak of 2006 information regarding potential vector species was scarce (Carpenter *et al.*, 2009). Extensive monitoring of biting midges following the outbreak identified five potential vector species: *C. obsoletus sensu stricto.*, *C. scoticus*, *C. dewulfi*, *C. chiopterus* and *C. pulicaris* (Meiswinkel *et al.*, 2008a).

The occurrence of BTV in northern Europe is primarily dependent on the virus' ability to overwinter in this temperate region. Unfortunately the mode by which BTV overwinters is still unknown (Wilson *et al.*, 2008a). However, several theories have been suggested: vertical transmission in the vector or host, overwintering in adult insects, prolonged viremia in host-animals or alternative vectors (Wilson *et al.*, 2008b).

## **Box 1. Continue**

### **Host-vector interaction**

In order to transmit BTV, *Culicoides* females must ingest a blood meal from an infected host, followed by replication of the virus inside the vector. Eventually the virus travels to the salivary glands and is then transmitted to a new host through the ingestion of a second blood meal (Mellor *et al.*, 2000). Although not all *Culicoides* species transmit BTV (Saegerman *et al.*, 2008; Wilson & Mellor, 2008), an increase in temperature has been shown to enhance the susceptibility of otherwise non-transmitting species. Moreover, increased temperature enhances the virus's ability to survive and replicate. Hence, global warming imposes a noticeable risk for viruses vectored by *Culicoides* biting midges to emerge in regions otherwise not affected by certain vector borne diseases (Maclachlan & Mayo, 2013; Wilson & Mellor, 2008).

## 2 Introduction to *Culicoides* biology, ecology and taxonomy

A greater understanding of the biology, ecology and taxonomy of *Culicoides* biting midges is essential to protecting humans and animals from the diseases they transmit. The emergence of BTV in northern Europe in 2006 demonstrated that little was known about potential vector species present in this region. Following the outbreak, there has been an increased effort to understand the life cycle, potential breeding habitats and adult behaviours associated with e.g. blood-feeding in biting midges. This work is essential to developing effective control strategies which will limit further disease transmission (Thompson *et al.*, 2013; Zimmer *et al.*, 2010; Takken *et al.*, 2008).

### 2.1 Life-cycle

*Culicoides* eggs generally hatch after two days, and the insects then go through four larval stages before pupation. In temperate regions, *Culicoides* biting midges overwinter as fourth instar larvae (Mellor *et al.*, 2000). The completion of all the larval stages may take as little as five days or up to several weeks, depending on temperature and humidity. The duration of the pupal stage is also temperature dependent, and can last from a few days to several weeks (Mellor *et al.*, 2000; Kettle, 1977). The adult insect has a lifespan of up to 90 days. This is the stage in the life-cycle when female biting midges ingest a blood-meal in order to develop her eggs, and potentially transmit viruses and other pathogens (Mellor *et al.*, 2000)

### 2.1.1 Breeding habitat

Biting midges are ecotone species, i.e. develop in substrates that are characterized as being in between land- and aquatic environments (Maclachlan & Mayo, 2013; Thompson *et al.*, 2013; Mellor *et al.*, 2000). The insects are not dependent on running water but they do require a moist environment. Scientists have only recently begun to understand the environmental conditions required of breeding sites utilized by different *Culicoides* species. In general, biting midges thrive in moist habitats with a high quantity of decaying material, e.g. dung, rotten vegetation, tree holes, swamps and river beds. Key environmental factors regulating breeding habitat selection by *Culicoides* are the moisture level in the soil, temperature, pH, salinity, exposure to sun as well as the amount of vegetation present (Kluiters *et al.*, 2013; Thompson *et al.*, 2013; Blackwell *et al.*, 1999; Kettle, 1977; Uslu & Dik). Different midge species appears to be associated with different environmental conditions (Takken *et al.*, 2008; Kettle, 1977). The nuisance species *Culicoides impunctatus* (Goetghebeuer) prefers an acidic and very moist environment, found for example in the highlands of Scotland, where this species thrives (Blackwell *et al.*, 1999). The BTV vectors *Culicoides obsoletus* (Meigen), *Culicoides scoticus* (Downes and Kettle), *Culicoides chiopterus* (Meigen), *Culicoides dewulfi* (Goetghebeuer) and *Culicoides pulicaris* (L.), are often associated with the products of animal husbandry (Zimmer *et al.*, 2013a; Ander *et al.*, 2012; Viennet *et al.*, 2011; Bartsch *et al.*, 2009; Casati *et al.*, 2009; Vorsprach *et al.*, 2009; Carpenter *et al.*, 2008c; De Deken *et al.*, 2008), especially animal dung (Thompson *et al.*, 2013; Zimmer *et al.*, 2013a; Ninio *et al.*, 2012; Takken *et al.*, 2008). Recent studies have also revealed that silage residues of maize, sugar beets and grass are suitable breeding habitats for these vector species (Zimmer *et al.*, 2013b). Manipulation of these habitats might therefore help in reducing local midge populations. As a consequence of the patch distribution of suitable breeding habitats for midges, population sizes and species composition often vary over short distances (Kluiters *et al.*, 2013; Ninio *et al.*, 2012; Takken *et al.*, 2008; Kettle, 1977). Identifying the likely location of these patches could enable more targeted management of vector species.

## 2.2 Activity pattern of adult female *Culicoides*

The behavioural activity patterns of adult female biting midges include mating, sugar feeding, host seeking and oviposition (Mellor *et al.*, 2000). In this

section, the focus will be on the blood-feeding behaviour, as this is essential to virus transmission.

### 2.2.1 Diurnal activity linked to host-seeking in biting midges

Female biting midges are generally crepuscular (Kettle, 1977), and will actively search for a blood-host at dawn and/or dusk. In cases when environmental conditions are not optimal for flight, biting midges will take shelter in trees, shrubs, high grasses or animal houses (Thompson *et al.*, 2013; Carpenter *et al.*, 2008b; Kettle, 1977).

Onset of host-seeking is primarily triggered by changes in light intensity (Sanders *et al.*, 2012; Viennet *et al.*, 2012; Gerry *et al.*, 2009), although different species (including BTV vectors) appear to vary in their sensitivity to light (Sanders *et al.*, 2012). Moreover, onset of host seeking is affected by environmental factors such as temperature, precipitation and wind (Sanders *et al.*, 2012; Viennet *et al.*, 2012; Kettle *et al.*, 1998).

Live baits and truck trapping have shown that vector species of BTV, including *C. chiopterus*, *C. dewulfi* (Sanders *et al.*, 2012) and *C. obsoletus* (Viennet *et al.*, 2012; Gerry *et al.*, 2009), have an onset of flight prior to sunset. This contrasts with results obtained with UV light traps where insects are not caught until after dark. Another plausible explanation for this difference could be that UV light only attracts phototactic insects, i.e. insects that are able to respond to this visual stimuli (Kettle *et al.*, 1998). The early onset of flight prior to sunset is also apparent for other species of *Culicoides* (Bellis *et al.*, 2004), and is also dependent on seasonal variation as well as lunar and tidal phases (Viennet *et al.*, 2012; Kettle *et al.*, 1998; Lillie *et al.*, 1987). Together, these studies highlight the need to develop alternative methods for surveillance to obtain a more reliable reflection of the biting population of biting midges (Carpenter *et al.*, 2008).

### 2.2.2 Long distance dispersal and its epidemiological importance

Adult biting midges are usually confined to habitats offering optimal breeding conditions, and rarely migrate more than a few hundred meters (Kettle, 1977). However, biting midges can engage in long distance dispersal, and have been suggested to travel as far as 700 km as wind borne aerial plankton (Hendrickx *et al.*, 2008). This enables the insects to transmit viruses over a large area. For example, biting midges have been suggested to be able to cross the English Channel from France to the UK, and thereby potentially introduce viruses to new areas (Burgin *et al.*, 2013). Long distance dispersal creates difficulties when predicting the epidemiology of BTV. Because of this, scientists have

started to develop models based on climate data and the distribution of potential hosts and vectors to anticipate potential outbreaks of BTV (Eagles *et al.*, 2014; Burgin *et al.*, 2013; Gloster *et al.*, 2008)

## 2.3 Systematics

Little is known about the phylogenetic relationships of the species within the genus *Culicoides* (Borkent, 2012). To date, roughly 1250 species of biting midges have been described worldwide (Wilson & Mellor, 2008), but this number is increasing as new species are discovered continuously. This is partly due to advances in the use of molecular markers that have aided scientists to distinguish between morphologically similar species (Garros *et al.*, 2011; Nolan *et al.*, 2007; Perrin *et al.*, 2006; Cetre-Sossah *et al.*, 2004). The use of these markers has also helped in unveiling the phylogenetic relationships of the species within the genus (Ander *et al.*, 2013; Perrin *et al.*, 2006).

The taxonomy of biting midges has traditionally been based on morphological characteristics. One of the more prominent features of *Culicoides* biting midges is their wing markings, which still form the basis of keys by which scientists often identify *Culicoides* species (Meiswinkel *et al.*, 2004). On the basis of morphology, 31 subgenera and 38 species groups have been identified. These species groups have not been placed at the subgenera level due to differences in morphological characteristics, as compared with already described subgenera (Borkent, 2012).

At present, the phylogeny of *Culicoides* is vast and does not provide a full account of the relationships of the species within the genus. However, considerable progress has been made recently in determining the phylogenetic relationships of the vector species of BTV, as well as of African horse sickness virus, using molecular markers (Bellis *et al.*, 2014; Ander *et al.*, 2013; Lassen *et al.*, 2012; Nolan *et al.*, 2007; Gomulski *et al.*, 2006; Dallas *et al.*, 2003; Linton *et al.*, 2002). As these methods have become more readily available and less expensive, recent studies have started to include non-vector *Culicoides* species, in order to address the phylogenetic relationships within the genus more thoroughly (Ander *et al.*, 2013; Perrin *et al.*, 2006)

Approximately thirty species are known to transmit BTV worldwide (Dijkstra *et al.*, 2008; Meiswinkel *et al.*, 2007; Meiswinkel *et al.*, 2004) and all but one of the BTV vectors in northern Europe are placed within the subgenus *Avaritia*: *C. obsoletus*, *C. scoticus*, *C. chiopterus* and *C. dewulfi*. These species cannot be easily distinguished by wing pattern alone (Meiswinkel *et al.*, 2004), hence identification is difficult and time consuming. The major

vectors of BTV in Africa and Australia, *C. imicola* (Kieffer) and *C. brevitarsis* (Kieffer), respectively, are also found in this subgenus (Meiswinkel *et al.*, 2004). The only BTV vector in northern Europe outside Avaritia, *C. pulicaris*, is found in the subgenus *Culicoides* (Meiswinkel *et al.*, 2004). This subgenus also includes *Culicoides impunctatus*, known for its nuisance behaviour in the Highlands of Scotland (Mordue & Mordue, 2003). Monoculicoides contains *Culicoides sonorensis* (Wirth and Jones), the major vector of BTV in north America, as well as *Culicoides variipennis* (Coquillett) and *Culicoides nubeculosus* (Meigen) (Borkent, 2012). Interestingly, these are the only species to be successfully reared in a laboratory environment (Hunt & Schmidtman, 2005; Boorman, 1974), and are extensively used for molecular (Veronesi *et al.*, 2013; Carpenter *et al.*, 2011; Beckenbach & Borkent, 2003) and behavioural studies (Ansari *et al.*, 2011; Papadopoulos *et al.*, 2010; Papadopoulos *et al.*, 2009; Bhasin *et al.*, 2000a). Two other subgenus of interest is Oecacta, which contains a large number of biting midge species, several of them known to northern Europe e.g. *Culicoides festivipennis* (Kieffer), *Culicoides brunnicans* (Edwards), *Culicoides vexans* (Staeger), *Culicoides duddingstoni* (Kettle and Lawson) as well as Beltranmya e.g. *Culicoides circumscriptus* (Kieffer) and *Culicoides salinarius* (Kieffer). Molecular studies suggest that the described species within Aviritia are monophyletic, which is also the case for the subgenus *Culicoides*, *Monoculicoides* as well as Beltranmya. In contrast, the subgenus Oecacta is believed to be paraphyletic (Ander *et al.*, 2013)

The continuous development of molecular markers to identify the phylogenetic relationships between *Culicoides* species will likely lead to a better understanding of how certain biological and ecological traits within the genus have evolved. This includes characteristics of epidemiological significance, e.g. susceptibility to different viruses. These molecular markers may also increase our understanding of the genetic determinants of host-preference in *Culicoides* biting midges. Moreover, an increased understanding of the phylogeny of *Culicoides* species may help us to target control efforts at species that are most likely to be involved in virus transmission.

### 3 Host preference of *Culicoides* biting midges

Haematophagous insects typically show a preference for one or a narrow range of host species, i.e. they are poly-specialists (Lehane, 2005). Host choice has evolved to ensure optimal reproduction and survival of the insect and is a trade-off between the costs and benefits in the nutritional values gained from feeding on one or several hosts (Lyimo & Ferguson, 2009).

Host preference in *Culicoides* biting midges has been investigated using live animal baits (Viennet *et al.*, 2013; Martinez-de la Puente *et al.*, 2009; Votypka *et al.*, 2009; Mullens & Dada, 1992). More recently, molecular techniques have enabled scientists to identify the hosts on which engorged female midges have blood fed (Lassen *et al.*, 2012; Pettersson *et al.*, 2012; Ninio *et al.*, 2011; Bartsch *et al.*, 2009). Results from these studies show that some biting midge species are mammalophilic, i.e. prefer to feed on large vertebrate hosts, and others ornithophilic, i.e. prefer to feed on birds. However there are also species that are opportunistic, i.e. feed on both vertebrates and birds (Table 3). Whether host preference in biting midges is genetically determined, as has been indicated for some mosquito species (Takken & Verhulst, 2013), is so far unknown. Host preference may also be dictated by geographical and environmental conditions, as observed for mosquitoes (Takken & Verhulst, 2013). To prevent transmission of BTV and other pathogens by biting midges, we need to better understand the ecology of individual *Culicoides* species, including host preference, in order to develop efficient integrated vector management strategies.

Blood meal analysis of vector species of BTV in northern Europe has shown that these species are mammalophilic, primarily feeding on cattle, horse or sheep (Pettersson *et al.*, 2012). They may also feed on rabbits, deer, hens, pigs and wild boars when available (Ninio *et al.*, 2011; Bartsch *et al.*, 2009).

Whether biting midges are hierarchical in their choice, and prefer one of these hosts over another is unknown since most studies have been performed in specific habitats with only one or a subset of these hosts present. An increasing number of studies, however, suggest that the availability and abundance of hosts are key determinants of host preference in *Culicoides* biting midges (Garcia-Saenz *et al.*, 2011; Kettle, 1977), a fact also supported by studies on mosquitoes (Takken & Verhulst, 2013). In fact, host diversity and abundance may override predetermined genetically traits as observed in mosquitoes (Takken & Verhulst, 2013). This enhances the difficulty in determining the host preference of biting midge species commonly found in farming environments.

Several species of biting midges are known to feed on birds and are often found closely associated with birds' nests (Votupka *et al.*, 2009). Blood meal analyses have shown that biting midges can feed on a variety of bird species (Lassen *et al.*, 2012; Pettersson *et al.*, 2012), with some biting midges potentially being specific in their preference (Pettersson *et al.*, 2012). Identifying ornithophilic species and the hosts they feed on is of significance to prevent transmission of avian diseases such as avian malaria (Mullens *et al.*, 2006; Garvin & Greiner, 2003).

Some species, including *C. impunctatus* (Boorman & Goddard, 1970) and species found in swamps in the south-eastern part of USA, are autogenous, i.e. they are able to go through a gonotrophic cycle without taking a blood meal (Koch & Axtell, 1978). This behaviour is likely linked to a low abundance and availability of hosts, and can lead to extremely high populations when biting midges are not reliant on blood to develop their eggs (Kettle, 1977). This type of behaviour is also observed amongst several species of mosquitoes (McIver, 1982), which emphasises the importance of this trait also in other haematophagous insects. When reaching high population levels even less BTV susceptible species could impose a threat, as seen for *C. brevitarsis*, the main vector of BTV in Australia.

### 3.1 Endophily in BTV vector species

Housing has traditionally been used as a mean of protecting animals from biting midges. However, there is now evidence indicating that vector species of BTV, including *C. obsoletus*, *C. scoticus*, *C. dewulfii* and *C. chiopterus* readily enters buildings, i.e. are endophilic, and display an endophagic feeding behaviour, i.e. feeding indoors (Viennet *et al.*, 2012; Meiswinkel *et al.*, 2008b). Infected vector species present indoors in temperate regions in the late autumn

enhances the possibility of animals being infected with virus in the winter months (Wilson *et al.*, 2008a). Implementing means of preventing biting midges to enter buildings could potentially decrease disease transmission. A similar result has been seen in strategies targeting the main African malaria vector, *Anopheles gambiae* (Giles), a highly endophilic and endophagic mosquito species (Takken & Verhulst, 2013).

## 4 Peripheral olfactory organs

Most insects, including *Culicoides* biting midges, rely primarily on their sense of smell to orientate within their environment. Olfaction plays an important role in several aspects of the behaviour of adult insects, including the location of a potential mate, food resources and oviposition sites (de Bruyne & Baker, 2008; Keil, 1999). Odours are detected by sensilla, sensory hairs present on the insect antennae, as well as on secondary olfactory organs, such as the maxillary palps (de Bruyne & Baker, 2008; Keil, 1999). Olfactory sensilla are classified according to morphological characteristics, such as length, width, and shape, as well as the basal attachment of the sensilla to the antennae (Hallberg & Hansson, 1999; Keil, 1999; Steinbrecht, 1996).

### 4.1 Antennae

The antennae of all female biting midges share a common basic structural design: a basal segment, scapa, followed by the pedicel and the flagellum, consisting of 13 subsegments (Figure 1a) (Isberg *et al.*, 2013; Kline & Axtell, 1999; Blackwell *et al.*, 1992; Chu-Wang *et al.*, 1975). The flagellum is covered with microtrichia and five morphological types of sensilla: *sensilla trichodea*, *s. chaetica*, *s. ampullacea*, *s. coeloconica*, and grooved peg sensilla (Figure 1b). Sensilla trichodea may be sub-divided into two types; blunt and sharpened tip sensilla trichodea. Moreover, two subtypes of sharpened-tipped trichodea, long- and short-sharp-tipped trichodea, and three subtypes of blunt-tipped trichodea, blunt-tipped trichodea as well as long- and short-blunt-tipped trichodea, have been described (Isberg *et al.*, 2013). Grooved peg sensilla have often been referred to as *sensilla basiconica* (Kline & Axtell, 1999; Blackwell *et al.*, 1992; Chu-Wang *et al.*, 1975). However, they bear more resemblance to grooved peg sensilla (Steinbrecht, 1996; McIver, 1982), in that they are double

walled, housing sensory neurons with unbranched dendrites (Blackwell *et al.*, 1992; Chu-Wang *et al.*, 1975). *S. basiconica* are commonly characterized as single walled sensilla, housing sensory neurons with branched dendrites (Keil, 1999).

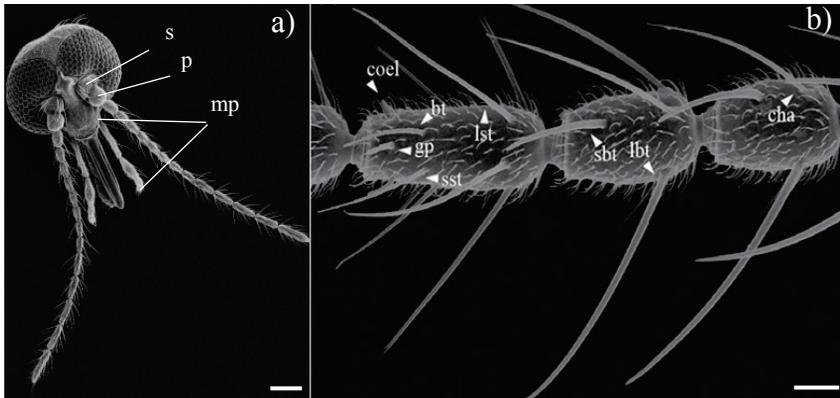


Figure 1. Scanning electron microscopy images of a) a detached head of *C. pulicaris* with the scape (s) and pedicel (p) of the antennae and the two maxillary palps (mp). b) The ovoid subsegments 6 and 7 as well as the elongated subsegment 8 on *C. chiopterus* are covered with different morphological sensilla; coel, *s. coeloconica*; gp, grooved peg sensillum; bt, blunt-tipped *s. trichodea*; sst, short sharp-tipped *s. trichodea*; lst, long sharp-tipped *s. trichodea*; sbt, short blunt-tipped *s. trichodea*; lbt, long blunt-tipped *s. trichodea*; and cha, *s. chaetica*. Scale bars a) 100  $\mu\text{m}$  and b) 20  $\mu\text{m}$ . b) is adapted after Isberg *et al.* (2013).

The sensillum types are distributed in specific patterns along the antennae (Isberg *et al.*, 2013; Kline & Axtell, 1999; Blackwell *et al.*, 1992; Chu-Wang *et al.*, 1975). For example, sharp-tipped *s. trichodea* and grooved peg sensilla are only found on the distal five segments (Isberg *et al.*, 2013; Kline & Axtell, 1999; Felipe-Bauer *et al.*, 1989; Chu-Wang *et al.*, 1975). *S. chaetica* are located on the proximal eight segments, as well as the most distal segment. Moreover, *s. ampullacea* are only found on the two proximal segments (Isberg *et al.*, 2013; Blackwell *et al.*, 1992; Felipe-Bauer *et al.*, 1989), while *s. coeloconica* (Isberg *et al.*, 2013; Braverman & Hulley, 1979) and blunt-tipped *s. trichodea* are found on all subsegments (Isberg *et al.*, 2013; Kline & Axtell, 1999; Chu-Wang *et al.*, 1975). The distributions of *s. coeloconica* and blunt-tipped *s. trichodea* vary between different species.

The function of the individual sensillum types in midges are to date unknown. However, functional analysis of *s. trichodea*, *s. coeloconica*, and grooved peg sensilla from other haematophagous insects suggest that these sensilla detect odours, including host-derived volatiles (Harraca *et al.*, 2010; Hill *et al.*, 2010; Hill *et al.*, 2009; Syed & Leal, 2009; Ghaninia *et al.*, 2008;

Qiu *et al.*, 2006; van den Broek & den Otter, 1999). Electrophysiological recordings are needed to verify the functional characteristics of the individual sensillum types in *Culicoides* biting midges.

## 4.2 Maxillary palp

The maxillary palp consists of four segments, with the third segment distally enlarged containing one or several sensory pits (Figure 2) (Isberg *et al.*, 2013; Blackwell, 2004; Kline & Axtell, 1999; McKeever *et al.*, 1994). These pits house *basiconic sensilla*, also referred to as capitata peg sensilla because of their structure, a stalk with a bulb-shaped tip. These sensilla are either completely submerged into the pit or protrude out of the pit at various degrees (Isberg *et al.*, 2013; Blackwell, 2004).

Previous studies on *Culicoides* have shown that these sensilla house olfactory sensory neurons that detect carbon dioxide, CO<sub>2</sub> (Grant & Kline, 2003), similar to that found in mosquitoes (Grant *et al.*, 1995). These compounds are known to elicit host-seeking behaviours in biting midges as well as mosquitoes (Logan & Birkett, 2007).

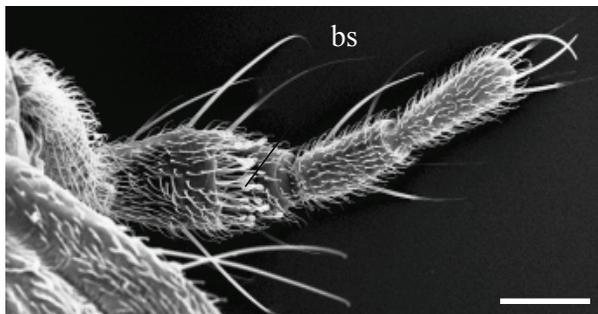


Figure 2. Scanning electron microscopy image of the enlarged third segment on the maxillary palp on *C. punctatus* housing basiconic sensilla (bs) in a pit. Scale bar 20  $\mu$ m.

## 4.3 Antennal and maxillary palp sensilla as a tool to determine host preference

Previous studies have shown a tentative correlation between the number of antennal *s. coeloconica* (Blackwell, 2004; Braverman & Hulley, 1979; Jamnback, 1965) and of maxillary palp basiconic sensilla (Blackwell, 2004; McKeever *et al.*, 1994; Braverman & Hulley, 1979; Rowley & Cornford, 1972) and the host preference of *Culicoides* species. In addition, Isberg *et al.* (2013)

found higher numbers of blunt-tipped *s. trichodea* in the ornithophilic *C. festivipennis* compared to the mammalophilic *C. obsoletus*, *C. chiopterus*, *C. pulicaris*, aside from an increase in the number of antennal *s. coeloconica* and maxillary palp basiconic sensilla. Similar correlations have been observed in *Culicine* mosquitoes where ornithophilic species have a higher number of antennal blunt-tipped *s. trichodea* and maxillary palp basiconic sensilla (McIver, 1982). Studies on both mosquitoes and biting midges emphasise the importance of making morphological comparisons at the genus level since differences in sensillar numbers may reflect phylogeny and not behaviour. Olfactory sensilla are easily visualized under a light microscope, hence morphological data could be an important tool (Braverman & Hulley, 1979) for determining the host preference of biting midges. However, additional behavioural studies and blood meal analyses are needed to further expand our understanding of sensillar density and host preference of individual species.

The increased number of *s. coeloconica* as well as of maxillary palp *basiconic sensilla* in ornithophilic species (Isberg *et al.*, 2013) could signify a higher sensitivity to volatiles from host animals. Sensitivity could be of greater importance to blood-feeding insects that feed on small or dispersed vertebrates such as solitary birds or reptiles, compared to those that feed on larger or gregarious vertebrates, for example, cattle and sheep.

## 5 Volatile cues involved in host-seeking

Female *Culicoides* biting midges primarily use olfactory cues to locate potential blood hosts. Moreover, biting midges are able to differentiate among vertebrate hosts (Viennet *et al.*, 2013; Mands *et al.*, 2004; Mullens & Dada, 1992) through differences in their odour profile (Mands *et al.*, 2004). The odour profile of a given blood host is a combination of volatiles emitted by their breath, hair, skin (either gland secretions or volatiles associated with microbial activity), urine or faeces (Logan & Birkett, 2007).

Only a handful of host-derived volatiles have so far been shown to elicit a behavioural response in *Culicoides* biting midges in laboratory bio-assays and field studies (Harrup *et al.*, 2012; Venter *et al.*, 2011; Logan *et al.*, 2009; Grant & Kline, 2003; Cilek & Kline, 2002; Bhasin *et al.*, 2001; Bhasin *et al.*, 2000b; Bhasin *et al.*, 2000a; Kline *et al.*, 1994; Ritchie *et al.*, 1994). Compounds which have been shown to elicit a response include carbon dioxide (CO<sub>2</sub>), 1-octen-3-ol and various phenolic compounds.

Carbon dioxide, present in the expired breath of vertebrates, is a key kairomone cue for haematophagous insects (Logan & Birkett, 2007). It activates the host-seeking behaviour of biting midges and other blood feeding insects. CO<sub>2</sub> has been shown to act synergistically with a number of other host odours (Syed & Leal, 2009; Takken & Kline, 1989; Vale & Hall, 1985), increasing the behavioural response of insects significantly compared to that elicited by compounds presented individually.

1-Octen-3-ol has been assessed extensively for its attraction of female biting midges (Harrup *et al.*, 2012; Kline *et al.*, 1994; Ritchie *et al.*, 1994; Kline *et al.*, 1990). This compound was initially identified in cattle breath (Hall *et al.*, 1984) and is to date a well-documented attractant for haematophagous insects, including biting midges, mosquitoes and tsetse flies (Logan & Birkett, 2007). 1-Octen-3-ol acts in synergy with CO<sub>2</sub>, and significantly increases trap

captures of many, but not all, mosquito and biting midge species (Cilek & Kline, 2002; Kline *et al.*, 1994; Ritchie *et al.*, 1994; Takken & Kline, 1989).

Phenolic compounds tested in combination with CO<sub>2</sub>, or as blends combined with 1-octen-3-ol, acetone and CO<sub>2</sub>, have also been shown to be potential attractants for biting midges (Cilek & Kline, 2002; Bhasin *et al.*, 2001). These compounds are found in aged urine (Bursell *et al.*, 1988), and were initially identified as attractants for tsetse flies (Bursell *et al.*, 1988; Gough *et al.*, 1988), against which they are used in combination with 1-octen-3-ol and acetone (Vale *et al.*, 1988), in control strategies (Pickett *et al.*, 2010). The ecological relevance of these compounds to biting midges is yet to be revealed. My hypothesis is that urine could be part of a long range olfactory cue signifying a suitable breeding habitat and/or the presence of blood hosts.

Host volatiles previously identified as attractants for other haematophagous insects may well not be the only odours responsible for host recognition and discrimination by *Culicoides* biting midges. To address this question, coupled gas chromatography and electroantennographic detection (GC-EAD) (Box 2) analysis offers relative fast screening and identification of bioactive volatile compounds present in complex host odour blends. This technique allows for an efficient selection of compounds that later can be evaluated in laboratory bioassays (Box 3), as well as in the field, for their efficacy to attract or repel host-seeking biting midges.

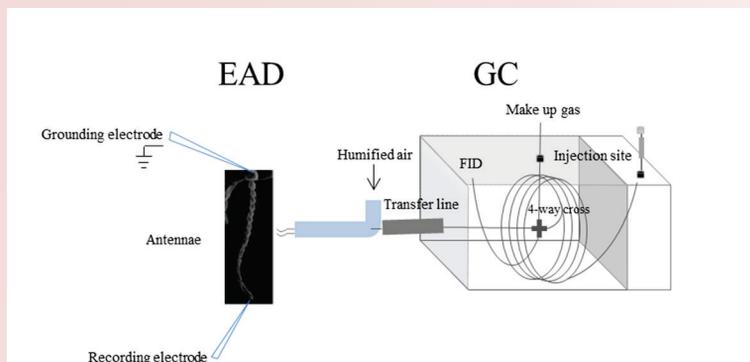
Host-volatiles identified by GC-EAD analyses of air entrainments of cattle hair and urine, as well as of whole human body odour, have been shown to elicit dose dependent behavioural responses in *C. nubeculosus* (III, IV) and *C. impunctatus* (Logan *et al.*, 2009), respectively. These studies have validated previous observations of 1-octen-3-ol as an attractant for biting midges. Moreover, they show that host-derived aldehydes, released from several large vertebrates, including humans, cattle and sheep, appear to mediate dose-dependent attraction or repellence of biting midges. The amounts of aldehydes released vary between vertebrate hosts (Tchouassi *et al.*, 2013), and blends of these compounds have been shown to increase the number of mosquitoes caught in traps, compared to single-host odour components. This result highlights the importance of developing blends of natural host odours as attractants for biting insects.

6-methyl-5-hepten-2-one (also referred to as sulcatone), another GC-EAD active compound, has been shown to be involved in the differential attractiveness of biting midges and mosquitoes to humans (Logan *et al.*, 2009; Logan *et al.*, 2008), as well as of horn flies to cattle (Birkett *et al.*, 2004). Several other ketones have been suggested to play a part in the non-

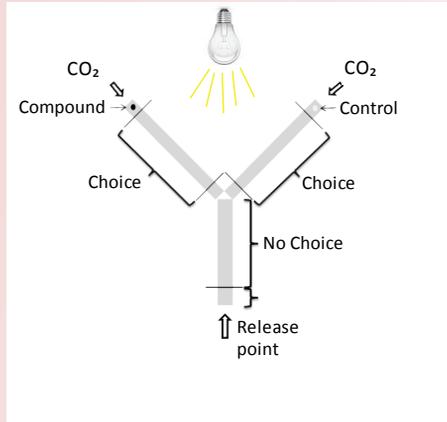
attractiveness of waterbuck to tsetse flies, suggesting that these compounds may be used as repellents for haematophagous insects (Gikonyo *et al.*, 2002).

Phenolic compounds identified as GC-EAD active compounds in cattle urine elicit dose-dependent behavioural responses in *C. nubeculosus* (III, IV) and *C. impunctatus* (Bhasin *et al.*, 2000a). In addition, 2-ethylhexanol, a non-phenolic compound identified in urine has been shown to attract biting midges (III). Biting midges also have a close association with dung, not least the vector species of BTV that utilize this habitat for breeding. Together, this suggests that biting midges may use excrement volatiles to locate habitats for both host location and oviposition.

Electrophysiological and behavioural studies suggest that haematophagous insects, including biting midges, tsetse flies, mosquitoes, bed bugs as well as triatomid bugs, have evolved to respond to a set of generic host-derived volatiles that could be exploited in future control strategies. Increased knowledge of host odours used by *Culicoides* biting midges will allow us to exploit these odours in monitoring and control strategies to target vector, as well as nuisance, species.



**Box 2.** Coupled gas chromatography and electroantennographic detection (GC-EAD) analysis is used to identify bioactive compounds in complex odour extracts (Bjostad, 1998). An odour extract is injected onto a GC column heated within an oven. Increasing the temperature of the oven allows for the separation of compounds in the extract on the basis of their physical and chemical properties. The separated compounds migrate through the column after which they reach a four way split situated at the end of the column. At this point, the GC effluent is split between the flame ionisation detector (FID) and the EAD. The GC effluent capillary for the EAD is passed through a Gerstel ODP-2 transfer line, which tracks the GC oven temperature, into a glass tube, where it is mixed with charcoal-filtered, humidified air. The antennae are placed close to the outlet of this tube. For EAD recordings, the tips of the antennae are placed in a recording electrode filled with Beadle-Ephrussi ringer solution and connected to a high impedance amplifier interface box, while a grounding electrode is inserted into the back of the detached insect head. As the separated compounds pass over the antenna the difference in the potential between the two electrodes is recorded. The change in potential corresponds to the sum of all olfactory sensory neurons on the antennae detecting the compound. By comparing the parallel recordings of the chromatogram obtained from the GC with the recorded signal from the EAD, bioactive compounds can be identified.



**Box 3.** Schematic drawing of a Y-tube olfactometer. Y-tube olfactometer assays are a simple and efficient way to evaluate the behavioural response of female biting midges to host odours. One arm of the assay contains a host compound, and the second arm contains either a control or a second compound. Insects are released, given a time to choose between the two arms and the choice is noted. The assay is scored by obtaining a response factor (RF):

$$\frac{n_{\text{compound}} - n_{\text{control}}}{n_{\text{compound}} + n_{\text{control}} + n_{\text{no choice}}} = RF$$

where n is the number of biting midges making a choice, either to the compound arm or to the control arm, as well as the number of insects not making a choice between the two arms. RFs from different compounds tested, can be used to compare the behavioural responses between different compounds, or different doses of one compound, compared to a blank response.

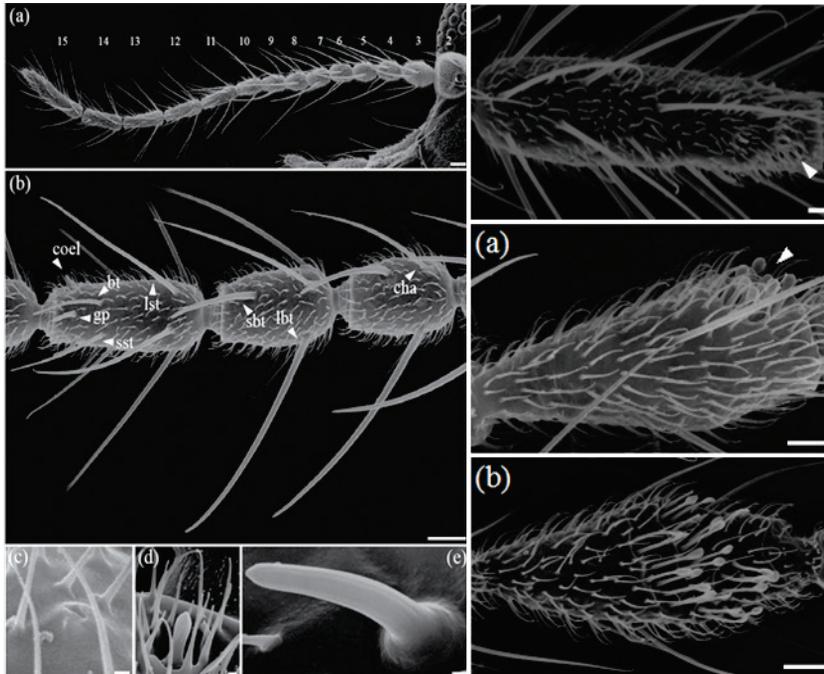
## 6 Summary of results

### 6.1 Numbers of antennal and maxillary palp sensilla in the context of phylogeny and host preference of biting midges (*Culicoides*: *Ceratopogonidae*) (I)

Olfactory sensilla are the physical sites for the detection of volatiles used by *Culicoides* biting midges to find a suitable host. Previous studies have suggested a correlation between the number and placement of antennal and maxillary palp sensilla of female biting midges and the host preference of different species (Braverman & Hulley, 1979; Rowley & Cornford, 1972; Jamnback, 1965). However, there have been few studies showing complete records of the number and types of antennal and maxillary palp sensilla of biting midges. Moreover, these studies have often not considered the phylogenetic relationship between species (Blackwell, 2004; Kline & Axtell, 1999; Chu-Wang et al., 1975).

Using Scanning Electron Microscopy (SEM), the aim of this study was first to characterize the olfactory sensilla of the peripheral olfactory organs in five species of *Culicoides* biting midges, *C. obsoletus*, *C. chiopterus*, *C. pulicaris*, *C. punctatus*, belonging to the subgenus *Aviritia* and *C. festivipennis* belonging to the subgenus *Oecacta*. Secondly, to determine if the number and placement of specific sensillum types was correlated with host preference. Five different morphological sensillum types were recorded: *sensilla trichodea*, *s. chaetica*, *s. ampullacea*, *s. coeloconica* and grooved peg sensilla. These sensilla were further divided into sub-classes depending on their external morphology (Figure 3, Table 1). *Sensilla basiconica* was present on the third enlarged segment of the maxillary palps of all species examined. These sensilla were either placed together in one large or several small pits protruding from the pit at different degrees (Figure 3g-h). Results from this study showed significant

differences in the number and placement of the different sensillum types between the five species examined (Table 2).



**Figure 3.** (a) The pedicel (sub-segment 2) and flagellum (sub-segments 3-15) of the antenna of a female *Culicoides obsoletus*. (b) The ovoid sub-segments 6 and 7 as well as the elongated sub-segment 8 of *C. chiopterus* are covered by different morphological sensillum types: coel.: *sensilla coeloconica*; gp.: grooved peg sensillum; bt.: blunt-tipped *s. trichodea*; sst.: short sharp-tipped *s. trichodea*; lst.: long sharp-tipped *s. trichodea*; sbt.: short blunt-tipped *s. trichodea*; lbt.: long blunt-tipped *s. trichodea*; and cha.: *s. chaetica*. (c) *Sensillum ampullacea*. (d) *Sensillum coeloconica*. (e) Grooved peg sensillum. Scale bars: (a) 20  $\mu\text{m}$ , (b) 10  $\mu\text{m}$ , and (c-e) 0.5  $\mu\text{m}$ . (f) *Sensilla coeloconica* of *Culicoides pulicaris* are grouped distally on sub-segment 14 (arrow head). Scale bar: 10  $\mu\text{m}$ . The third sub-segment of the maxillary palp of *Culicoides chiopterus* (g) and *C. punctatus* (h). Note that *sensilla basiconica* are located either in a single pit (g; arrow head) or several shallow pits (h). Scale bars (g) 5  $\mu\text{m}$ , and (h) 10  $\mu\text{m}$

Our results are in line with previous studies showing that ornithophilic species of female biting midges possess a higher number of maxillary palp *s. basiconica* and antennal *s. coeloconica* than mammalophilic and opportunistic species (Table 3). The ornithophilic *C. festivipennis* had *s. coeloconica* on all antennal subsegments, while this sensillum type was only present on subsegments 3 and 11-15 in the other species. *C. festivipennis* also had a higher number of short-blunt tipped *sensilla trichodea*, which previously has not been recorded in ornithophilic species. The phylogenetic relationships between the

ornithophilic species presented in (Table 3) suggest that the increase in number of *s. coeloconica*, short-blunt tipped *s. trichodea* and *s. basiconica* is a consequence of host preference and not phylogeny.

Table 1. (a) Length, area, and total number of sensilla ( $\pm$  SE;  $n = 8$ ) on the antennal flagellum of female *Culicoides*. Species with similar letters in the same column are not significantly different ( $p < 0.001$ ). (b) Length ( $l$ ;  $\mu\text{m} \pm$  SE) and basal width ( $w$ ;  $\mu\text{m} \pm$  SE) of the different morphological sensillum types present on the antennae of female *Culicoides obsoletus* (*obs*), *C. chiopterus* (*chi*), *C. pulicaris* (*pul*), *C. punctatus* (*pun*), and *C. festivipennis* (*fest*). Short sharp-tipped *s. trichodea* ( $N=40$ ); long sharp-tipped *s. trichodea* ( $N=40$ ); blunt-tipped *s. trichodea* ( $N=17$ ); short blunt-tipped *s. trichodea* ( $N=12$ ); long blunt-tipped *s. trichodea* ( $N=21$ ); *s. chaetica* ( $N=24$ ) and grooved peg ( $N=40$ )

<b>a)</b>		Length ( $\mu\text{m}$ )	Area ( $\mu\text{m}^2$ )	Sensilla
<i>C. obsoletus</i>		514 $\pm$ 20.5c	28849 $\pm$ 1411c	205 $\pm$ 4.5c
<i>C. chiopterus</i>		422 $\pm$ 14.2d	24404 $\pm$ 955c	182 $\pm$ 3.8c
<i>C. pulicaris</i>		797 $\pm$ 15.5a	55561 $\pm$ 2019a	352 $\pm$ 4.1ab
<i>C. punctatus</i>		638 $\pm$ 17.3b	44982 $\pm$ 2046b	303 $\pm$ 7.4b
<i>C. festivipennis</i>		679 $\pm$ 20.0b	48596 $\pm$ 2205ab	383 $\pm$ 5.8a

<b>b)</b>	<i>obs</i>		<i>chi</i>		<i>pun</i>		<i>pul</i>		<i>fest</i>	
	<i>l</i>	<i>w</i>								
short sharp-tipped sensilla <i>trichodea</i>	29.8 $\pm$ 0.5	1.4 $\pm$ 0.02	26.0 $\pm$ 0.4	1.3 $\pm$ 0.04	32.1 $\pm$ 0.6	1.4 $\pm$ 0.02	34.6 $\pm$ 0.5	1.7 $\pm$ 0.06	33.3 $\pm$ 0.4	1.7 $\pm$ 0.03
long sharp-tipped <i>s. trichodea</i>	44.7 $\pm$ 1.0	1.8 $\pm$ 0.02	40.1 $\pm$ 0.7	1.8 $\pm$ 0.03	47.3 $\pm$ 0.7	1.8 $\pm$ 0.03	52.9 $\pm$ 0.9	2.5 $\pm$ 0.09	54.2 $\pm$ 0.8	2.2 $\pm$ 0.04
blunt-tipped <i>s. trichodea</i>	20.6 $\pm$ 0.7	1.2 $\pm$ 0.03	16.6 $\pm$ 0.5	1.1 $\pm$ 0.03	23.0 $\pm$ 0.7	1.1 $\pm$ 0.04	21.0 $\pm$ 0.7	1.3 $\pm$ 0.07	21.8 $\pm$ 0.6	1.4 $\pm$ 0.04
short blunt-tipped <i>s. trichodea</i>	22.7 $\pm$ 0.8	1.9 $\pm$ 0.20	23.9 $\pm$ 0.8	1.9 $\pm$ 0.10	28.6 $\pm$ 1.5	2.1 $\pm$ 0.10	31.0 $\pm$ 1.1	2.8 $\pm$ 0.10	26.1 $\pm$ 0.7	2.1 $\pm$ 0.10
long blunt-tipped <i>s. trichodea</i>	55.7 $\pm$ 1.9	2.7 $\pm$ 0.09	52.4 $\pm$ 1.7	2.2 $\pm$ 0.07	60.2 $\pm$ 1.5	2.6 $\pm$ 0.09	75.4 $\pm$ 1.6	5.3 $\pm$ 2.00	77.7 $\pm$ 2.4	3.0 $\pm$ 0.06
<i>s. chaetica</i>	52.3 $\pm$ 2.5	1.3 $\pm$ 0.05	34.8 $\pm$ 1.1	1.2 $\pm$ 0.05	57.7 $\pm$ 1.6	1.5 $\pm$ 0.04	70.1 $\pm$ 1.6	1.7 $\pm$ 0.07	61.2 $\pm$ 1.5	1.4 $\pm$ 0.03
grooved peg <i>s.</i>	6.8 $\pm$ 0.10	1.7 $\pm$ 0.03	6.70 $\pm$ 0.1	1.8 $\pm$ 0.05	7.30 $\pm$ 0.2	2.2 $\pm$ 0.04	8.0 $\pm$ 0.20	2.6 $\pm$ 0.10	7.90 $\pm$ 0.2	2.4 $\pm$ 0.05

Table 2. The number ( $\pm$  SE;  $n = 8$ ) and distribution of the different morphological sensillum types present on sub-segments 3-15 of the antennae of female *Culicoides obsoletus* (obs), *C. chiopterus* (chi), *C. pulicaris* (pul), *C. punctatus* (pun), and *C. festivipennis* (fest). The total numbers of each sensillum type are significantly different ( $p < 0.001$ ) between each species as indicated by different letters.

sub-segment	short sharp-tipped sensilla trichodea					long sharp-tipped sensilla trichodea					
	obs	chi	pul	pun	fest	obs	chi	pul	pun	fest	
15	13.8 ± 0.3	14.5 ± 0.5	27.5 ± 1.0	29.7 ± 0.9	24.0 ± 0.6	15.3 ± 0.5	12.5 ± 0.4	29.5 ± 0.4	23.0 ± 0.9	31.0 ± 0.8	
14	8.3 ± 0.4	6.8 ± 0.3	14.5 ± 0.5	10.5 ± 0.5	14.0 ± 0.4	9.0 ± 0.5	7.5 ± 0.2	21.0 ± 0.5	16.8 ± 0.8	21.8 ± 0.7	
13	7.8 ± 0.4	7.8 ± 0.5	14.0 ± 0.4	11.8 ± 0.5	11.8 ± 0.4	7.8 ± 0.2	7.0 ± 0.3	16.8 ± 0.3	13.0 ± 0.7	20.5 ± 0.3	
12	7.0 ± 0.4	5.7 ± 0.4	13.3 ± 0.5	13.0 ± 0.7	10.8 ± 0.4	7.3 ± 0.3	6.8 ± 0.4	16.0 ± 0.5	13.7 ± 0.5	20.5 ± 0.3	
11	6.3 ± 0.2	5.7 ± 0.3	10.8 ± 0.5	13.8 ± 0.9	10.3 ± 0.5	6.5 ± 0.5	6.8 ± 0.4	15.8 ± 0.8	13.0 ± 0.4	17.5 ± 0.2	
Σ	43 ± 1.1b	40.5 ± 1.4b	80.0 ± 1.7a	78.7 ± 2.7a	70.8 ± 0.8a	45.8 ± 1.5c	40.5 ± 1.2c	99.0 ± 1.3a	78.5 ± 2.7b	111.3 ± 3.6a	
		blunt-tipped sensilla trichodea					grooved peg sensilla				
15	4.3 ± 0.3	1.8 ± 0.2	4.3 ± 0.6	3.0 ± 0.4	8.0 ± 0.3	14.0 ± 0.5	11.0 ± 0.2	19 ± 0.9	16.7 ± 0.9	19.6 ± 0.9	
14	3.0 ± 0.2	1.8 ± 0.1	2.8 ± 0.2	2.5 ± 0.3	1.3 ± 0.3	6.5 ± 0.5	6.3 ± 0.2	9.5 ± 0.4	11 ± 0.6	12.0 ± 0.5	
13	2.3 ± 0.1	1.5 ± 0.2	2.0 ± 0.2	2.7 ± 0.2	1.5 ± 0.3	6.3 ± 0.3	4.8 ± 0.2	7.8 ± 0.6	7.3 ± 0.3	9.3 ± 0.3	
12	2.3 ± 0.1	0.8 ± 0.2	1.5 ± 0.3	1.5 ± 0.3	1.5 ± 0.3	4.5 ± 0.3	3.5 ± 0.2	7.0 ± 0.4	5.0 ± 0.7	8.0 ± 0.2	
11	1.8 ± 0.1	0.8 ± 0.2	1.5 ± 0.3	0.8 ± 0.2	0.8 ± 0.2	3.3 ± 0.2	3.0 ± 0.2	5.8 ± 0.4	3.3 ± 0.4	5.1 ± 0.4	
Σ	13.5 ± 0.5a	6.5 ± 0.6a	12.0 ± 1.1a	10.4 ± 0.8a	13.0 ± 0.5a	34.5 ± 1.2bc	28.5 ± 0.5c	49.0 ± 2.4a	43.2 ± 2.3ab	53.9 ± 1.3 a	
		short blunt-tipped sensilla trichodea					long blunt-tipped sensilla trichodea				
10	1.5 ± 0.2	1.5 ± 0.2	1.3 ± 0.2	1.5 ± 0.2	3.2 ± 0.2	2.3 ± 0.1	2.3 ± 0.1	2.4 ± 0.1	2.5 ± 0.2	3.2 ± 0.1	
9	1.5 ± 0.2	1 ± 0.2	1.5 ± 0.2	1.5 ± 0.2	3.3 ± 0.2	2.3 ± 0.1	1.0 ± 0.2	2.5 ± 0.2	2.5 ± 0.2	3.0 ± 0.2	
8	1.5 ± 0.2	1.3 ± 0.2	1.3 ± 0.2	1.5 ± 0.2	3.3 ± 0.2	2.0 ± 0.0	2.3 ± 0.1	2.5 ± 0.1	2.3 ± 0.2	2.8 ± 0.2	
7	1.3 ± 0.2	1.3 ± 0.2	1.5 ± 0.2	1.3 ± 0.2	3.3 ± 0.2	2.3 ± 0.1	2.0 ± 0.2	2.5 ± 0.2	2.0 ± 0.2	3.0 ± 0.2	
6	1.5 ± 0.3	1.3 ± 0.2	2.0 ± 0.0	1.5 ± 0.5	3.0 ± 0.2	2.3 ± 0.1	3.0 ± 0.2	0.1 ± 0.1	2.5 ± 0.2	2.8 ± 0.2	
5	1.3 ± 0.2	1.3 ± 0.2	1.8 ± 0.1	1.5 ± 0.2	3.0 ± 0.2	2.5 ± 0.2	2.5 ± 0.2	2.3 ± 0.1	2.5 ± 0.2	2.3 ± 0.1	
4	1.5 ± 0.3	1.3 ± 0.2	1.8 ± 0.1	1.3 ± 0.2	2.8 ± 0.2	2.0 ± 0.0	2.5 ± 0.3	2.3 ± 0.1	2.0 ± 0.1	2.5 ± 0.2	
3	0.0 ± 0.1	0.0 ± 0.0	0.5 ± 0.2	0.1 ± 0.1	4.0 ± 0.3	2.0 ± 0.0	2.3 ± 0.1	2.0 ± 0.0	2.8 ± 0.0	2.8 ± 0.0	
Σ	10 ± 1.2b	8.8 ± 1b	11.8 ± 0.9b	10 ± 1.1b	25.7 ± 1.3a	17.5 ± 0.4a	17.8 ± 0.9a	18.5 ± 0.9a	18 ± 0.8a	22.2 ± 0.9a	
		sensilla chaetica					sensilla ampullacea				
15	0.8 ± 0.2	0.8 ± 0.2	0.9 ± 0.1	0.8 ± 0.2	0.8 ± 0.2						
10	4.0 ± 0.3	4.0 ± 0.3	5.8 ± 0.2	5.5 ± 0.3	4.3 ± 0.3						
9	3.0 ± 0.2	1.8 ± 0.2	6.8 ± 0.2	6.8 ± 0.2	4.5 ± 0.4						
8	3.5 ± 0.3	3.8 ± 0.2	7.0 ± 0.2	3.8 ± 0.2	3.8 ± 0.3						
7	2.8 ± 0.2	3.3 ± 0.2	7.3 ± 0.2	3.3 ± 0.2	4.0 ± 0.3						
6	4.3 ± 0.2	3.5 ± 0.2	8.5 ± 0.3	6.8 ± 0.2	4.3 ± 0.2						
5	3.0 ± 0.2	3.3 ± 0.2	8.5 ± 0.3	3.3 ± 0.3	4.8 ± 0.3						
4	3.8 ± 0.2	3.3 ± 0.2	7.5 ± 0.2	7.8 ± 0.2	4.3 ± 0.3	0.3	0.0	0.3	1.0	0.8	
3	4.8 ± 0.1	5.5 ± 0.3	7.8 ± 0.5	9.5 ± 0.1	6.0 ± 0.4	0.3	0.0	0.8	1.0	0.3	
Σ	29.8 ± 1.1b	29.0 ± 1.1b	59.9 ± 1.3a	47.3 ± 1.6a	36.5 ± 2b	0.6	0.0	1.1	2.0	1.1	
		sensilla coeloconica									
14	2.0 ± 0.2	1.8 ± 0.1	5.5 ± 0.7	2.8 ± 0.3	6.5 ± 0.3						
13	1.3 ± 0.2	1.5 ± 0.2	2.0 ± 0.3	2.0 ± 0.3	2.5 ± 0.3						
12	1.8 ± 0.2	1.3 ± 0.2	2.3 ± 0.2	2.0 ± 0.2	2.0 ± 0.0						
11	1.5 ± 0.2	1.8 ± 0.1	2.0 ± 0.2	1.8 ± 0.1	1.8 ± 0.1						
10					3.8 ± 0.2						
9					3.5 ± 0.3						
8					2.5 ± 0.3						
7					3.3 ± 0.2						
6					3.5 ± 0.3						
5					3.3 ± 0.3						
4					2.5 ± 0.3						
3	2.5 ± 0.5	3.6 ± 0.5	4.4 ± 0.7	3.0 ± 0.6	9.8 ± 0.5						
Σ	10.8 ± 0.7c	11.1 ± 0.9c	21.9 ± 1.4b	16.2 ± 0.7bc	50.3 ± 1a						

Table 3. Compiled data of the different morphological sensillum types present on the antennae and maxillary palps of female *Culicoides* biting midges. The host preference of the different species is indicated

	<i>sensilla trichodea</i>			<i>grooved peg sensilla</i>	<i>sensilla coeloconica</i>	<i>palpal sensilla basiconica</i>	<b>Host preference</b>
	Sharp- tipped	Blunt- tipped	Total <i>sensilla trichodea</i>				
<b><i>Avaritia</i></b>							
<i>C. imicola</i> (Kieffer) <sup>1</sup>	NR	NR	NR	24.6	7.1	10.4	M
<i>C. obsoletus</i> <sup>a</sup>	88.8	41	129.8	34.5	10.8	11.5	M <sup>2</sup>
<i>C. chiopterus</i> <sup>a</sup>	81	33.1	114.1	28.5	11.1	6.8	M
<b><i>Culicoides</i></b>							
<i>C. pulicaris</i> <sup>a</sup>	179	42.3	221.3	49	21.9	27.3	M <sup>2</sup>
<i>C. punctatus</i> <sup>a</sup>	157.2	38.4	195.6	43.2	16.2	26.8	O <sup>2</sup>
<i>C. impunctatus</i> <sup>3</sup>	96	33	129	16.5	7	8.3 <sup>4</sup>	M <sup>5</sup>
<b><i>Oecacta</i></b>							
<i>C. festivipennis</i> <sup>a</sup>	182.1	60.9	243	53.9	50.3	50	OR <sup>2</sup>
<i>C. pectipennis</i> <sup>6</sup>	NR	NR	NR	NR	32	NR	OR
<i>C. duddingstoni</i> <sup>6</sup>	NR	NR	NR	NR	37.2	NR	OR <sup>2</sup>
<i>C. furens</i> (Poey) <sup>8</sup>	92.7	48.1	140.8	42.2	10	10.5	O <sup>9</sup>
<b><i>Beltranmyia</i></b>							
<i>C. circumscriptus</i> <sup>6</sup>	NR	NR	NR	NR	49.4	24 <sup>7</sup>	OR <sup>2</sup>
<i>C. hollensis</i> (Melander and Brues) <sup>8</sup>	100.3	42	142.3	9.5	8.3	9	O <sup>9</sup>
<i>C. crepuscularis</i> <sup>10</sup>	NR	NR	NR	NR	NR	64	OR
<b><i>Unplaced</i></b>							
<i>C. melleus</i> (Coquillett) <sup>8</sup>	96.5	35.8	132.3	12.6	8.6	18	O <sup>11</sup>
<b><i>Heamatomyidium</i></b>							
<i>C. paraensis</i> (Goeldi) <sup>12</sup>	62	31	93	30	8	10.5	M

<sup>a</sup> This study; No records (NR); mammalophilic (M); opportunistic (O); ornithophilic (OR); <sup>1)</sup> (Braverman & Hulley, 1979); <sup>2)</sup> (Pettersson et al., 2012); <sup>3)</sup> (Blackwell et al., 1992); <sup>4)</sup> (Blackwell, 2004); <sup>5)</sup> (Blackwell et al., 1995); <sup>6)</sup> (Delecolle, 1985); <sup>7)</sup> (McKeever et al., 1994); <sup>8)</sup> (Kline & Axtell, 1999); <sup>9)</sup> (Koch & Axtell, 1979); <sup>10)</sup> (Rowley & Cornford, 1972); <sup>11)</sup> (McKeever et al., 1988); <sup>12)</sup> (Felippe-Bauer et al., 1989)

## 6.2 Evaluation of light- and odour-baited traps for capturing *Culicoides* biting midges (II)

With the emergence and increased incidence of arboviruses in Europe there is a dire need to evaluate and consequently standardize the methods used to monitor and control *Culicoides* biting midges. At present, UV-light traps is the most commonly used tool for monitoring vector species of BTV in Europe (Carpenter *et al.*, 2008a). Recent studies, however, implies that this method is not effective in trapping all vector species (Carpenter *et al.*, 2008c).

The aim of this study was to compare the efficacy of four trap types, with respect to the number of insects and diversity of species caught, as well as the physiological stage of the females trapped. Three Center for Disease Control and Prevention (CDC) incandescent light traps were used; one using incandescent light only as an attractant, one combined with CO<sub>2</sub> and one combined with CO<sub>2</sub> and 1-octen-3-ol. A fourth CDC trap employed a UV-light as an attractant. These four trap types were used with a bottle rotator collection system, to divide midge captures over a 24 h day into eight 3 h periods. This allowed us to evaluate trapping efficacy and activity period of the biting midges throughout the day-night cycle.

### 6.2.1 Trap efficacy is increased by host volatiles

The smallest number of biting midges was caught in incandescent light traps. Incandescent light traps baited with CO<sub>2</sub> and 1-octen-3-ol trapped a higher number of biting midges than UV-light traps, whereas there was no significant difference in capture between UV light traps and incandescent light traps baited with CO<sub>2</sub> (Figure 4). These results show the potential of using host odours as a mean of attracting host-seeking biting midges.

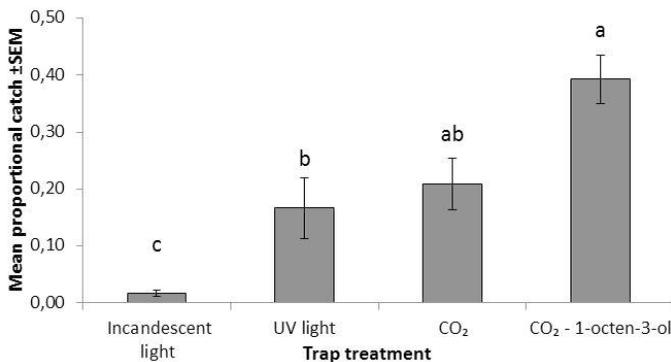


Figure 4. The mean proportional catch ( $\pm$  SEM) of female *Culicoides* biting midges in the different trap types used in the study ( $p < 0.05$ ). N=14

## 6.2.2 Species diversity and physiological state of female *Culicoides*

Qualitative analysis of trap captures revealed differences in the species caught by the different trap types. *Culicoides obsoletus s.l.*, *C. impunctatus*, *C. grisescens*, *C. pictipennis*, *C. brunnicans* and *C. poperighensis* were caught in all trap types (Table 4). However, the number of individuals of each species differed significantly between trap types (Table 5). *Culicoides impunctatus* and *C. poperighensis* were trapped in higher numbers in traps baited with either CO<sub>2</sub> or CO<sub>2</sub> and 1-octen-3-ol. *Culicoides obsoletus*, on the other hand, were trapped in equal numbers in UV light traps and in traps baited with either CO<sub>2</sub> or CO<sub>2</sub> and 1-octen-3-ol. In contrast, *C. pectipennis*, an ornithophilic species (Table 5), were trapped almost exclusively in UV light traps.

Table 4. Presence of biting midge species in the four different CDC suction traps; UV light trap, incandescent light trap, incandescent light trap baited with CO<sub>2</sub> or a combination of CO<sub>2</sub> and 1-octen-3-ol.

Species	Incandescent light	UV light	CO <sub>2</sub>	CO <sub>2</sub> -1-octen-3-ol
<i>C. obsoletus s.l.</i>	x	x	x	X
<i>C. impunctatus</i>	x	x	x	x
<i>C. pulicaris</i>		x		x
<i>C. punctatus</i>	x	x		
<i>C. grisescens</i>	x	x	x	x
<i>C. pictipennis</i>	x	x	x	x
<i>C. brunnicans</i>	x	x	x	x
<i>C. poperighensis</i>	x	x	x	x
<i>C. segnis</i>		x		x
<i>C. festipennis</i>		x		
<i>C. fascipennis</i>				x
<i>C. pallidicornis</i>				x

Table 5. Mean proportional daily catches ( $\pm$  SEM) of the different biting midge species caught by the four traps types used in the study. Means with similar letters for the different species are not significantly different from each other ( $p < 0.05$ ).  $N=14$

Trap	<i>C. obsoletus s.l.</i> *	<i>C. impunctatus</i> <sup>†</sup>	<i>C. grisescens</i> <sup>†</sup>	<i>C. brunnicans</i> *	<i>C. poperighensis</i> <sup>†</sup>	<i>C. pectipennis</i> *
<b>Incandescent light</b>	0.02 $\pm$ 0.01 b	0.03 $\pm$ 0.07 b	0.04 $\pm$ 0.03 b	0.15 $\pm$ 0.11 a	0.01 $\pm$ 0.01 b	0.02 $\pm$ 0.02 b
<b>UV light</b>	0.42 $\pm$ 0.11 a	0.14 $\pm$ 0.09 b	0.29 $\pm$ 0.10 ab	0.26 $\pm$ 0.14 a	0.19 $\pm$ 0.10 b	0.76 $\pm$ 0.21 a
<b>CO<sub>2</sub></b>	0.18 $\pm$ 0.07 a	0.32 $\pm$ 0.14 a	0.23 $\pm$ 0.06 ab	0.32 $\pm$ 0.14 a	0.21 $\pm$ 0.11 b	0.01 $\pm$ 0.01 b
<b>CO<sub>2</sub>1-octen-3-ol</b>	0.30 $\pm$ 0.09 a	0.51 $\pm$ 0.15 a	0.44 $\pm$ 0.13 a	0.27 $\pm$ 0.16 a	0.59 $\pm$ 0.11 a	0.04 $\pm$ 0.03 b

<sup>†</sup>One way ANOVA with a Turkey's multiple comparisons,  $p < 0.05$  \*Dunn's test, adjusting for ties,  $p < 0.05$

The different trap types attracted female biting midges differentially depending on their physiological state (Figure 5). Incandescent light traps baited with CO<sub>2</sub> or CO<sub>2</sub> and 1-octen-3-ol, as well as UV light traps, caught higher numbers of nulliparous and parous biting midges compared to incandescent light traps. In addition, incandescent light traps baited with CO<sub>2</sub> and 1-octen-3-ol caught higher numbers of parous biting midges than the other trap types. Interestingly, UV light traps caught the highest numbers of gravid biting midges. The results of this study suggest that differentially baited traps, baited with either host-odours or UV light, could be used to target different physiological stages of female biting midges.

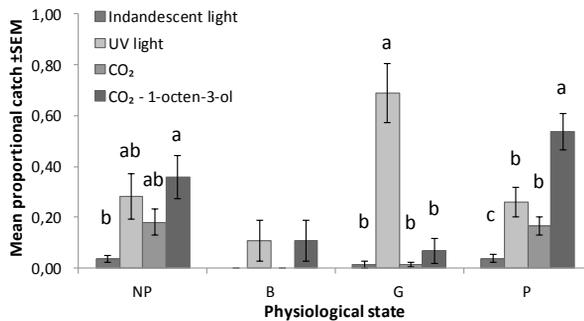


Figure 5. The mean proportional catch of nulliparous (NP), blood fed (B), gravid (G) and parous (P) female biting midges in the different CDC suction traps; UV light trap, incandescent light trap, incandescent light trap baited with CO<sub>2</sub> or a combination of CO<sub>2</sub> and 1-octen-3-ol, ( $\pm$ SEM). Trap types with similar letters within the different physiological states are not significantly different from each other ( $p < 0.05$ ). N=14

### 6.2.3 Diurnal activity and trap efficacy

Diurnal capture of female biting midges showed that they have an activity peak around sunset (Figure 6), in which they engage in host-seeking activity. Incandescent light traps baited with either CO<sub>2</sub> or CO<sub>2</sub> and 1-octen-3-ol revealed a distinct peak of activity around sunset. These results are in concordance with previous studies on diurnal activity of biting midges (Viennet *et al.*, 2011; Gerry *et al.*, 2009). Incandescent light traps baited with CO<sub>2</sub> and 1-octen-3-ol also caught higher numbers of biting midges at and around sunset compared to UV light and incandescent light traps baited with CO<sub>2</sub> (Table 6). Host-derived volatiles are natural agents targeting the host-seeking behaviour of the female biting midges, which is primarily driven by olfactory cues, as compared to UV-light. Our results also imply that females engage in other types of behaviours not driven by host volatiles, such as nectar

feeding or oviposition, as suggested by the prolonged trapping period as seen for the UV light trap.

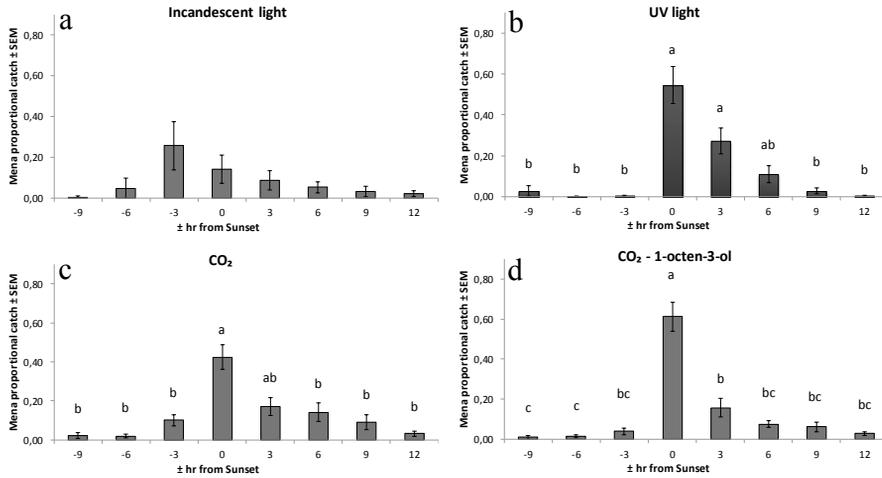


Figure 6. Mean proportional daily catch of biting midges ( $\pm$  SEM) in **a.** incandescent light traps, **b.** UV-light traps, **c.** incandescent light traps baited with  $\text{CO}_2$  and **d.** incandescent light traps baited with  $\text{CO}_2$  and 1-octen-3-ol, over a 24 hr period, with three hour increments. 0 signifies the onset of sunset. Columns with different letters indicate significant differences between the different time periods ( $p < 0.05$ ).  $N=14$ . Due to the low number of biting midges trapped in the incandescent light trap, no statistical analysis was performed.

Table 6. Statistical comparison of the mean proportional catch ( $\pm$ SEM) of *Culicoides* biting midges during 24 hours, during eight 3 hour trapping periods, using four different CDC suction traps; UV light trap, incandescent light trap, incandescent light trap baited with  $\text{CO}_2$  or a combination of  $\text{CO}_2$  and 1-octen-3-ol. 0 signifies the onset of sunset. Rows with similar letters are not significantly different from each other ( $p < 0.05$ ).  $N=14$

Period	Incandescent light	UV light	$\text{CO}_2$	$\text{CO}_2$ -1-octen-3-ol
-9	0.02 $\pm$ 0.02a	0.21 $\pm$ 0.10a	0.18 $\pm$ 0.08a	0.16 $\pm$ 0.09a
-6	0.05 $\pm$ 0.05a	0.14 $\pm$ 0.10a	0.22 $\pm$ 0.11a	0.23 $\pm$ 0.11a
-3	0.10 $\pm$ 0.04b	0.09 $\pm$ 0.08b	0.39 $\pm$ 0.08a	0.28 $\pm$ 0.09ab
0	0.01 $\pm$ 0.01c	0.24 $\pm$ 0.06b	0.21 $\pm$ 0.04b	0.53 $\pm$ 0.07a
3	0.02 $\pm$ 0.01b	0.35 $\pm$ 0.07a	0.27 $\pm$ 0.07a	0.36 $\pm$ 0.07a
6	0.03 $\pm$ 0.02a	0.30 $\pm$ 0.09a	0.21 $\pm$ 0.09a	0.39 $\pm$ 0.10a
9	0.02 $\pm$ 0.02b	0.20 $\pm$ 0.10a	0.37 $\pm$ 0.12a	0.26 $\pm$ 0.10a
12	0.04 $\pm$ 0.04b	0.07 $\pm$ 0.05b	0.28 $\pm$ 0.10ab	0.46 $\pm$ 0.12a

In conclusion, this work has shown that host-derived volatiles could be a potential tool for targeting host-seeking female biting midges at their peak activity periods at dusk and dawn. However, our results also reveal that biting midge species with different host preferences do not respond to the same attractants. This emphasizes the importance of identifying novel host volatiles that can be incorporated in integrated vector control strategies against *Culicoides* biting midges.

### 6.3 Identification and evaluation of host-derived volatiles in *Culicoides* biting midges (III, IV)

Host-derived volatiles have been used successfully as baits to monitor and control other haematophagous insects (Pickett *et al.*, 2010; Logan & Birkett, 2007). Several generic host-derived volatiles have been shown to elicit behavioural response in biting midges, including 1-octen-3-ol and several phenolic compounds. These volatiles have often been used in combination with CO<sub>2</sub>, a key kairomone for haematophagous insects (Logan & Birkett, 2007). However, few attempts have been made to incorporate host volatiles in control strategies against biting midges (Venter *et al.*, 2011; Cilek *et al.*, 2003; Bhasin *et al.*, 2001). This may be due to the absence of bioactive host compounds that attract *Culicoides* biting midges.

Using *C. nubeculosus*, a known research species which shares the same host preference as the vectors of the Bluetongue and Schmallenberg viruses (Pettersson *et al.*, 2012; Lassen *et al.*, 2011; Nielsen & Christensen, 1975; Mellor & McCaig, 1974), our aim was to identify bioactive host-derived volatiles from cattle hair and urine. Further, to evaluate the behavioural response of host-seeking *C. nubeculosus* both in the laboratory as well as in a field environment to identify host odours that could be incorporated into surveillance and control strategies of biting midges.

#### 6.3.1 Identification of bioactive host-derived volatiles

Through GC-EAD analysis we identified 16 bioactive compounds from odour entrainments of cattle hair and urine (Figure 7, Table 7). Several of these compounds have previously been shown to elicit electrophysiological responses at the peripheral olfactory level in biting midges, mosquitoes, tsetse flies, bed bugs and triatomid bugs (Hill *et al.*, 2009; Logan *et al.*, 2009; Syed & Leal, 2009; Ghaninia *et al.*, 2008; Logan *et al.*, 2008; Qiu *et al.*, 2006; Gikonyo *et al.*, 2003; Guerenstein & Guerin, 2001; Bhasin *et al.*, 2000a; Blackwell *et al.*, 1996; Denotter *et al.*, 1988).

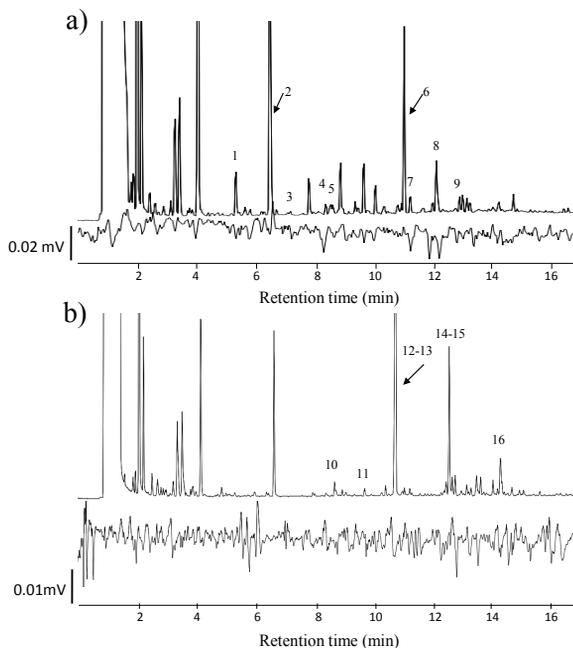


Figure 7. GC-EAD recording from cattle hair a) and urine b). The peak number corresponds to the peak number in table 7.

Table 7. Compounds in odour collections from cattle hair and urine that elicit GC-EAD activity in *C. nubeculosus*. Kovats' Index (KI) calculated for an HP5 column for cattle hair and DB-wax column for cattle urine

Cattle hair			Cattle urine		
Peak	KI	Compound	Peak	KI	Compound
1	846	<i>E</i> -2-Hexenal	1	846	<i>E</i> -2-Hexenal
2	898	Heptanal	11	2006	Phenol
3	937	Unknown	12	2084	<i>p</i> -cresol
4	977	1-Octen-3-ol	13	2092	<i>m</i> -cresol
5	986	Sulcatone	14	2177	4-Ethylphenol
6	1107	Nonanal	15	2184	3-Ethylphenol
7	1119	Unknown	16	2269	3-Propylphenol
8	1168	<i>E</i> -2-Nonenal			
9	1212	Decanal			

### 6.3.2 Behavioural relevance of bioactive host-derived volatiles

To analyse the behavioural response of *C. nubeculosus* to the GC-EAD active compounds, a two-choice bioassay was used. Carbon dioxide was added to both test arms of the olfactometer, and a host volatile eliciting an electrophysiological response was added to one of the arms, in a series of decadic concentrations.

The analysis showed that the bioactive compounds elicited dose-dependent behavioural responses in *C. nubeculosus* (Figure 8). Decanal, 1-octen-3-ol, 2-ethylhexanol, phenol, 3-ethylphenol and 4-methylphenol elicited significant dose-dependent attraction. In contrast, *C. nubeculosus* were significantly repelled by heptanal, octanal, nonanal, *E*-2-hexenal, and 3-propylphenol. Moreover, 6-methyl-5-hepten-2-one, 3-methylphenol and 4-ethylphenol elicited both attraction and inhibition depending on the dose tested.

1-octen-3-ol has been assessed extensively for its effect on the behaviour of *Culicoides* biting midges and other haematophagous insects (Logan & Birkett, 2007). We show that *C. nubeculosus* is attracted to a lower concentration of 1-octen-3-ol than previously shown for this and other species, suggesting a species-specific behavioural response to 1-octen-3-ol, which is dependent on dose. An alternative explanation is that variation in response is a product of differences in experimental design.

The aldehydes tested elicited both attraction and repellence of *C. nubeculosus* (Figure 8). The behavioural responses to decanal, octanal and nonanal differ from that observed in *C. impunctatus*, again suggesting a species-specific response to host-derived compounds.

Phenolic compounds, when tested at natural doses, elicited attraction of *C. nubeculosus* (Figure 8). This is also in line with results obtained for *C. impunctatus* (Bhasin et al., 2000a). At high concentrations these host odours are repellent.

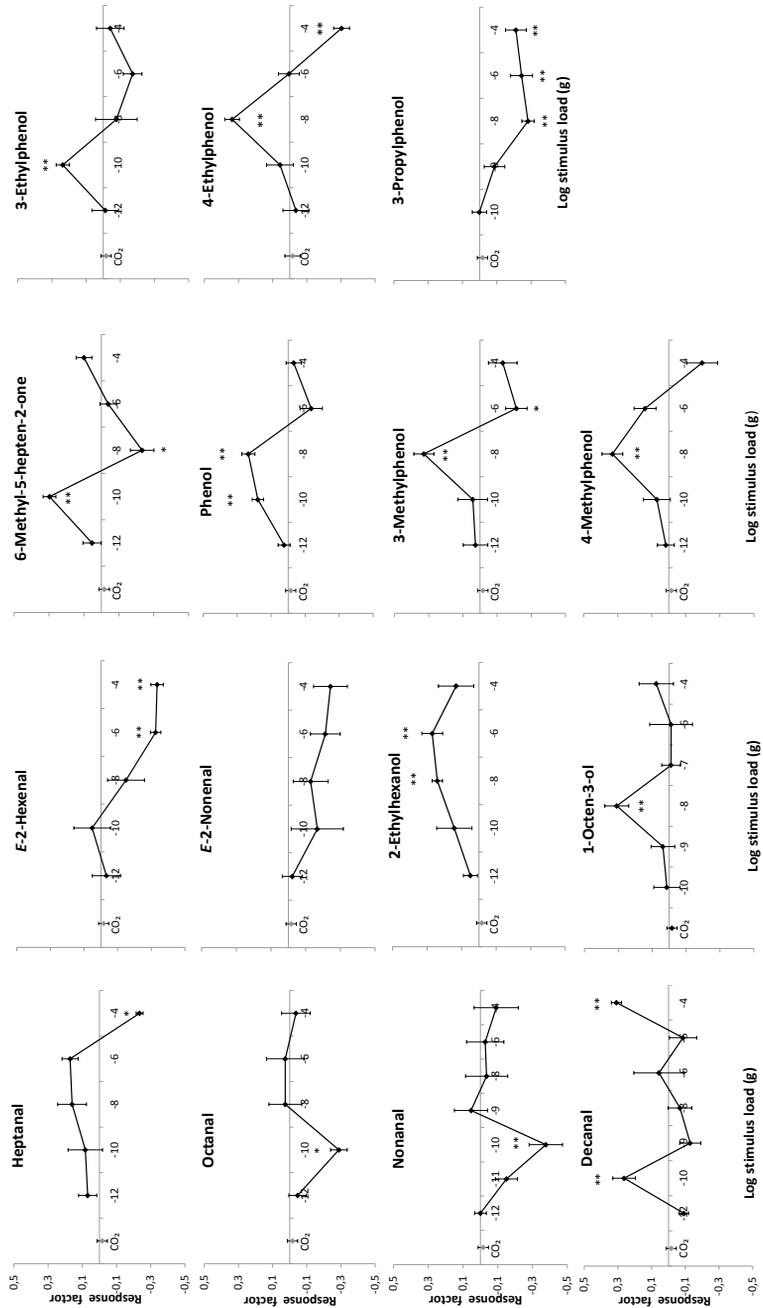


Figure 8. The behavioural response of *C. nubeculosus*, given as a response factor ( $\pm$ SEM), to different doses of cattle-derived volatiles and a hexane control in a two-choice behavioural assay. A Dunnett's test, comparing with a control, reveal significant attraction of the biting midges to the tested volatiles(positive values) or less attractive(negative values)thanthe CO<sub>2</sub> only control. \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001. N=10

### 6.3.3 Field evaluation of host-derived volatiles

Nine of the behaviourally active host-odours were evaluated in the field for their efficacy in trapping *Culicoides* biting midges. Center for Disease Control and Prevention (CDC) standard miniature light traps with a CO<sub>2</sub> delivery system were used to trap biting midges. Three different release rates of the tested compounds were used to determine the optimal rate for trapping female biting midges: 0.1 mg/h, 1 mg/h and 10 mg/h. The individual compounds were released from lures made from polyethylene caps. Different sized holes in the cap lid were used to obtain the required release rates. CO<sub>2</sub> were released from all traps at 500 ml/h.

A control trap, releasing CO<sub>2</sub> only, was placed together with the three traps baited with the different doses of an odour compound, together forming a test group. One test group was placed at one of the field sub-sites in the form of a square, 1.5 m from the ground, three meters apart. Trapping took place at the peak of host-seeking activity, 2 hours before sunset until 1 hour after sunset.

*C. impunctatus* represented the majority of biting midges caught in the traps. Our results showed a dose-dependent behavioural attraction or inhibition of *C. impunctatus* to the tested host-derived volatiles (Figure 9). CO<sub>2</sub> traps baited with 1-octen-3-ol, nonanal, decanal, phenol, 4-methylphenol and 3-propylphenol caught significantly higher number of *C. impunctatus*, compared to the CO<sub>2</sub> control trap. In contrast, CO<sub>2</sub> traps baited with octanal and *E*-2-nonenal trapped a significantly lower number of biting midges compared to the CO<sub>2</sub> control trap. 2-Ethylhexanol did not show any significant result.

1-Octen-3-ol has repeatedly been shown to attract *Culicoides*, although different species vary in their attraction to different release rates (Harrup *et al.*, 2012; Kline *et al.*, 1994; Ritchie *et al.*, 1994). The current study was the first field based experiment to evaluate the impact host-derived aldehydes impact on biting midges host-seeking behaviour. Further, phenolic compounds have previously been evaluated in the field for several species of biting midges, including *C. impunctatus*, as blends or in combination with 1-octen-3-ol as well as CO<sub>2</sub>, however these results are inconclusive and further research is needed.

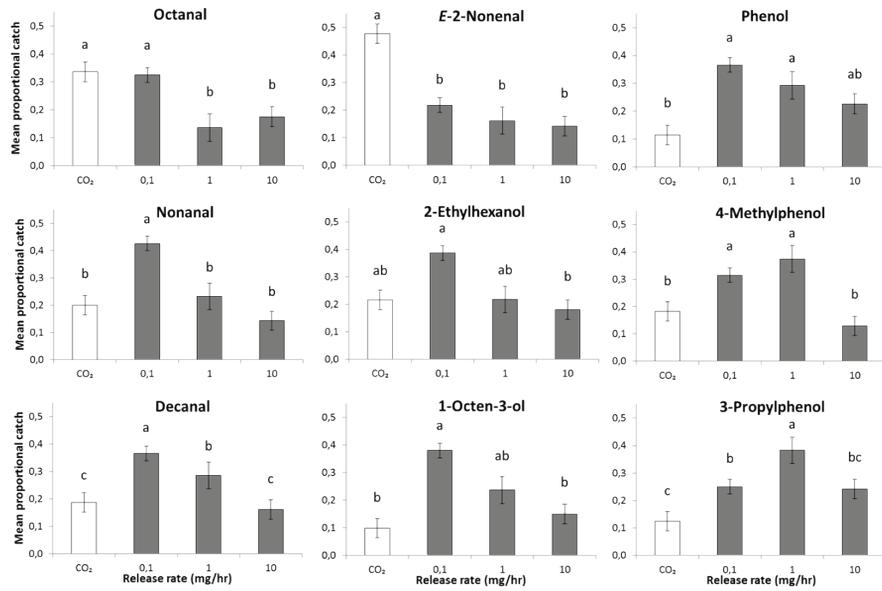


Figure 9. Mean proportional catch ( $\pm$ SEM) of biting midges caught by traps baited with nine different host-related compounds, at three release rates, together with CO<sub>2</sub> and by a CO<sub>2</sub> control trap. Release rates within the different compounds with similar letter are not significantly different ( $p < 0.05$ ). N=7

## 7 Conclusion and future perspective

The exploitation of host odours for use against biting midges is still at an early stage. Little research has gone into identifying novel bioactive compounds involved in host-seeking and other odour-mediated behaviours of female biting midges. However, there is great potential in exploiting these natural cues for the development of novel control methods against *Culicoides*.

Through the results obtained from my thesis work, our understanding of the volatile cues driving the host-seeking behaviour of female biting midges has increased (III, IV). I have identified several bioactive compounds in cattle odour that could be incorporated into monitoring and control strategies. Additional research is however required to develop a commercial product. For example, development and optimisation of host odour blends could further increase the attraction of biting midges, as shown for other haematophagous insects (Tchouassi *et al.*, 2013; Pickett *et al.*, 2010). Moreover, several of the bioactive compounds showed potential as repellents. If used together with an attractant, these repellents could be used in a push-pull system, and thereby decrease vector-host interactions.

Results from my thesis work (II) also demonstrate that species-specific differences, including host-preference, and physiological stage, are important factors to consider when developing an attractant lure. Identifying synthetic blends of host-odours could be a way in the future to tailor lures that target species with different host-preferences, e.g. those of BTV, avian malaria or African horse sickness. A complementary strategy would be to identify non-host volatiles that repels the insects (Pickett *et al.*, 2010), as shown for waterbuck repellents and tsetse flies (Gikonyo *et al.*, 2003).

This thesis also includes the first complete morphological characterisation of the peripheral olfactory organs of some of the most important midge vector species in Europe. The results show a link between the distribution and

numbers of sensilla on the antenna and maxillary palps in different *Culicoides* species and their host preference. The next step will be to conduct functional characterisation of individual sensilla, in order to elucidate the full range of host-derived volatiles detected by biting midges.

In conclusion, results from this thesis work have given us new tools that could be incorporated in integrated management strategies of biting midges. Furthermore, we have opened up several new research questions to be answered, regarding the ecology of the host-seeking behaviour of the biting midges, e.g. what is the difference in the host volatiles utilized by species with different host preference? This could enable us to develop methods targeting specific species of biting midges. Another interesting question is the potential use of host habitat cues as a mean for midges to locate sites to find potential hosts. This could be an important factor for vector-host interactions.

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