Ovipositional responses of *Culex tarsalis* to fish-associated semiochemicals in laboratory bioassays

Adena M. Why, Dong-Hwan Choe and William E. Walton

Department of Entomology, University of California, Riverside, CA 92521
Email: awhy001@ucr.edu

ABSTRACT: We isolated and characterized semiochemicals associated with the Western mosquitofish, *Gambusia affinis*, that influence oviposition by *Culex tarsalis*. Semiochemicals were isolated from water containing mosquitofish using solid-phase microextraction (SPME) and liquid-phase chromatography and were analyzed using a variety of chemistry techniques, mainly gas-chromatograph mass spectrometry (GCMS). These compounds, as well as the natural blend of semiochemicals, were presented to gravid female *Cx. tarsalis* to determine their affect on ovipositional site-selection. The volatile compounds identified to date appear to act as an oviposition attractant, indicating to the female that a suitable oviposition site is present. The non-volatile class of compounds appears to be acting as an ovipositional deterrent.

INTRODUCTION

Culex tarsalis, the Western encephalitis mosquito, is one of the important vectors of arboviruses in western North America. It is responsible for the maintenance, amplification and epidemic transmission of Western Equine Encephalomyelitis (WEEV) virus, St. Louis Encephalitis (SLEV) virus, and is currently a predominant vector of West Nile virus (WNV) in the western U.S. The USDA-APHIS has highlighted WEE and Eastern Equine Encephalomyelitis viruses as “Animal Health Emerging Issues” because these arboviruses have the potential to impact United States animal agriculture. *Culex tarsalis* is also the predominant vector of WEEV in the U.S and breeds readily in large bodies of water, such as natural and man-made wetlands.

While searching for oviposition sites, female insects will most likely encounter a range of microhabitats over which survival of offspring varies. Several components of habitat quality could influence offspring survival, such as the density of competitors, seasonal duration (e.g. vernal or temporary pools), overall productivity of the habitat (e.g. available food resources) and the risk of predation present (Angelon and Petranka 2002). Natural selection should then favor ovipositing females that can assess habitat quality and choose microhabitats that would maximize offspring survival. Gravid female mosquitoes use a combination of cues from the environment, including physical, biological and chemical, to select oviposition sites (Bentley and Day 1989; Isoe and Millar 1995).

More recently investigators have begun looking at the semiochemical(s) produced by fish, specifically kairomones, and how they affect site selection during oviposition by female mosquitoes (Ritchie and Laidlaw-Bell 1994; Angelon and Petranka 2002; Van Dam and Walton 2008; Pamplona et al. 2009; Walton et al. 2009). A kairomone is defined as a semiochemical that mediates interactions between individuals of different species, where the information transfer is beneficial for the receiver but not the individual producing the signal, also called the sender (Brönmark and Hansson 2012).

To date, the vast majority of these chemical compounds have yet to be identified and their efficacy as potential control agents has not been evaluated. Previous research has shown that female mosquitoes respond to the chemical signature(s) put off by certain fish species in breeding sites, leading to a decrease in number of egg rafts/eggs laid (Van Dam and Walton 2008; Pamplona et al. 2009; Walton et al. 2009; Why et al. 2016). This response was seen in both laboratory and field trials. Van Dam and Walton (2008) found that *Cx. tarsalis* responds strongly to the presence of fish-associated chemicals in oviposition sites. On average, four times as many egg rafts were laid on control water when compared with water conditioned with fish in the laboratory.

In our current study, we isolated and characterized semiochemicals associated with the Western mosquitofish, *Gambusia affinis*. Semiochemicals were isolated from water containing *Gambusia*-semiochemicals using solid-phase microextraction (SPME) and liquid-phase chromatography and analyzed using a variety of chemistry techniques, mainly gas-chromatograph mass spectrometry (GCMS). These compounds, as well as the natural blend of semiochemicals, were presented to gravid female *Cx. tarsalis* to determine their affect on ovipositional site-selection.

METHODS

Culex tarsalis adults were reared from a colony derived from wild individuals collected from San Jacinto, CA in 2001. *Culex tarsalis* larvae were reared in enamel pans under standard laboratory conditions. The resulting adults were allowed to feed on a 10% sucrose mixture and hydrated raisins sprinkled with 5 ml of granulated sugar. Once each week, female mosquitoes were fed overnight on a 2–5-d-old restrained chick.

Mosquito oviposition preference was tested in binary choice assays. Semiochemical-laden water was made during 3-day incubations in the laboratory. Ten fish were fed ad libitum on flaked fish food for 24 h in a 18.5 liter plastic bucket containing 10 liters of aged tap water. On day 2, the fish were moved to a clean bucket containing 10 liters of aged tap water and allowed to empty their guts. On day 3, the fish were moved to a new, clean container holding 10 liters of aged tap water. The fish were removed after 24 h and the latter fish-conditioned water was used to test ovipositional responses of the mosquitoes. The control consisted of 10 liters of tap water that had been aged using an aerator for 24 h in same type of 18.5 liter plastic bucket. The tap water was not treated in any way, nor was anything added to it.

Within 18 h of ingesting a bloodmeal, 30 blood-fed female *Cx. tarsalis* were aspirated into cages measuring 30 by 30 by 30 cm^3 (Model # 1450B; Bioquip Products, Rancho Dominguez, CA). After 3-4 days had elapsed, gravid females in each cage were presented with two white 200-ml wax-lined cups (Solo Cup Co.), containing either 150 ml of fish-conditioned water or the control. Oviposition cups were replaced daily. Three to six replicate cages were used in each trial. Mosquitoes were allowed to lay egg rafts for three successive nights. The number of egg rafts in each cup was counted. Each gravid female mosquito was used only once.

Semiochemicals were isolated from water containing mosquitofish using solid-phase microextraction (SPME) and liquid-phase chromatography and were analyzed using a variety of chemistry techniques, mainly gas-chromatograph mass spectrometry (GCMS). These compounds, as well as the natural blend of semiochemicals, were presented to gravid female *Cx. tarsalis* to determine their affect on ovipositional site-selection.

RESULTS

Our current results indicate that there are two classes of chemical compounds associated with mosquitofish that affect oviposition behavior: volatile and non-volatile. In laboratory bioassays the natural blend of these compounds led to a reduction in the number of egg rafts laid by female *Cx. tarsalis*, (N=7, $P < 0.036$). Three volatile compounds appeared to act as an oviposition attractant, indicating to the female that a suitable oviposition site is present with adequate larval resources, i.e. food source. The non-volatile class of compounds appeared to act as an ovipositional deterrent. It is only when female *Cx. tarsalis* land on the water and taste the chemicals present that she was deterred from ovipositing.

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