Review of semiochemicals that mediate the oviposition of mosquitoes: a possible sustainable tool for the control and monitoring of Culicidae

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ABSTRACT. Review of semiochemicals that mediate the oviposition of mosquitoes: a possible sustainable tool for the control and monitoring of Culicidae. The choice for suitable places for female mosquitoes to lay eggs is a key-factor for the survival of immature stages (eggs and larvae). This knowledge stands out in importance concerning the control of disease vectors. The selection of a place for oviposition requires a set of chemical, visual, olfactory and tactile cues that interact with the female before laying eggs, helping the localization of adequate sites for oviposition. The present paper presents a bibliographic revision on the main aspects of semiochemicals in regard to mosquitoes’ oviposition, aiding the comprehension of their mechanisms and estimation of their potential as a tool for the monitoring and control of the Culicidae.

KEYWORDS. Attractancy; repellency; infochemical; mosquito control.

RESUMO. Revisão dos semioquímicos que mediam a oviposição em mosquitos: uma possível ferramenta sustentável para o monitoramento e controle de Culicidae. A seleção de locais adequados pelas fêmeas de mosquitos para depositarem seus ovos é um fator chave para a sobrevivência de seus imaturos (ovos e larvas). O conhecimento das relações ecológicas implicadas neste processo é de grande importância quando se refere a vetores de agentes patogênicos. A determinação do local de oviposição pelas fêmeas grávidas envolve uma rede de mensagens químicas, visuais, olfativas e táteis que facilitam a localização de lugares adequados para depositarem seus ovos. Neste trabalho é apresentada uma revisão bibliográfica dos principais aspectos relacionados com semioquímicos presentes na oviposição dos mosquitos auxiliando no entendimento dos mecanismos de atuação dos mesmos e potencializando a aplicação destes semioquímicos como uma possível ferramenta de monitoramento e controle de Culicidae.

PALAVRAS-CHAVE. Ação atraente; ação repelente; infoquímicos; controle de mosquitos.

For the control of the Culicidae there are several methods of chemical and biological control that can be applied with relative success for a rapid decrease in this insect family, which is linked to public health matters. Among them, the ones that provide higher effectiveness and reasonable safety for the relationship environment-mankind have the recommendation of the World Health Organization (WHO) for the application in control activities. Then, the most frequently applied methods in the struggle against vectors are the biological insecticides, Bacillus thuringiensis (Bti) and Bacillus sphaericus (Bs), together with chemicals, such as the organophosphorates and pyrethrroids (Forattini 2002). However, these approaches confront a great deal of problems that endanger these pests’ control, particularly the short effectiveness period, the mosquitoes’ resistance to the insecticides’ active principles, the environments contamination (Stenersen 2004, WHO 1992) and also for not being self-sustainable strategies.

In the last decades, in search for new methodologies that supply the deficiency of the traditional methods, emphasis has been given to methods supported by the theoretical foundations of chemical ecology, especially concerning the semiochemicals. ‘Semiochemical’ include infochemicals, toxins and nutrients (Dicke & Sabelis 1988). Infochemicals are substances that, in their natural context, carry information or chemical cues for a given interaction between organisms, triggering a behavior or a physiological response in the receiving individual. The infochemicals are subdivided into allelochemicals, related to interspecific communications, and pheromones, in intraspecific communications (Vilela & Della Lucia 2001).

The allelochemicals are subdivided into allomones, whose information’s exchange favors the emitting species; kairomones favor the receiving species; synomones favor both species. Also considered allelochemicals are the antimonies; substances produced or acquired by an organism that, when in encounter to another individual of a different species in the natural environment, activate in the receiving individual a repellent response to the emitting and receiving individuals. The apneumones are chemical substances emitted by non-living material that evokes a behavioral or physiological reaction that is adaptively favorable to a receiving organism (Vilela & Della Lucia 2001).

Pheromones are defined as substances secreted by an individual for the environment, which are received by a second individual of the same species provoking a specific reaction or defined physiological process. These may include different
kinds of behavior, such as sexual, aggregation, dispersion, alarm, territoriality, trail, oviposition and others (Mordue 2003).

The aim of the present work is to provide a bibliographical revision concerning the main aspects related to semiochemicals, especially pheromones, allelochemicals and chemical compounds, that act on the chemical communication and play an important role in the choice of oviposition sites. This should allow a better understanding of the mechanisms involved in the attractancy and repellency of females regarding the oviposition sites, allied to the great potential that the use of semiochemicals provide as monitoring and vector control tools.

Behavior and selection of oviposition sites. The selection of sites for oviposition is a critical factor for the survival and population dynamics of the species. It initiates with the reception of environmental (visual, tactile, and olfactory) stimuli, which may either attract or repel, limiting the possibilities of finding oviposition sites. The cues include color and optical density water, texture and moisture, temperature and reflectance of the oviposition substrate (Bates 1940, Beckel 1955, Fay & Perry 1965, Hazard et al. 1967, Snow 1971, Benzon & Apperson 1988, Bentley & Day 1989, Davis & Bowen 1994, Kline 1994, McCall & Cameron 1995, Bandano & Regidor 2002). Dethier et al. (1960) provided a more accurate description of this behavior. Those authors showed that an attractant ensures to the insect the direction towards a suitable place, inducing oviposition. A repellent is a stimulus that unleashes movements towards an oviposition site, restricting egg-laying.

This behavior occurs because the insect’s sensorial system is a complex composed by chemoreceptors, mechanoreceptors, higroreceptors and thermoreceptors. This system can detect a wide range of volatile compounds in the environment that inform qualitative aspects, such as food source, presence of mating partners or suitable oviposition sites (Mordue 2003). These receptors are connected to neurons by specialized setae known as olfactory and gustatory sensillae. The olfactory sensillae occur in pairs and may be observed on the head, antennae and maxillary palpus, including internal and external buccal parts, wing margin and female ovipositors (Romoser & Stoffolano 1998, Dahanukar et al. 2005, Hallem et al. 2006).

Identification of semiochemicals involved in the selection of oviposition sites. At first several studies performed different experiments trying to evaluate the influence of physic-chemical factors in the oviposition, such as light reflection, odor, temperature, humidity, substrate texture and other breeding sites’ features (Gjullim 1961, Gjullim et al. 1965, Fay & Perry 1965, Perry & Fay 1967). Yet, the first scientists to erect a hypothesis concerning the existence of a pheromone that should stimulate oviposition in mosquitoes were Hudson & Mcintosh (1967). Later, Osgood (1971) verified this hypothesis studying the behavior of gravid females of Culex tarsalis Coquillett which displayed a preference to lay eggs in water with conspecific larvae, instead of distilled water. With the use of gas-liquid chromatography, Starratt & Osgood (1972) detected the presence of a mixture of 1,3-diglycerides in the active fraction associated with egg oviposition of the mosquito Cx. tarsalis. Acid-catalyzed methanolation of the mixture yielded methyl esters of mono- and dihydroxy fatty acids being the erythro-5,6-dihydroxyhexadecanoic acid the major component among the dihydroxy ones (Fig. 1).

Bentley et al. (1979) identified p-cresol through gas chromatography coupled with mass spectrometry (GC-MS) in wood infusions that showed to be attractant to females of Aedes triseriatus (Say) (Fig. 2).

Hwang et al. (1980) proved the repellency of the carboxylic acids isobutyric, butyric, isovaleric and hexanoic in the oviposition of Culex quinquefasciatus (Say) (Fig. 3).

Hwang et al. (1982) assessed the repellency and attractancy of a series of carboxylic acids, from pentanoic to tridecanoic, in several concentrations, in Cx. quinquefasciatus, Cx. tarsalis Coquillett and Aedes aegypti (Linnaeus).

Bruno & Laurence (1979) inferred that the increment observed in the oviposition of Cx. pipiens could occur due to droplets present on the egg’s apex, though without specificity, as they were equally attractant to females of Cx. molestus and Cx. tarsalis. Afterwards, Laurence & Pickett (1982), through gas chromatography-mass spectrometry, proved that the active compound in the droplets observed by Bruno & Laurence (1979) was erythro-6-acetoxy-5-hexadecanolate, supporting the existence of a mosquito oviposition pheromone (MOP) (Fig. 4).

Millar et al. (1992) identified five compounds in an infusion prepared with grass: phenol, 4-methylphenol, 4-ethylphenol, isobutyric acid, butyric acid, isovaleric acid and hexanoic acid.
indole and 3-methylindole, indicating a synergistic action in the oviposition stimuli in *Cx. quinquefasciatus* (Fig. 5).

The 3-methylindole presented higher effectivity to attract mosquitoes when used in concentrations between 1 and 10 ng/L. Millar *et al.* (1994) also evaluated the effects of the 3-methylindole added to the synthetically derived oviposition pheromone 6-acetoxy-5-hexadecanolide, using combinations of the two products. A mixture of these compounds significantly increased the oviposition of *Cx. quinquefasciatus* between 0.01 and 0.1 mg; higher to this level, a repellent trend was observed. When separately applied, under this same concentration range, the compounds presented distinct attractancy, thus, determining an additive behavior, not synergistic. Barbosa *et al.* (2007) demonstrated that high concentrations of synthetic oviposition pheromone (SOP) act as a repellent for oviposition in *Cx. quinquefasciatus* in laboratory.

Mendki *et al.* (2000) identified other five compounds linked to *Ae. aegypti*, analyzing the water in their larvae’s breeding site. The compounds are octadene, isopropyl myristate, heneicosane, docosane and nonacosane, being the heneicosane the most attractant to oviposition (Fig. 6).

Torres–Estrada *et al.* (2005) noted that in *Anopheles albimanus* Wiedemann the oviposition is mediated by the effect of some plants, such as *Cynodonton dactylon*, *Jouveau straminea*, *Fimbristylis spadicea*, *Ceratophyllum demersum* and *Brachiaria mutica*. In that study no significant statistical differences in the mosquitoes’ attraction to the plant’s extracts was observed, nevertheless, a repellent effect was evident with the extracts in high concentrations. As a result, guaiacol, phenol, isoeugenol, longifolene, caryophyllene, phenylethyl alcohol and *p*-cresol were identified, which did not have their biological activities separately determined (Fig. 7).

Ganesan *et al.* (2006), using *Ae. aegypti* eggs’ extracts, identified the dodecanoic, tetradecanoic, hexadecanoic, (Z)-9-hexadecenoic, octadecanoic and (Z)-9-octadecenoic acids, the esters methyl dodecanoate, methyl tetradecanoate, methyl hexadecanoate, methyl octadecanoate, methyl (Z)-9-hexadecenoate, methyl-(Z)-9-octadecenoate and 6-hexanolate. In the experimental tests, the dodecanoic and (Z)-9-hexadecenoic acids showed positive response to oviposition, whereas the esters showed repellent ovipositional response (Fig. 8).

Sharma *et al.* (2008) evaluated the oviposition responses of *Ae. aegypti* and *Ae. alpopictus* to several C_{21} fatty acid esters. They observed that hexadecyl pentanoate, tetradecyl...
heptanoate and tridecyl octanoate presented significant oviposition repellent activity against the two mosquito species, while propyl octadecanoate was found to attract \textit{Ae. aegypti} to oviposition substrates (Fig. 9).

Ponnusamy \textit{et al.} (2008) showed that \textit{Ae. aegypti} females direct most of their eggs to bamboo (\textit{Arundinaria gigantean}) leaf infusions, due to the oviposition-stimulating kairomones produced by microorganisms. The methanol extract obtained from lyophilized bacteria revealed the presence of a mixture of carboxylic acids from nonanoic to octadecanoid and carboxylic acids methyl esters. Most fatty acids and esters were ineffective, however, others, namely nonanoic acid, tetradecanoic acid and methyl tetradecanoate, were highly effective at inducing egg laying but at extremely narrow dosage ranges (Fig. 10).

**Validation of semiochemicals under field conditions.** The activity observed under laboratory conditions allowed the advancement towards field work. Beehler \textit{et al.} (1994) were the first to perform a study in real field conditions using traps with semiochemicals in California, USA. That study confirmed the validity of the laboratory results with skatole (3-methylindole) as an attractant to females of \textit{Cx. quinquefasciatus}, \textit{Cx. stigmatosoma} Dyar, and \textit{Cx. tarsalis}. In Tanzania, Mboera \textit{et al.} (1999) also evaluated the skatole’s residual time for \textit{Cx. quinquefasciatus}, \textit{Cx. tigripes} Grandpré & de Chamosy and \textit{Cx. cinereus} Theobald, besides its effectiveness. This study determined that the pheromone is active for up to nine days with a decrease in its activity after this period. It demonstrated, for the first time, the attraction of \textit{Cx. cinereus} Theobald to the skatole. Moreover, Mboera \textit{et al.} (2000a) evaluated the ovipositional behavior using skatole and the synthetic oviposition pheromone (SOP) (5R,6S)-6-acetoxy-5-hexadecanolid in field conditions in Tanzania, concluding that both intervene in the selection of sites for oviposition under natural conditions. Mboera \textit{et al.} (2000b) included the synthetic oviposition pheromone in traps designed by Reiter (1983) for the capture of adult mosquitoes and composed of grass infusion, confirming this pheromone’s effectivity as an attractant for gravid females of \textit{Cx. quinquefasciatus}. This demonstrated its potential to monitor the mosquito. As for other species, such as \textit{Ae. albopictus}, the results did not yield significant statistical differences either in field or laboratory for the synthetic compounds indole, 3-metylindole and 4-metylindole, suggesting that these compounds are highly specific attractants for \textit{Culex} (Trexler \textit{et al.} 2003) (Fig. 11).

**New perspectives that can be explored with ovipositional semiochemicals.** As a large amount of the documentation found refers to semiochemicals that act in the oviposition of \textit{Culex},
a study with other culicids is required for a better understanding on the semiochemicals’ role in the selection of oviposition sites.

The determination of kairomones in the predator-prey systems may be a vast area to explore new and promising compounds, since females may detect a predator and search for another place. It could work as a females’ remover which are ready to lay their eggs in risky areas with the presence of arboviruses like dengue, or other culicid vectors. An example that illustrates this line of investigation is the capacity of Culex spp. to detect predators, such as Notonecta irrorata Fabricius (Hemiptera: Notonectidae) and Culiceta longiareolata Macquart (Diptera; Culicidae) (Blaustein et al. 2004, Blaustein et al. 2005). Studies that could lead to the identification of synomones, antimones and apneumones would be necessary, due to the lack of records on the semiochemicals and their respective mechanisms of activity (Eiras 2001). The development of methodologies to detect and synthesize new compounds should be a priority, most importantly if they are meant to be employed as a large scale tool for monitoring and control (Fuganti et al. 1982, Olagbemiro et al. 1999, Olagbemiro et al. 2004, Michaelakis et al. 2005).

Integrated systems for pest management, which include strategies of attraction towards predefined places for the capture of mosquitoes (“Push – Pull strategy”) might be the best way of sustainable control of mosquitoes in the future. Under this perspective, compounds with repellent effect would act pushing away vectors from places close to their hosts and attractant compounds would guide them to specific traps for their capture. Such control strategies would require little insecticide or they could even become unnecessary (Cook et al. 2007).

**Final considerations.** Chemical cues undoubtedly play a crucial role in the selection of oviposition sites and, when adequately applied, may provide promising results in the control and monitoring of mosquitoes’ populations. Notwithstanding, the utilization of semiochemicals must be done cautiously in order to avoid undesired repellent effects to the mosquitoes. This could be an unwanted consequence as such populations could disperse to new places, carrying with them aetiological agents that cause diseases.

The semiochemicals may be perceived as a tool that ought to be integrated to other control methodologies with advantages, such as faster detection of circulation sites of arboviruses and a higher selectivity in the monitoring and capture of targeted species. Further, with the utilization of traps for adults, the number of egg-laying females can be inferred, as well as it employs a dynamic control of these populations.

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**REFERENCES**


Fig. 11. Attractants of several species of Culex.


