

## Oviposition Responses of *Culex pipiens* to a Synthetic Racemic *Culex quinquefasciatus* Oviposition Aggregation Pheromone

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The oviposition pheromone of *Culex quinquefasciatus* was synthesized in a racemic form in a simple (five steps), efficient, high yielding (45% total yield), and low cost way (use of relatively low cost reagents). Our synthetic racemic pheromone (SRP) was tested in the laboratory for its bioactivity on *Culex pipiens* biotype *molestus*, which is a member of the species complex that *Culex quinquefasciatus* belongs. In the testing conditions, bioactivity at the doses of 0.01, 0.1, 1, and 10  $\mu\text{g}$  per cage was found with the best bioactivity achieved at 1  $\mu\text{g}$  per cage. The effectiveness of our SRP offers a capable tool for improving mosquito oviposition traps for surveillance or even control programs.

**KEYWORDS:** Mosquito; oviposition; pheromone; *Culex pipiens* biotype *molestus*; racemic synthesis; 6-acetoxy-5-hexadecanolid

### INTRODUCTION

*Culex pipiens* represents a mosquito species complex whose taxonomical status is still a matter of argument. According to the recent generic and subgeneric changes, the formerly known two separated species or subspecies *Cx. pipiens pipiens* and *Cx. pipiens molestus* are now considered as a single species with two different biotypes (1). The most common and widespread members of the complex are as follows: *Cx. pipiens* biotype *pipiens*, *Cx. pipiens* biotype *molestus*, and *Culex quinquefasciatus*. The aforementioned two biotypes possess significant ecological and physiological differences. The *molestus* biotype prefers hypogeous habitats (underground urban environments, such as cellars, sanitary spaces under buildings, and septic tanks), it is autogenous (does not require a blood meal to produce its first batch of eggs), it is stenogamous (able to mate in confined spaces), it is homodynamous (does not hibernate), and it is mammophilous (feeds from mammals). In contrast, the *pipiens* biotype prefers epigeous habitats (breeds in rural, open air collection of water), it is anautogenous (requires blood meal to produce its first batch of eggs), it is eurygamous (incapable of mating in cages in the laboratory and other confined spaces), it is heterodynamous (able to hibernate during the winter), and it is primarily ornithophilous (tendency to feed on birds). *Cx.*

*quinquefasciatus* has many similarities with the *molestus* biotype (homodynamous, stenogamous, and anautogenous), but it is usually found in tropical areas whereas *Cx. pipiens* is mainly found in temperate or even colder climates (2).

The *Cx. pipiens* complex has a worldwide distribution, and members of the complex play a significant role in the transmission of arboviruses and other vector-borne diseases (3). Thus, any minimal change in local climates could force the population as well as the pathogens that they transmit to move to new areas (4, 5). One very important flavivirus disease is the West Nile (WN) virus, which is transmitted in natural cycles between birds and mosquitoes but also can infect humans (6). WN occurs in Middle East, Africa, India, the United States, and also in Europe (1). *Cx. quinquefasciatus* as well as *Cx. pipiens* biotype *pipiens* are the most likely WN vectors, but Lundström (3) suggested that biotype *molestus* should also be collected and processed for isolation of WN virus in order to evaluate the occurrence of the virus in an area.

Female *Culex* mosquitoes deposit their eggs in the form of egg rafts on the water surface. Some of these species form a droplet at the apex of each egg in the egg raft, which affects the oviposition behavior of intraspecific gravid females (7–9). The main volatile compound present in the apical droplets of the *Cx. quinquefasciatus* egg rafts is (–)-(5*R*,6*S*)-6-acetoxy-5-hexadecanolid, which acts as an attractant to other gravid females in order to oviposit the pheromone release nearby (10, 11). The interspecific activity of the previously mentioned pheromone was demonstrated by Bruno and Laurence in 1979

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(7) when they reported that a *Cx. quinquefasciatus* egg raft acted as an attractant to gravid *Cx. pipiens* biotype *pipiens* females.

The isolation and characterization of the (–)-(5*R*,6*S*)-6-acetoxy-5-hexadecanolide from *Cx. quinquefasciatus* egg rafts provided the impetus for several attempts toward its syntheses. Sugars or amino acids (12), chiral auxiliaries (13), as well as Sharpless asymmetric dihydroxylation (14) and epoxidation reactions (15) have been used for the introduction of the asymmetry (16) while Wittig coupling, Knoevenagel condensation, and Julia olefination have been applied for the construction of the carbon skeleton. However, most of these syntheses suffer from various drawbacks as they require either uncommon reagents and/or usually expensive intermediates or entail many steps giving low overall yields.

Hwang et al. in 1987 (17) reported that the only enantiomer bioactive to *Cx. quinquefasciatus* was the (–)-(5*R*,6*S*) isomer whereas the other three were inactive but not repellent. Sakakibara et al. in 1984 (18) first tested all four stereoisomers with the *molestus* biotype and reported that they all showed a significant bioactivity, but Clements in 1999 (19) stated that these results could not be taken as proven, since there was not a clear specification of their synthetic procedure.

The unclear evidence for the bioactivity possessed by all four isomers in combination with the need to simplify the synthetic procedure and reduce the cost led us to produce the racemic pheromone in a simple, efficient, high yielding, and less expensive way. Toward that, a synthetic racemic oviposition pheromone (SRP) of *Cx. quinquefasciatus* was synthesized in a five step procedure and its bioactivity was evaluated against *Cx. pipiens* biotype *molestus* in laboratory bioassays.

## MATERIALS AND METHODS

**General.** All chemical reactions were carried out under anhydrous conditions and argon atmosphere using dry, freshly distilled solvents, unless otherwise noted. Tetrahydrofuran (THF) was distilled from sodium/benzophenone, dichloromethane ( $\text{CH}_2\text{Cl}_2$ ) was distilled from  $\text{CaH}_2$ , and pyridine was first dried over solid sodium hydroxide and distilled through an efficient fractionating column. Yields refer to chromatographically and spectroscopically ( $^1\text{H}$  NMR) homogeneous materials, unless otherwise stated. All reagents were purchased at the highest commercial quality and used without further purification, unless otherwise mentioned. All reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm Merck silica gel plates (60 F<sub>254</sub>) by using UV light as the visualizing agent and ethanolic phosphomolybdic acid or *p*-anisaldehyde solution followed by heat as developing agents. Merck silica gel (60; particle size, 0.040–0.063 mm) was used for flash column chromatography. NMR spectra were recorded on a Bruker AC-250 instrument at 25 °C. IR spectra were recorded on a Perkin-Elmer 1600 series FT-IR. Gas chromatograms were recorded on a Fisons GC 8000 instrument, and high-resolution mass spectra (HRMS) were recorded on a VG ZAB-ZSE mass spectrometer under fast atom bombardment (FAB) conditions.

**Synthesis of Racemic Pheromone.**  $\delta$ -Valerolactol. To a solution of  $\delta$ -valerolactone (**2**) (4 g, 40 mmol) in  $\text{CH}_2\text{Cl}_2$  (60 mL) cooled at –78 °C was added dropwise DIBAL-H (44 mL, 44 mmol, 1 M in hexane) over 15 min. After 15 min at –78 °C, the reaction mixture was quenched with MeOH. EtOAc (130 mL) and saturated sodium potassium tartrate (10 mL) were added, and the whole mixture was stirred at room temperature for 3–4 h until complete decomposition of the aluminum complex. The granular precipitate of alumina was filtered off through Celite, and the product was purified by flash column chromatography (silica gel, using ether as eluent) to afford  $\delta$ -valerolactol as a colorless oil (3.6 g, 88%). The volatile lactol was used in the following reaction without further analysis.  $R_f$  = 0.23 (hexanes/EtOAc 8:2).

5-(*Z,E*)-Hexadecenol (**3** and **4**). A solution of *n*-butyllithium (1.6 M solution in hexane, 2.94 mL, 4.7 mmol) was added to a stirred

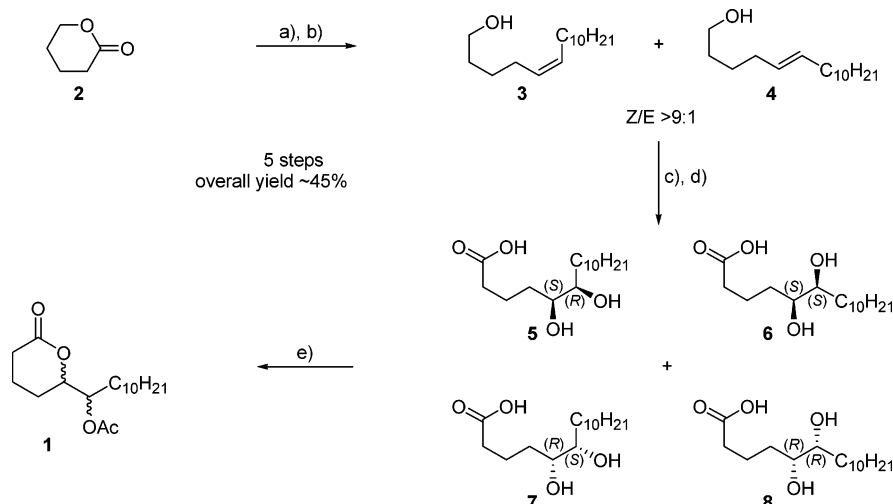
solution of triphenylundecylphosphonium bromide (2.4 g, 5 mmol) in THF at 0 °C and was stirred under argon. After 1 h at 0 °C, a solution of the above lactol (0.2 g, 1.96 mmol) in dry THF (1 mL) was added. The reaction mixture was allowed to reach room temperature. After 1 h of stirring, the reaction mixture was poured into a saturated solution of  $\text{NH}_4\text{Cl}$  and extracted with  $\text{Et}_2\text{O}$ . The combined organic extracts were washed with water and brine, dried over  $\text{Na}_2\text{SO}_4$ , and concentrated in vacuo. Subjection of the crude mixture to flash chromatography (silica gel, hexanes/EtOAc 6:4) yielded pure 5-hexadecenol (**3**, **4**) (0.42 g, 89%) as a mixture of isomers with a ratio of *Z/E* > 9:1 (determined by GC analysis). The mixture was used in the following reactions without further purification.  $R_f$  = 0.58 (hexanes/EtOAc 5:5).  $^1\text{H}$  NMR (250 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.88 (t,  $J$  = 6.7 Hz, 3 H,  $\text{CH}_3$ ), 1.1–1.5 (m, 18 H,  $\text{CH}_2$ ), 1.5–1.75 (m, 2 H,  $\text{CH}_2\text{CH}_2\text{OH}$ ), 1.9–2.2 (m, 4 H,  $\text{CH}_2\text{-CH=CHCH}_2$ ), 3.65 (t,  $J$  = 6.25 Hz, 2 H,  $\text{CH}_2\text{OH}$ ), 5.25–5.5 (m, 2 H,  $\text{CH=CH}$ ).

5-(*Z,E*)-Hexadecenoic Acid. To a stirred solution of 5-(*Z,E*)-hexadecenol (**3** and **4**) (0.42 g, 1.75 mmol) in acetone at 0 °C was added portionwise a solution of Jones' reagent until no alcohol was traced by TLC. The reaction was then quenched with 2-propanol, filtered through Celite, and poured in EtOAc/ $\text{H}_2\text{O}$  1:1. The organic phase was separated, the aqueous phase was washed twice with EtOAc, and the combined organic extracts were washed successively with water and brine, dried over  $\text{Na}_2\text{SO}_4$ , and concentrated in vacuo. The crude mixture was subjected to flash column chromatography (silica gel, hexanes/EtOAc 6:4) to afford pure 5-(*Z,E*)-hexadecenoic acid (0.35 g, 80%) as a yellow oil.  $R_f$  = 0.50 (hexanes/EtOAc 5:5).  $^1\text{H}$  NMR (250 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.88 (t,  $J$  = 6.7 Hz, 3 H,  $\text{CH}_3$ ), 1.05–1.45 (m, 16 H,  $\text{CH}_2$ ), 1.55–1.85 (m, 2 H,  $\text{CH}_2\text{CH}_2\text{COOH}$ ), 1.85–2.2 (m, 4 H,  $\text{CH}_2\text{-CH=CHCH}_2$ ), 2.35 (t,  $J$  = 6.7 Hz, 2 H,  $\text{CH}_2\text{COOH}$ ), 5.2–5.55 (m, 2 H,  $\text{CH=CH}$ ). IR (neat):  $\tilde{\nu}$  3500–2500, 2920, 2856, 1710, 1460, 1410, 935  $\text{cm}^{-1}$ . HRMS (FAB):  $m/z$  calcd for  $\text{C}_{16}\text{H}_{30}\text{O}_2$  [ $\text{M} + \text{H}$ ] $^+$ , 255.2324; found, 255.2317.

(5,6)-Dihydroxyhexadecanoic Acid (**5–8**). A solution of  $\text{K}_3\text{Fe}(\text{CN})_6$  (1.94 g, 5.91 mmol),  $\text{K}_2\text{CO}_3$  (0.816 g, 5.91 mmol), and  $\text{K}_2\text{OsO}_2(\text{OH})_4$  (7.36 mg, 0.02 mmol) in *t*-BuOH/ $\text{H}_2\text{O}$ /acetone 4.2 mL:6 mL:1.5 mL was cooled to 0 °C. 5-(*Z,E*)-Hexadecenoic acid (0.5 g, 1.97 mmol) was added, and the reaction mixture was stirred vigorously at 0 °C for 12 h. After the addition of  $\text{Na}_2\text{SO}_3$ , the mixture was allowed to warm to 25 °C and ethyl acetate was added. The organic layer was washed with saturated  $\text{NH}_4\text{Cl}$  and saturated NaCl, dried over  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated under reduced pressure. Purification by recrystallization afforded (5,6)-dihydroxyhexadecanoic acid (**5–8**) (0.52 g, 90%) as a white solid.  $R_f$  = 0.58 (hexanes/EtOAc 5:5); mp 135–138 °C.  $^{13}\text{C}$  NMR (62.5 MHz, acetone- $d_6$ ):  $\delta$  13.9, 21.8, 22.8, 26.2, 28.7, 29.0, 29.4, 32.0, 32.1, 32.8, 33.9, 74.5, 74.7, 174.4. IR (KBr):  $\tilde{\nu}$  3650–3000, 2922, 2848, 1600, 1467, 1417, 1404, 1069  $\text{cm}^{-1}$ .

6-Acetoxy-5-hexadecanolide (**1**). To a stirred solution of (5,6)-dihydroxyhexadecanoic acid (**5–8**) (0.10 g, 0.35 mmol) in dry pyridine (6 mL) at 0 °C was added  $\text{Ac}_2\text{O}$  (1.18 mL, 12.5 mmol) (**16b**). The reaction mixture was allowed to stir at 0–5 °C for 3 h. EtOAc was added to the reaction mixture, and the organic layer was washed with saturated  $\text{CuSO}_4$ ,  $\text{NaHCO}_3$ , water, and brine, dried over  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated under reduced pressure. Purification by flash column chromatography (silica gel, hexanes/EtOAc 6:4) afforded 6-acetoxy-5-hexadecanolide (**1**) (0.087 g, 80%) as a clear yellow oil.  $R_f$  = 0.50 (hexanes/EtOAc 6:4).  $^1\text{H}$  NMR (250 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.88 (t,  $J$  = 6.7 Hz, 3 H,  $\text{CH}_3$ ), 1.1–1.4 [m, 16 H,  $(\text{CH}_2)_8\text{CH}_3$ ], 1.5–2.0 [m, 6 H,  $\text{CH}_2\text{CH}(\text{O-})$  and  $\text{CH}_2\text{CH}_2\text{C}(\text{O})$ ], 2.1 (s, 3 H, Ac), 2.35–2.7 [m, 2 H,  $\text{CH}_2\text{C}(\text{O})$ ], 4.3–4.42 [m, 1 H,  $\text{CH}(\text{OAc})$ ], 4.95–5.05 [m, 1 H,  $\text{CH}(\text{O-})$ ].  $^{13}\text{C}$  NMR (62.5 MHz,  $\text{CDCl}_3$ ):  $\delta$  14.0, 18.1, 20.8, 22.5, 23.4, 25.1, 29.2, 29.3, 29.4, 31.8, 74.3, 80.5, 170.0, 170.6; major peaks correspond to *erythro* (5*R*,6*S* and 5*S*,6*R*) isomers whereas less than 10% of the mixture, according to the spectrum, corresponds to *threo* (5*S*,6*S* and 5*R*,6*R*) isomers. IR (neat):  $\tilde{\nu}$  2920, 2860, 1740, 1460, 1370, 1230, 1040  $\text{cm}^{-1}$ . HRMS (FAB):  $m/z$  calcd for  $\text{C}_{18}\text{H}_{32}\text{O}_4$  [ $\text{M} + \text{H}$ ] $^+$ , 313.2379; found, 313.2369.

**Mosquito Rearing.** A *Cx. pipiens* biotype *molestus* colony that was maintained in the laboratory of Benaki Phytopathological Institute (Kifissia, Greece) for more than 2 decades was used. Adults were kept in wooden framed cages (33 cm  $\times$  33 cm  $\times$  33 cm) with 32  $\times$  32

Scheme 1<sup>a</sup>

<sup>a</sup> Reagents: (a) DIBAL,  $\text{CH}_2\text{Cl}_2$ ,  $-78^\circ\text{C}$ , 30 min, 88%. (b)  $\text{Ph}_3\text{P}^+(\text{Br}^-)\text{C}_{11}\text{H}_{23}$ ,  $n\text{-BuLi}$ , THF,  $0^\circ\text{C}$ , 1 h; and then addition of lactol,  $0\text{--}25^\circ\text{C}$ , 1 h, 89%. (c) Jones' reagent, acetone,  $0^\circ\text{C}$ , 80%. (d)  $\text{K}_3\text{Fe}(\text{CN})_6$ ,  $\text{K}_2\text{OsO}_2(\text{OH})_4$ ,  $\text{K}_2\text{CO}_3$ ,  $t\text{-BuOH}/\text{H}_2\text{O}/\text{acetone}$  2.8:4:1,  $0^\circ\text{C}$ , 12 h, 90%. (e)  $\text{Ac}_2\text{O}$ , pyr,  $0\text{--}5^\circ\text{C}$ , 3 h, 80%.

mesh at  $20 \pm 2^\circ\text{C}$ ,  $80 \pm 2\%$  relative humidity, and a photoperiod of 14:10 (L:D) h. Cotton wicks saturated with 10% sucrose solution were provided to the mosquitoes as a food source. Females laid eggs in round, plastic containers (10 cm diameter  $\times$  5 cm depth) filled with 150 mL of tap water. Egg rafts were removed daily and placed in cylindrical enamel pans in order to hatch (35 cm diameter  $\times$  10 cm depth). Larvae were reared under the above temperature and light conditions and were fed daily with baby fish food (TetraMin, Baby Fish Food) at a concentration of 0.25 g/L of water until pupation. Pupae were then collected and introduced into the adult rearing cages.

**Laboratory Oviposition Bioassays.** Two-choice oviposition experiments were set in sieve-covered wooden framed cages (33 cm  $\times$  60 cm  $\times$  33 cm). Two to three days old male and female adult mosquitoes were removed from the maintenance cages (not containing oviposition pots) and introduced into the bioassay cages. The bioassay cages were kept under the normal rearing conditions. Adult mosquitoes were left for one more day in order to achieve as more mating as possible. Afterward, two glass pots (10 cm diameter  $\times$  5 cm depth), one containing 100 mL of distilled water and the other containing 100 mL of distilled water plus SRP, were placed into the cages in approximately 40 cm distance between each other as more centrally as possible in order to provide oviposition sites.

SRP was diluted in hexane, and the appropriate volume was applied on glass cover slips with a Gilson micropipet and allowed to evaporate as the solvent for an hour at room temperature. SRP doses that tested were 0.01, 0.1, 1, 10, and 100  $\mu\text{g}$  per cage. The cover slips were then placed on small square plastic floating pads, and the system floating pad plus cover slip were placed on the water surface. That was designed in order to achieve the best possible imitation of nature where the pheromone is released from the top of the floating egg rafts.

The number of egg rafts in the oviposition pots was recorded 24 h after the introduction of mosquitoes into the cages. The counted egg rafts were converted to percentages of the total number of rafts in both pots for each cage. Five bioassays were carried out for each dose, and data (percentages of egg rafts laid in test pot) were subjected to nonlinear regression analysis (SPSS 11.0).

## RESULTS AND DISCUSSION

The synthetic procedure of SRP is demonstrated in **Scheme 1**. Compound **1** was synthesized by using the following reaction sequence: reduction, Wittig coupling, oxidation, dihydroxylation, and finally lactonization. According to Couladouros et al. in 1999 (14b) following the methodology for the synthesis of  $\gamma$ - and  $\delta$ -lactones, the key intermediates **3** and **4** were easily produced from  $\delta$ -valerolactone (**2**) in two steps involving reduction of the molecule with 1.1 equiv of diisobutylaluminum

hydride (DIBAL-H) leading to the corresponding lactol and subsequent enlargement of the carbon skeleton by Wittig reaction. Oxidation, *cis*-dihydroxylation on the 9:1 mixture of *cis* and *trans* double bonds, and consequent lactonization led to the formation of the *erythro* and *threo* isomers, respectively (see **Scheme 1**). Thus, a mixture of four stereoisomers of 6-acetoxy-5-hexadecanolide (**1**) containing 45% of the natural oviposition aggregation pheromone [( $-$ )-(5*R*,6*S*)], 45% of its enantiomer [( $+$ )-(5*S*,6*R*)], and 10% of the respective *threo* enantiomeric pair [( $-$ )-(5*S*,6*S*) and ( $+$ )-(5*R*,6*R*)] was synthesized.

By this approach, a bioactive mixture (see following bioassays) was prepared using a short and high yielding procedure (five steps, with 45% total yield) employing a less expensive, commercially available starting material. Olagbemiro et al. (20) also reported a semibiotechnological method to prepare racemic pheromone (as a mixture of *erythro* isomers) employing glycerides of (*Z*)-5-hexadecenoic acid as the starting material, which has to be derived after cultivation of *Kochia scoparia* (Chenopodiaceae) and extraction of the seeds.

According to behavioral and electrophysiological studies, it is suggested that the oviposition pheromone acts as both an attractant and a stimulant in *Cx. quinquefasciatus* (21). In *Culex* mosquitoes, the oviposition attractant pheromone is stereospecific and the receptor accepts only the ( $-$ )-(5*R*,6*S*) enantiomer and not the other three isomers, which are inactive (17).

Our SRP of *Cx. quinquefasciatus* was tested as an oviposition attractant for gravid *Cx. pipiens* biotype *molestus* at the doses of 0.01, 0.1, 1, 10, and 100  $\mu\text{g}$  per testing cage. Oviposition was elicited with the presence of the SRP at the doses of 0.01, 0.1, 1, and 10  $\mu\text{g}$  per cage (see **Table 1**). The highest response was recorded at the dose of 1  $\mu\text{g}$  per cage resulting in an oviposition attraction level of 72.3%. From these data, it is obvious that SRP is effective on *Cx. pipiens* biotype *molestus* even though it is the oviposition pheromone of the *Cx. quinquefasciatus* and it is also demonstrated that the pheromone of one species could be active to other species from the same complex. In contrast, the 100  $\mu\text{g}$  per cage dose did not elicit any oviposition response. In **Figure 1**, the oviposition response in relation to doses is shown. The fitting curve was

$$\text{response} = 0.73 (\pm 0.0079) - 0.024 (\pm 0.0036) \times$$

$$\text{Log}(\text{dose}) - 0.039 (\pm 0.003) \times \text{Log}^2(\text{dose})$$

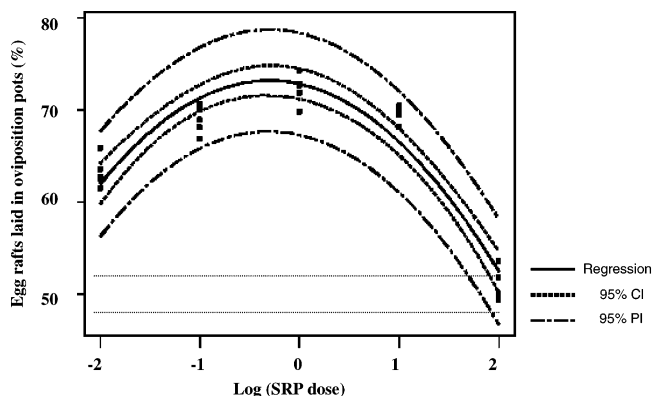
where  $R_{(\text{adjustment})}^2$  is 0.9.



**Table 1.** Mean Percentage of Egg Rafts ( $\pm$ SE) in Oviposition Pots with Different Doses of SRP<sup>a</sup>

SRP dose ( $\mu$ g)	mean % egg rafts laid in oviposition pots	SE ( $\pm$ )	no. of trials
control	50.1	2.1	11
0.01	63.3	1.6	5
0.1	68.9	1.5	5
1	72.3	1.6	5
10	69.6	0.9	5
100	51	1.7	5

<sup>a</sup> Eleven bioassays for control were carried out in contrast with SRP bioassays, which were carried out five times.

**Figure 1.** Response to different doses of SRP. The broken lines represent the upper and lower values of the control mean  $\pm$  SE ( $50.1 \pm 2.1\%$ ,  $n = 11$ ).

The high pheromone concentration ( $100 \mu\text{g}$  per cage) could result in either confusion of the gravid females for the oviposition sites or even repulsion of the gravid females of laying their eggs. Although Millar et al. in 1994 (22) reported that attraction was stable to very high pheromone concentrations ( $1000 \mu\text{g}$  per cage), similar results to ours were stated from Hwang et al. in 1987 (17) and Blackwell et al. in 1993 (21) when they recorded the same pattern of behavior in the oviposition pheromone of *Cx. quinquefasciatus* gravid females. Specifically, Blackwell et al. in 1993 (21) found that the synthetic oviposition pheromone, with the two isomers (erythro-6-acetoxy-5-hexadecanolide), increased egg laying by *Cx. quinquefasciatus* females in a dose-dependent manner over a dose range of  $0.01$ – $80 \mu\text{g}$ . With  $100 \mu\text{g}$  of pheromone added to the laying pot, there was a statistically significant reduction in the oviposition behavior. The attraction (% eggs laid in test pot) in this experiment was almost 85%.

Our attraction was almost 72%, although another member of the same species complex is used and these results indicated that there is a very good response of *Cx. pipiens* biotype *molestus* to the SRP of *Cx. quinquefasciatus*. In addition, the presence of the three other isomers into our SRP did not cause any reverse effect on its bioactivity, which is in agreement with previously cited studies (8, 23).

These results are very promising toward the use of our SRP in field conditions. To achieve that, further studies could be contacted as to determine the best possible way of using it. Fields of studies could be the search of possible synergy with other reported attractants such as chemical substances, grass infusions, etc. (21, 22, 24, 25). Effective doses in open air, stability of the SRP in natural conditions, ways of applying it, etc. are also research areas. The development of a pheromone oviposition mosquito trap, which is not species specific, could

be of great value for surveillance or even control programs if it could be combined with larvicide.

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