

## Oviposition Responses of the Malaria Mosquito *Anopheles gambiae sensu stricto* to Hay Infusion Volatiles in Laboratory Bioassays and Investigation of Volatile Detection Methods

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**Abstract :** The responses of individual gravid *Anopheles gambiae sensu stricto* (s.s.) to hay infusion volatiles were evaluated under laboratory conditions. Such infusions have long been known to be effective baits for monitoring mosquitoes that vector arboviral and filarial diseases but have previously not been tested for malaria vectors. Hay infusions were prepared by adding sun-dried Bermuda grass to lake water and leaving the mixture in a covered bucket for three days. The proportions of eggs laid by gravid *An. gambiae* s.s. in diluted (10%) and concentrated infusions ( $\geq 25\%$ ) was compared to that laid in lake water in two-choice egg-count bioassays. Furthermore, with the aim to develop a method that can be used to collect volatiles that influence the egg-laying behavior of malaria mosquitoes, different volatile trapping methods were investigated. Two different polymer-traps eluted using two different desorption methods and three parameters were investigated. Porapak®-Q traps and solvent desorption was compared to Tenax®-TA traps and thermal desorption. The parameters investigated were: collection time (1h vs. 20h), addition of salt (0.15 g/ml sodium chloride (NaCl) vs. no NaCl), and stirring the infusion (0 vs. 300 rpm). Sample analysis was with gas chromatography-mass spectrometry (GC-MS). *An. gambiae* s.s. was ten times less likely to lay eggs in concentrated hay infusion than in lake water. The volatiles were best characterized by thermally desorbed Tenax traps, collected for 20 hours from infusion aliquots with sodium chloride added. Ten volatiles identified from headspace and previously indicated as putative oviposition semiochemicals for *An. gambiae* s.s. or confirmed semiochemicals for other mosquito species were tested in egg-count bioassays. Six of these (3-methylbutanol, phenol, 4-methylphenol, nonanal, indole and 3-methylindole), when added to lake water, were avoided for egg-laying when lake water was offered as the alternative in dual-choice egg count bioassays. These compounds likely contribute to the unfavorable oviposition responses towards hay infusions. This difference in oviposition response of different mosquito species should be considered when designing control measures.

**Keywords :** *Anopheles gambiae*, oviposition behaviour, egg-count cage bioassays, hay infusions, volatile detection, semiochemicals

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