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Research

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OXFORD

From the Field to the Laboratory: Quantifying Outdoor Mosquito Landing Rate to Better Evaluate Topical Repellents

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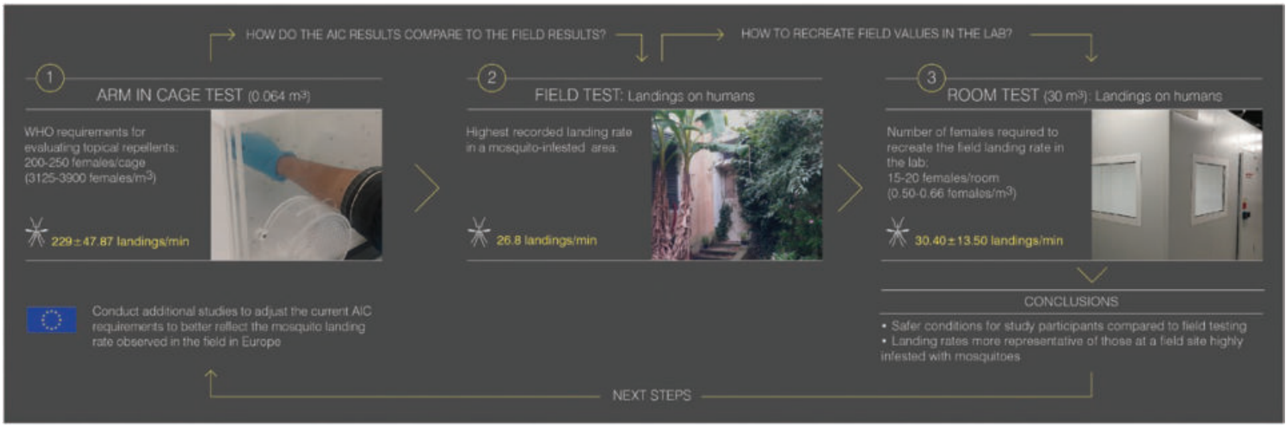
Abstract

Vector-borne diseases are a worldwide threat to human health. Often, no vaccines or treatments exist. Thus, personal protection products play an essential role in limiting transmission. The World Health Organization (WHO) arm-in-cage (AIC) test is the most common method for evaluating the efficacy of topical repellents, but it remains unclear whether AIC testing conditions recreate the mosquito landing rates in the field. This study aimed to estimate the landing rate outdoors, in an area of Europe highly infested with the Asian tiger mosquito (*Aedes albopictus* (Skuse, 1894, Diptera: Culididae)), and to determine how to replicate this rate in the laboratory. To assess the landing rate in the field, 16 individuals were exposed to mosquitoes in a highly infested region of Italy. These field results were then compared to results obtained in the laboratory: 1) in a 30 m^3 room where nine volunteers were exposed to different mosquito abundances (ranges: 15–20, 25–30, and 45–50) and 2) in a 0.064 m^3 AIC test cage where 10 individuals exposed their arms to 200 mosquitoes (as per WHO requirements). The highest mosquito landing rate in the field was 26.8 landings/min. In the room test, a similar landing rate was achieved using 15–20 mosquitoes (density: 0.50–0.66 mosquitoes/m^3) and an exposure time of 3 min. In the AIC test using 200 mosquitoes (density: 3,125 mosquitoes/m^3), the landing rate was 229 ± 48 landings/min. This study provides useful reference values that can be employed to design new evaluation standards for topical repellents that better simulate field conditions.

Graphical Abstract



HOW DO THE AIC RESULTS COMPARE TO THE FIELD RESULTS?

HOW TO RECREATE FIELD VALUES IN THE LAB?

① ARM IN CAGE TEST (0.064 m^3)

WHO requirements for evaluating topical repellents:
200-250 females/cage
(3125-3900 females/m^3)

229 ± 47.87 landings/min

② FIELD TEST: Landings on humans

Highest recorded landing rate in a mosquito-infested area:

26.8 landings/min

③ ROOM TEST (30 m^3): Landings on humans

Number of females required to recreate the field landing rate in the lab:
15-20 females/room
(0.50-0.66 females/m^3)

30.40 ± 13.50 landings/min

CONCLUSIONS

- Safer conditions for study participants compared to field testing
- Landing rates more representative of those at a field site highly infested with mosquitoes

Conduct additional studies to adjust the current AIC requirements to better reflect the mosquito landing rate observed in the field in Europe

NEXT STEPS

Key words: *Aedes albopictus*, arm in cage, laboratory testing, field testing, LRC

Over the last two decades, vector-borne diseases (VBDs) have been increasingly affecting human health in tropical, subtropical, and temperate areas (Weaver and Reisen 2010, Gould et al. 2017). Globalization and environmental shifts, including climate change, are key factors driving the emergence and spread of VBDs worldwide. At the same time, other intrinsic factors, such as vector competence and capacity, are significantly contributing to rises in incidence (Gould and Higgs 2009, Wilke et al. 2019).

The Asian tiger mosquito, *Aedes albopictus* (Skuse, 1894), is a prime example of a vector that has spread as a result of human activity (Lounibos 2002, Powell and Tabachnick 2013). The species naturally occurs in Asia, but it has successfully expanded its range to include parts of Africa, Europe, Australia, the Americas, and the Middle East during the 20th century (Gratz 2004, Benedict et al. 2007, Paupy et al. 2009).

Under normal circumstances, *Ae. albopictus* is exophilic and diurnal, with two main peaks of activity: one in the morning and one in the early evening (Paupy et al. 2009, Delatte et al. 2010). As a consequence, the species can disrupt outdoor recreational activities (Greenberg and Schneider 1997, Halasa et al. 2014). Over recent decades, such activities have taken on increasing societal importance (Bell et al. 2013, Outdoor Industry Association 2013), and thus humans are at greater risk of being bitten by *Ae. albopictus* and contracting VBDs. Laboratory experiments have shown that this species can transmit at least 26 arbovirosis causing diseases, including dengue, Zika, and chikungunya (Paupy et al. 2009). Indeed, *Ae. albopictus* has caused local outbreaks of arboviral diseases (mainly dengue and chikungunya) in various continents, including Europe (Grandadam et al. 2011, Lourenço and Recker 2014, Schaffner and Mathis 2014) and the Americas (Moore and Mitchell 1997, Ruiz-Moreno et al. 2012, Halstead 2015, Kraemer et al. 2015, Hennessey et al. 2016).

Due to the increasing incidence of VBDs for which vaccines are unavailable (Gossner et al. 2018), people are more frequently adopting preventive measures, such as the use of personal protection products. As a consequence, the demand for repellents and household insecticides during periods of high mosquito activity has risen significantly over the last few years (Chouhan and Deshmukh 2020). In Europe, insecticides and repellents are strictly regulated. Before being authorized for use and going on the market, such products must demonstrate compliance with the strict human health, environmental, and efficacy standards imposed by the Biocidal Products Regulation (BPR; ECHA 2019a). In 2012, the European Chemical Agency (ECHA) updated its guidelines for evaluating the efficacy of insecticides and repellents (ECHA 2011). Since 2017, the guidelines for product type 19 (PT19), the category that includes attractants and repellents, have been undergoing revision (ECHA 2018a, 2018b, 2018c, 2018d, 2019b, 2019c).

A commonly used methodology about which there is much discussion is the internationally recognized arm-in-cage (AIC) test, which has been formally described by WHO (2009) and the US Environmental Protection Agency (EPA 2010). This test is employed to estimate the complete protection time (CPT) of topical repellents (i.e., lotions, creams, wipes, or sprays) under laboratory conditions. In the WHO's methodological guidelines, 200–250 host-seeking female mosquitoes are placed in test cages (with sides measuring 35–40 cm), resulting in a density that is equivalent to 3,125–3,900 mosquitoes per m^3 (or one mosquito per 320 cm^3). In the EPA's methodological guidelines, mosquito number is similar—200 mosquitoes—but cage size is larger (61 cm × 61 cm × 61 cm), resulting in a density that is equivalent to 881 mosquitoes per m^3 (or one mosquito per 1,160 cm^3). The topical repellent to be tested is applied to the forearms of volunteers (similar numbers of women and men), who then place their arms into the cages for 3 min every 30–60 min; the experiment continues for 8 h or until the repellent no longer provides protection. During the 3-min exposure periods, mosquito landing rate and/or probing activity are quantified. In the WHO guidelines, CPT is defined as the time elapsed between the application of the product and the first instance of landing or probing by a mosquito. In the EPA guidelines, CPT is the time elapsed between the application of the product and the failure of its efficacy, which is defined in each study (e.g., the time between application and the first failure event when the latter is confirmed within 30 min by a second failure event).

Although the AIC test is a well-accepted and internationally recognized methodology for evaluating topical repellents, it may be underestimating CPT under field conditions (ECHA 2019b). Indeed, research has found that CPT estimates are shorter in AIC tests conducted in the laboratory than in alternative tests conducted in the field (Obermayr et al. 2010, Colucci and Müller 2018). As mentioned before, the AIC test employs a high density of mosquitoes, which may result in a higher landing rate than under field conditions. If the mosquito density required by WHO or EPA guidelines were extrapolated for use in testing rooms, which a priori more accurately simulate the conditions under which humans encounter mosquitos but obviously have larger volumes (e.g., 30 m^3), it would be necessary to place an absurdly high number of mosquitoes in the room, between 26,400 and 94,000 (depending on the specific guidelines being followed). That said, although field tests allow repellents to be evaluated under more realistic conditions in terms of landing rate, such tests should be avoided because of the risk of VBD transmission in areas where number of the infections are climbing and the infection status of wild mosquitoes is unknown, such in many parts of Europe (Seyler et al. 2009, Rocklöv et al. 2016).

Thus, there is a need to compare the landing rates obtained via the AIC test with those obtained in the field; latter may more accurately reflect the conditions that a consumer in Europe might encounter in an area highly infested with mosquitoes. To this end, the first step was to carry out a field test to quantify the mosquito landing rate in an area naturally infested with high levels of *Ae. albopictus*. Then, two laboratory tests were performed. First, a room test was conducted in which the objective was to recreate the field landing rate in a 30 m^3 testing room. Second, an AIC test was conducted in accordance with WHO guidelines requirements (WHO 2009) to assess the resulting landing rate.

This study seeks to meet the urgent need to develop new laboratory methodologies that can better recreate conditions of mosquito exposure in nature, but that are also safer for study participants than field testing. The results of this study can help guide the development of such techniques, by providing reference values that can be useful in defining new evaluation factors and conditions.

Materials and Methods

This research was carried out in two different settings—in the field and in the laboratory—using humans as study participants.

Participants were fully informed about the nature and purposes of the study and about any physical consequences that could foreseeably result from having taken part. We preferentially recruited nonsmokers; if participants were smokers, they were asked to refrain from using tobacco during the testing process and for 12 h prior. Participants were also asked to avoid alcohol consumption and the use of perfumes, body lotions, soap, and/or repellents during the testing process and for 12 h prior. In addition to these selection criteria, participants in the field test were chosen based on their ability to identify free-flying *Ae. albopictus* from its morphological characteristics.

In this study, human exposure to mosquito bites was quantified in the field test, the room test, and the AIC test using the landing rate count (LRC) method. It was important to use the same method to be able to compare results across tests and between the field and the laboratory. This method counts the number of landings that take place during a defined exposure period. A landing was defined in the following way: after a mosquito alights on a human, it probes the skin with its proboscis. At this point, the LRC method allows participants to gently disturb mosquitoes to reduce the risk of being bitten. The LRC method is often used during vector control programs by mosquito control agencies to evaluate the need for or the efficacy of adulticide treatments (Connelly and Carlson 2009). According to the Florida Coordinating Council on Mosquito Control, the following variables must be controlled when this method is used: the time of the observations, the duration of the observations, the part of body exposed to the mosquitoes, the number and type of nearby habitats, and the number of study participants. The LRC method is most effective when repeated measures are obtained for a given study participant at a given site because there is considerable interindividual variability in attracting and collecting mosquitoes (Connelly and Carlson 2009).

Although standard guidelines for its use have yet to be established, the LRC method is a recognized alternative to the human landing catch (HLC) method to measure the density of adult nuisance mosquitoes (Connelly and Carlson 2009, NCEMA 2016). In this study, an important consideration in the choice to use the LRC method rather than the HLC method was that the latter requires that mosquitoes be captured, while the former does not. One of the reasons for capturing mosquitoes is to identify specimens to species, which was unnecessary here since the only diurnal vector species in the field-testing area was *Ae. albopictus* (Marini et al. 2015). Furthermore, in this study, the landing rate in the field was recorded during the whole day. Consequently, capturing mosquitoes would have reduced the density of the mosquito population over the course of the field trials and could have skewed estimates of landing rate by the end of the field test. It was important to avoid the potential effects of removing members of the *Ae. albopictus* population, given its patchy distribution.

Aedes albopictus is an aggressive mosquito that may try to bite several times until it has fully fed; therefore, a given mosquito might land more than once to make multiple feeding attempts. The LRC method also account for this behavior.

Field Test

The field test was carried out in September 2018 in a green landscaped zone within a hospital complex (45°23′58.7″N 11°50′31.3″E) located to the southwest of Padua (Veneto region, Italy; Fig. 1). The first time that *Ae. albopictus* was observed in Padua was in the late summer of 1991 (Dalla Pozza et al. 1994). Despite the implementation of vector control programs, the mosquito has become well established in the region. Several studies have been conducted in this area because of the abundance of *Ae. albopictus* (Grisenti et al. 2015, Corcos et al. 2019, Marini et al. 2019). Furthermore, it has been found that *Ae. albopictus is* the only human-biting mosquito that is active at this location during the day (Marini et al. 2015). The study zone contained buildings with courtyard-like patios, which naturally formed independent 'experimental plots'. The vegetation present included grass; flowering plants, hedges, and bushes (height: 0.5–2 m); ornamental trees (height: 2–5 m); and nonornamental trees (height: 5–20 m; Figs. 1 and 2a–c).

Sixteen people (nine men and seven women) participated in the field test, which took place over two consecutive days between 9:00 and 18:00. On the first day, observations were made on 10 plots; there was one study participant per plot. On the second day, observations were made on 16 more plots; there was one study participant per plot. An observation was defined as the number of mosquito landings obtained for a single study participant within a given plot for a given 5-min exposure period. Although the total potential number of observations was 260, 221 observations were ultimately obtained because of the study participants' scheduling limitations. Given that 221 observations represent 85% of the potential number of observations, the study collected sufficient data to carry out robust analyses and allow us to address our research questions.

The maximum landing rate of *Ae. albopictus* was determined by carrying out measurements in 26 experimental plots (Fig. 1). The experimental plots were chosen to be as similar as possible to gardens or patios; all had comparable levels of sun exposure. Furthermore, as recommended in WHO guidelines (WHO 2009), the experimental plots measured approximately 3 × 3 m, and they were separated by at least 20 m to avoid the concurrent attraction of mosquitoes.

Participants were randomly assigned to the different experimental plots. *Aedes albopictus* preferentially targets the lower body (Shirai et al. 2002). Consequently, during the exposure period, participants stood in the middle of the plot and exposed the lower half of both their legs (from knee to ankle). Their bodies were otherwise protected from bites by a light beekeeper's suit (including a hat) that the mosquitoes could not penetrate. As noted above, there was one study participant per plot; this person counted the number of landings that occurred on her/his legs. The study participants remained standing during the entire 5-min exposure period. During this period, the number of mosquito landings was recorded. After it had ended, they rolled down their pant legs and went to a centralized meeting point, where they stayed until the next trial. The meeting point was located more than 50 m from the nearest plot.

The observed landing rates were grouped into different categories; the lowest category contained rates of <10 landings/min, and the highest category contained rates of >20–30 landings/min.

Over the course of the experiment, temperature and humidity were measured hourly using a portable digital weather station (TFA Dostmann) and a digital thermohygrometer (Lafayette TM-4).

Laboratory Room Test

Ten people (five men and five women) participated in the room test, which was carried out at Henkel's R&D Insect Control Laboratory (Spain). The mosquitoes used in the test came from a colony at the Entostudio Test Institute (Italy), which Henkel has maintained for the past 7 yr.

Mosquito rearing conditions were as follows: temperature of 25 ± 2°C, relative humidity of 60 ± 5%, and photoperiod of 12:12 (L:D). The test was conducted using 5- to 10-d-old, nonblood-fed females of *Ae. albopictus*. To ensure that they were active during the

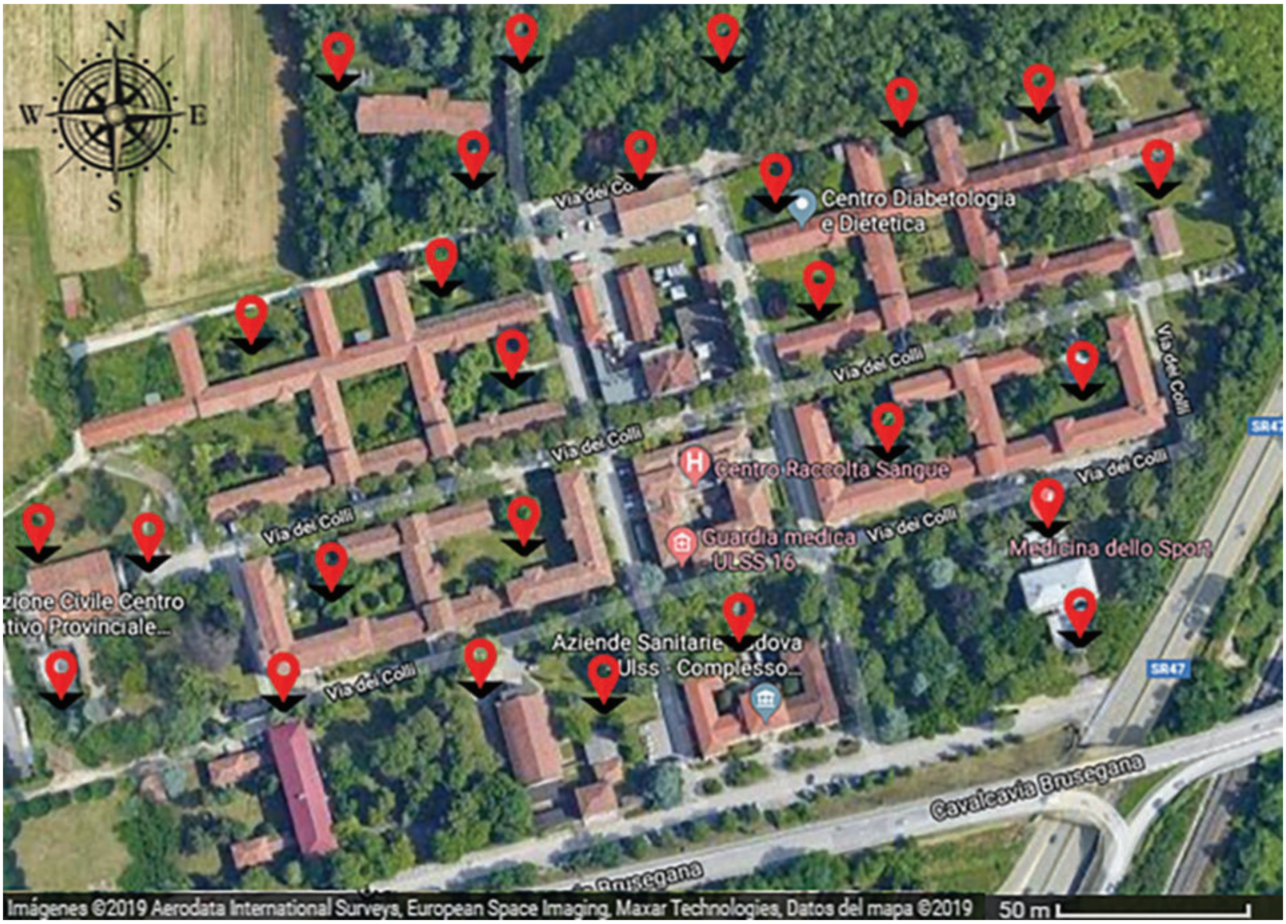


Fig. 1. Study zone (45°23′58.7″N 11°50′31.3″E) located to the southwest of Padua, Italy. The rounded markers with a translucid circle in the middle indicate the locations of the experimental plots. The field test was performed at this location over two consecutive days in September 2018. To the greatest degree possible, given the constraints of working in the field, plots were selected to be similar in temperature, relative humidity, and sun exposure. They measured approximately 3 × 3 m, and they were separated from each other by at least 20 m to avoid the concurrent attraction of mosquitoes by study participants on two plots. Source: Google Earth.

Fig. 2. (a) Study participant in an experimental plot wearing a protective suit and waiting for the exposure period to start; (b) experimental plot located in a courtyard; (c) experimental plot located in a covered patio. The vegetation consisted of ornamental plants, common garden shrubs, and trees.

experiment, the mosquitoes were given water and 10% sucrose solution ad libitum until the test began.

Testing took place in a 30 m³ room (4.0 m × 3.6 m × 2.3 m) that was illuminated by external overhead fluorescent lighting (150-W halogen bulbs). The walls were white to facilitate the counting of insects, and all the surfaces were made of a material that was sealed, waterproofed, and easy to clean. Using high-pressure extract ventilation, a decontamination rate of more than 99% could be obtained (Fig. 3a).

In the room, the temperature and relative humidity were always kept at 25 ± 2°C and 60 ± 5%, respectively; the ventilation rate was equivalent to renewing the air in the room 2.5 times per hour, simulating an 'open windows' situation (Bremmer at al. 2006).

Each day before starting the test, the room was checked for insecticide contamination. At least 10 mosquitoes were released into the chamber and left therein for 30 min. They were given cotton wool soaked in a 10% sugar solution. Any mortality during this period was noted, and the room was considered to be contaminated or in an unsatisfactory state if knockdown was higher than 10%. If no contamination was detected, the first set of mosquitoes was removed, and a second set of mosquitoes was released to initiate testing. These latter mosquitoes were given 30 min to acclimate to the room. Then, a study participant entered the room. As in the field test, only the lower part of the person's legs was exposed. The rest of the body was protected by a light beekeeper's suit. Furthermore, in the laboratory tests, participants wore gloves and white hospital booties (Fig. 3b).

To evaluate the relationship between mosquito number and landing rate, participants were exposed to different levels of mosquito abundance: 15–20 (density of 0.50–0.66/m³), 25–30 (density of 0.83–1.00/m³), and 45–50 (density of 1.50–1.66/m³). The maximum level of mosquito abundance was defined based on the results of preliminary research (Moreno et al., unpublished data) that employed the highest mosquito density represented in this study (1.50–1.66 mosquitoes/m³).

The primary goal of the room test was to quantify the number of mosquitoes needed to recreate the maximum rate observed during field test. Consequently, the first step in the laboratory was to use the same methodology as in the field to assess the landing rate over a 5-min exposure period. In the first set of trials, 45–50 mosquitoes were placed in the room. In the next set of trials, 25–30 mosquitoes were used. In the last set of trials, 15–20 mosquitoes were used. The next step was to see whether the landing rate could be adjusted downward by decreasing the length of the exposure period. Thus, trials were performed using 15–20 mosquitoes that lasted 3 min instead of 5 min. These trials were then repeated using 25–30 mosquitos. The landing rate obtained with 45–50 mosquitoes and 5 min of exposure was far above the maximum landing rate seen during the field test. Because no significant differences were found in relation to exposure duration (3 and 5 min) and landing rate at either of the lower levels of mosquito abundance (15–20 and 25–30 mosquitoes), it was considered unnecessary to perform the trials using 45–50 mosquitoes and a 3-min exposure period.

An observation was defined as the number of mosquito landings obtained for a single study participant at a given level of mosquito abundance and for a given exposure period (3 or 5 min).

Laboratory AIC Test

Ten people (six men and four women) participated in the AIC test, which was carried out at the Entostudio Test Institute. WHO AIC test guidelines were employed (WHO 2009).

The mosquitoes used in the test came from a colony that has been maintained at the institute for the last 10 yr. The rearing conditions were as described above (see the description of the room test). The AIC test was also conducted using 5- to 10-d-old, nonblood-fed females of *Ae. albopictus*. To ensure they were active during the experiment, the mosquitoes were given water and 10% sucrose solution ad libitum until the test began.

In the test, 200 mosquitoes were released in a 0.064 m³ cage (Fig. 4). A study participant then introduced her/his forearm (surface area: 600 cm²) into the cage; her/his hand was protected by a glove. The surface area of the skin being exposed was estimated in accordance with WHO recommendations (WHO 2009). The

Fig. 3. (a) Outside view of the 30 m³ testing room at Henkel's R&D Insect Control Laboratory; (b) participant wearing a protective suit while inside the testing room.

Fig. 4. Arm-in-cage test being conducted in a 0.064 m³ cabin. A study participant exposing a full forearm (surface area: 600 cm²).

mean circumference of the forearm was calculated by measuring the arm's circumference at the wrist and at the elbow. This value was then multiplied by the length of the forearm—the distance from the wrist to the elbow. For 1 min, the number of mosquito landings was recorded. Counting could not proceed for 3 min, as it had in the room test, because the high biting pressure impeded the accurately counting of the number of mosquitoes landing.

An observation was defined as the number of mosquito landings obtained for a single study participant for a given exposure period (1 min).

Statistical Analysis

All the statistical analyses were carried out using R and SPSS (Windows v. 12.0.1; SPSS, Chicago, IL). The glmmPQL function in the MASS package was used to perform generalized linear mixed models (GLMMs). In general, the Poisson error distribution was employed. In some cases, the Gaussian error distribution was needed; it was implemented via the lme function in the nlme package. In all the analyses, participant identity was included as a random factor.

When overall significant differences were detected, pairwise comparisons were performed using t-tests with pooled SD. Bonferroni corrections were applied.

Field Test

GLMMs were used to determine whether the number of mosquito landings (Poisson error distribution and log-link function) and the number of landings/min (Gaussian error distribution and identity link function) were affected by the hour of the day during which the data were recorded.

Using Pearson's correlation, the relationships between the values of the abiotic factors (ambient temperature and humidity) and the number of mosquito landings (absolute number of landings and landings/min) were evaluated for both sampling days.

Room Test

Patterns in the number of landings/min were examined using GLMMs (Gaussian error distribution and identity link function) in which the level of mosquito abundance and the duration of the exposure period were the explanatory variables.

AIC Test

For the AIC test, descriptive statistics were employed. The mean and the SD were calculated using the results for all the participants involved in the study.

Ethics Approval and Consent to Participate

The work conducted herein was approved by the ethics committee of Henkel AG & Co. KGaA. It meets the company's corporate standards, which ensure health, safety, and respect for the environment as well as the protection and ethical treatment of all study participants. Human volunteers were recruited and signed a written informed consent form that explained the study's purpose and procedures as well as the participants' roles and responsibilities; the form also notified participants of their right to withdraw or refuse to take part in the study at any point without being penalized in any way. People who were pregnant, breastfeeding, younger than 18 yr of age, or older than 55 yr of age were not allowed to take part in the study. Other types of vulnerable individuals were also excluded: people who were mentally incapable of giving their consent to participate, people in poor health or with weak immune systems, and people with sensitivity to insect bites.

Study participants formally consented to their photos being used in the article.

Results

Field Test

In total, 221 observations were recorded during the field test. There were 10,922 landings across all the trials with all the participants over the 2 d of the test. The mean landing rate was 9.50 ± 1.06 landings/min (range: 0–26.8; Fig. 5).

Mean temperature and relative humidity were 28.3°C (range: 23.7–30.7°C) and 56.2% (range: 47.0–71.0%), respectively (Fig. 6).

The number of mosquitoes landing was not affected by time of day. Over the course of a given day, no significant differences were found in the number of the landings that occurred within 5 min (GLMM: $F_{155} = 0.343$; $P = 0.731$) or in the number of landings/min (GLMM: $t_{155} = 0.298$, $P = 0.765$).

More than 80% of the observed rates fell into two categories: <10 landings/min and 10–15 landings/min. Only 0.9% were in highest category (Table 1).

Based on the Pearson's correlation coefficient, neither temperature nor relative humidity was associated with mosquito landing rate (landings/min or landings/5 min) on either test day (Table 2).

Laboratory Room Test

In total, 88 observations and 19,163 landings were recorded during the room test. The results are shown in Fig. 7. When participants were exposed to 15–20 mosquitoes for 3 min, the total number of landings ranged from 31 to 164; when the exposure time was 5 min, the total number of landings ranged from 46 to 328. When participants were exposed to 25–30 mosquitoes, landing number ranged from 53 to 320 for 3 min of exposure and from 79 to 470 for 5 min of exposure. Finally, when participants were exposed to 45–50 mosquitoes for 5 min, landing number ranged between 141 and 613.

The lowest landing rate was obtained using 15–20 mosquitoes (0.50–0.66 mosquitoes/m³). The means for 3 and 5 min of exposure were 30.4 ± 13.5 and 32.3 ± 14.0 landings/min, respectively. When 25–30 mosquitoes (0.83–1.00 mosquitoes/m³) were used, the means for 3 and 5 min of exposure were 49.7 ± 26.3 and 55.3 ±

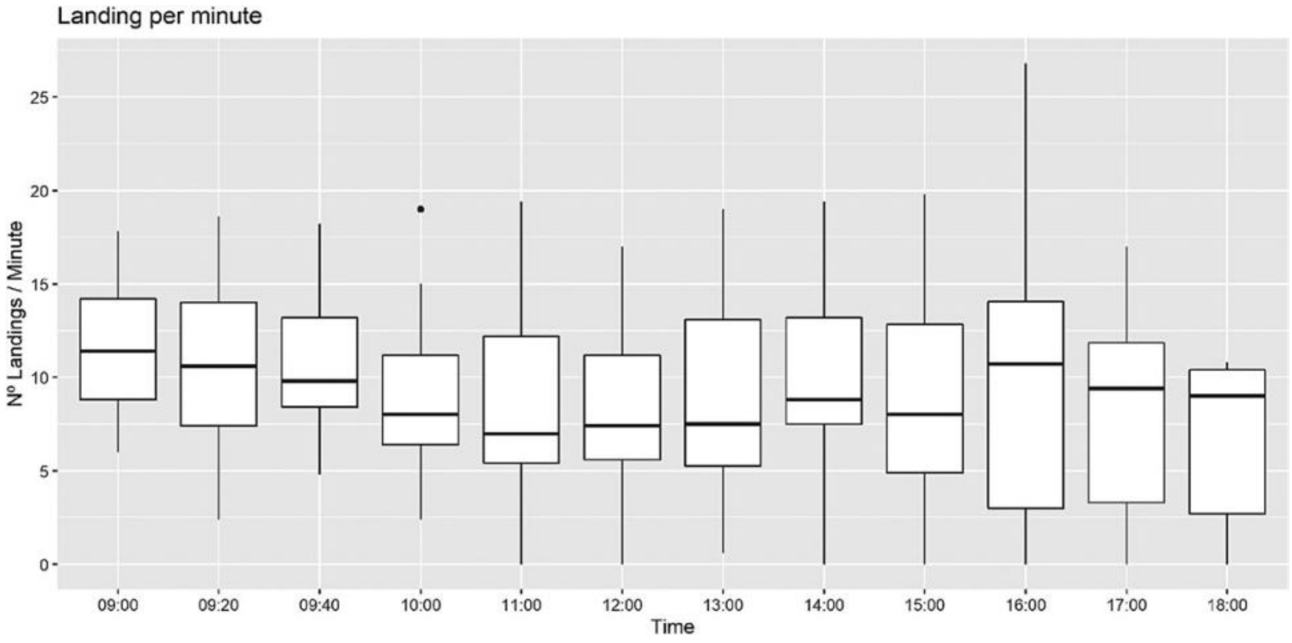


Fig. 5. Mean number of landings per minute by *Aedes albopictus* during the field test. Between 9:00 and 18:00 over two consecutive days, the number of mosquito landings taking place over a 5-min period was recorded hourly. Data were collected in 26 experimental plots, and a total of 221 observations were obtained. The whiskers represent the data that fell beyond the first and third quartiles (Q1 − 1.5 × IQR and Q3 + 1.5 × IQR), and the single point is an outlier.

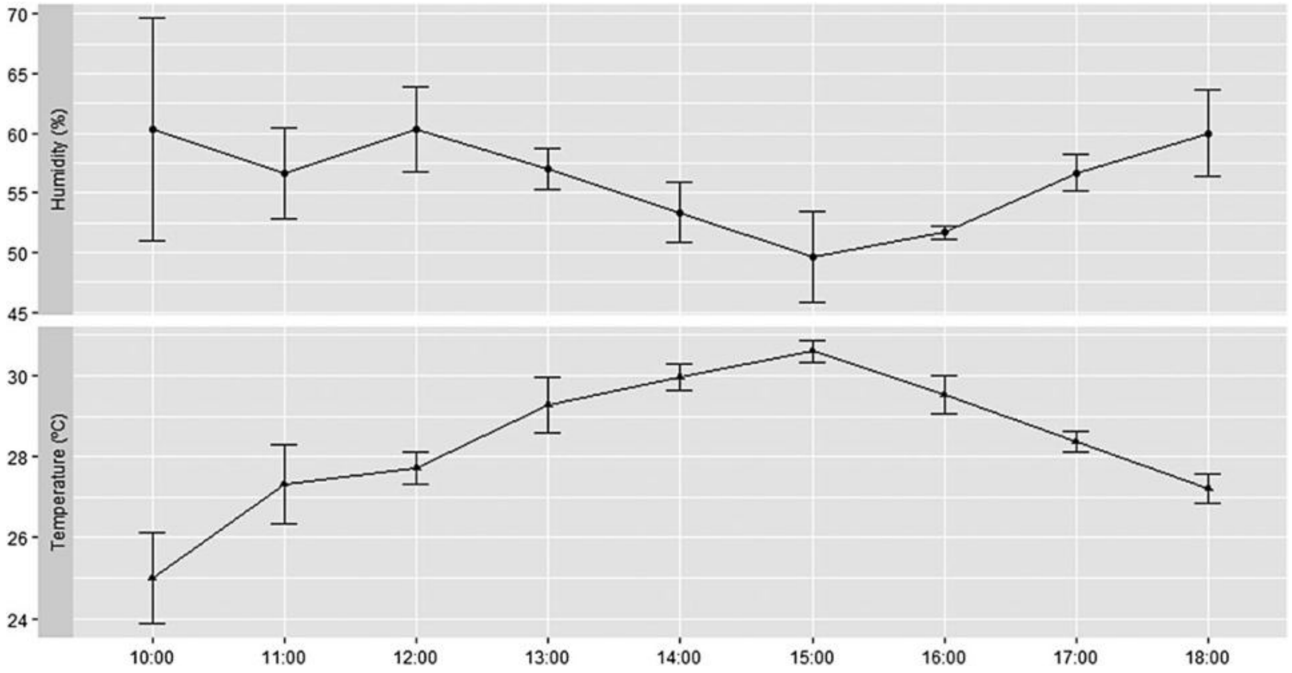


Fig. 6. Mean temperature and relative humidity recorded hourly from 10:00 to 18:00 over the two consecutive days of the field test during which the landing rate of *Aedes albopictus* was measured.

27.5 landings/min, respectively. When 45–50 mosquitoes (1.50–1.66 mosquitoes/m^3) were used, the mean for 5 min of exposure was 76.8 ± 36.9 landings/min.

The duration of the exposure period (i.e., 3 or 5 min) did not influence the landing rate (GLMM: $t_{76} = 1.292$; $P = 0.200$). In contrast, the landing rate was significantly higher at higher levels of mosquito abundance when the duration of exposure was 3 min (GLMM: $t_{76} = 6.27$); when the exposure period lasted 5 min, the landing rate differed significantly when the mosquito abundance level was 15–20 versus 30–35 ($P < 0.05$) or 15–20 versus 40–50 ($P < 0.0001$), but not when it was 30–35 versus 45–50 ($P = 0.052$).

Results of the Field Test Versus the Room Test

The mean landing rate obtained in the field test was 9.50 ± 1.06 landings/min. In total, 53.4% of the observations fell into the lowest category of >10 landings/min (range: 0–9.8 landings/min), while just two observations (0.9%)—20.2 and 26.8 landings/min—fell into the highest category of >20–30 landings/min. It was therefore considered that the maximum landing rate obtained during the field test (26.8 landings/min) should be the target rate, representing the highest landing rate that might be experienced in an area highly infested with *Ae. albopictus*.

The room test investigated how this field landing rate could be recreated by using different levels of mosquito abundance and exposure times. Using the mosquito abundance (15–20 mosquitoes in the 30 m^3 room or 0.5–0.66 mosquitoes/m^3) and an exposure time of 3 min, a mean landing rate of 30.4 ± 13.5 landings/min was obtained; this figure contains the field rate of 26.8 landings/min within the range of its SD.

Laboratory AIC Test

In total, 10 observations and 2,290 landings were recorded during the AIC test. The mean landing rate was 229 ± 48 landings/min.

Table 1. Categorization of the number of landings per minute observed during the field test

Landings/min	Number of observations	% Observations
<10	118	53.4
>10–15	67	30.3
>15–20	34	15.4
>20–30	2	0.9
Total	221	100

Table 2. Correlations of the abiotic factors with the total number of mosquito landings and mosquito landings per minute

Abiotic factors	Landings/min	
Temperature (°C)	$r = 0.095$	$P = 0.244$
Relative humidity (%)	$r = 0.063$	$P = 0.437$

Discussion

Mosquito repellents for personal protection against mosquito nuisance and VBD (Benedict et al. 2007) are usually evaluated under field and laboratory conditions based on international guidelines (WHO 2009, EPA 2010, ECHA 2018a).

One of the major challenges faced during the experimental design process was to identify a suitable parameter and/or method that would allow a link to be established among the field test, the room test, and the AIC test. The LRC method was found to be a valid alternative to the HLC method for quantifying the landing rate in such a way as to allow comparisons across field and laboratory settings. The HLC method is a widely used standard approach for evaluating mosquito density and species occurrence within defined areas. However, this method was inappropriate in the context of this study because it requires that mosquitoes be captured and would thus have yielded incomparable results between field and laboratory settings. Notably, in the standard AIC test, the mosquitoes landing on the forearms of study participants are never captured. The decision was thus made to employ the lesser-known LRC method, which is primarily used during vector control programs and which does not require mosquito capture.

To our knowledge, this is the first study of its kind to compare the landing rates of a mosquito species, *Ae. albopictus*, in both the field and the laboratory. This research successfully identified the laboratory conditions (room test) that could be used to recreate the maximum landing rate that was observed outdoors in an area highly infested with *Ae. albopictus*. More specifically, this maximum outdoor landing rate (26.8 landings/min, 5-min exposure period) was simulated in the laboratory by placing 15–20 mosquitoes in a 30 m^3 room (density: 0.50–0.66 mosquitoes/m^3, 3-min exposure period). The latter conditions yielded a rate of 30.4 ± 13.5 landings/min, which is 1.15-fold higher than the maximum field rate (26.8 landings/min) and 3-fold higher than the mean field rate (9.5 ± 1.06 landings/min). Based on the findings of this study, the LRC method appears to be a valid approach for measuring mosquito landing rates across field and laboratory settings even though it remains to be fully standardized.

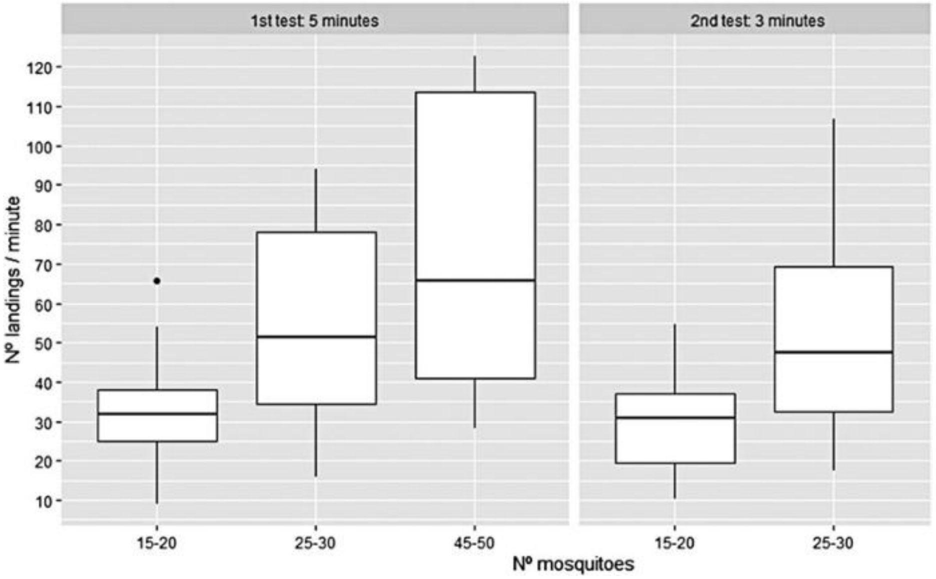


Fig. 7. Mean and SD of landing rate for the different levels of mosquito abundance and durations of exposure in the room test. The trial using 45–50 mosquitoes and a 3-min exposure period was not performed for ethical reasons (see Materials and Methods). The whiskers represent the data that fell beyond the first and third quartiles (Q1 − 1.5 × IQR and Q3 + 1.5 × IQR), and the single point is an outlier.

It is worth mentioning that temperature, relative humidity, and time of day did not significantly influence the landing rate in the field.

When conducting field testing of mosquito repellents, neither WHO guidelines (WHO 2009) nor EU guidelines (ECHA 2018a) specify a minimum landing rate for validating study plots. In contrast, US EPA guidelines recommend that a minimum of one mosquito landing be observed per minute on plots that are to be included in study trials (EPA 2010). The mean landing rate observed during the field test in this study was 9.5 ± 1.06 landings/min. Because mosquitoes were disturbed in this study instead of being captured as they would have been in an EPA study, it is not possible to compare absolute landing rates. Nevertheless, the results obtained suggest that our study zone is characterized by challenging conditions that are representative of those to which a consumer might be exposed in outdoor areas with high levels of mosquito abundance.

Furthermore, previous studies conducted in Europe on other mosquito species have found comparatively lower mean landing rates in the field compared with our field study that underscore that the study zone was in an area with a high abundance of *Ae. albopictus*. It is important to note that these studies also employed the HLC method, making it difficult to perform absolute comparisons. For example, the species *Aedes cinereus* (Meigen 1818) (Diptera: Culididae), *Aedes geminus* (Peus, 1970) (Diptera: Culididae), *Aedes vexans* (Meigen, 1830) (Diptera: Culididae), and *Anopheles plumbeus* (Stephens, 1828) (Diptera: Culididae) had mean landing rates of 0.276 landings/min (range: 0–0.432) in the Langholz forest and 0.0342 landings/min (range: 0–0.336) in the Thurauen Nature Reserve, based on research conducted by Colucci and Müller (2018) in Switzerland Similarly, a study conducted in southern England (Brugman et al. 2017) found that the species *Coquillettidia richiardii* (Ficalbi, 1889) (Diptera: Culididae), *Anopheles maculipennis* (Shute, 1936) (Diptera: Culididae), and *Culex modestus* (Ficalbi, 1889) (Diptera: Culididae) had landing rates of between 0.0084 and 0.1680 landings/min. A field study conducted in the United States, in California, also documented lower biting rates: 1.5 bites/min on the arm and 3 bites/min on the leg for *Ochlerotatus melanimom* (Dyar 1928) (Diptera: Culididae), *Ae. vexans*, and *Ochlerotatus increpitus* (Dyar, 1916) (Diptera: Culididae).

When interpreting the laboratory test results, it should be highlighted that, according to WHO guidelines for laboratory testing of topical repellents (WHO 2009), 200–250 female mosquitoes must be placed in an approximately 0.0064 cm^3 cabin, which results in a mosquito density equivalent to 3,125–3,900 mosquitoes/m^3 (WHO 2009). In this study, the AIC test used the minimum number of mosquitoes within the range specified in WHO guidelines (i.e., 200). Under these conditions, the mean landing rate was 229 ± 48 landings/min, which is more than seven times higher than the 30.4 ± 13.5 landings/min obtained in the room test trial that best recreated the maximum field landing rate.

Past studies have explored the relationship between field and laboratory mosquito landing rates and mosquito density (Obermayr et al. 2010, Colucci and Müller 2018). Tropical repellents were evaluated in the laboratory using the WHO AIC test and in the field using EPA guidelines (WHO 2009, EPA 2010). The results of the room test performed in this study were similar to the results of the above research (Obermayr et al. 2010, Colucci and Müller 2018): in both cases, there was a significant positive relationship between mosquito density and landing rate. Additionally, when these previous studies examined the association between mosquito density and CPT under both field and laboratory conditions, it was found that the AIC test, as a result of its high mosquito densities, created higher landing rates and shorter CPTs than those observed in the field. A study by Barnard et al. (1998) provides additional evidence of this relationship: for *Aedes aegypti* (Linnaeus, 1762) (Diptera: Culididae) and *Anopheles quadriannulatus* (Barnard et al. 1998), landing rates increased with mosquito density, leading to a decline in CPT. Nevertheless, it is worth noting that mosquito landing rates may be influenced by multiple factors, including mosquito species (Petrić et al. 2014, Brugman et al. 2017). In the research mentioned above (Obermayr et al. 2010, Colucci and Müller 2018), different mosquito species were used in the laboratory versus in the field, making difficult to compare the studies' respective results. These limitations were taken into consideration when our study was designed, which is why the landing rates of the same species were measured in both the field and the laboratory, thus reducing variability.

As previously stated, the main concern related to current guidelines for AIC testing (WHO 2009) is that the high mosquito density required could led to CPTs being significantly underestimated compared to what would be seen under field conditions in Europe. Notably, it must be highlighted that, at the European level, efficacy testing is undergoing a mandated shift to align with Human Health Risk Assessments guidelines for biocidal product approval in Europe, which apply stricter dosage standards (ECHA 2019a). As a result, only lower doses (<1 g) could be viewed as acceptable, especially for topical repellents containing higher concentrations of active substances (>15%). It is thus important to ensure that repellents are tested under conditions that more accurately reflect consumer use. If topical repellents are assessed under overly intense conditions, protection times could be underestimated, and consumers may risk overapplying chemicals to their skin.

Furthermore, although this study focused on Europe, it is essential to highlight that the conclusions herein are based on the rather high landing rate that was observed in the field (26.8 landings/min, which translates to 134 landings over a 5-min period). It is considered that this landing rate represents intense mosquito pressure for repellent users, regardless if one is in Europe or elsewhere.

The results of this study can help improve and refine the evaluation standards used for testing repellents in the laboratory by better simulating outdoor conditions and more accurately estimating CPT. Furthermore, this work is an essential part of effort to develop safer yet reliable alternatives to field testing, given that the latter exposes study participants to health risks. These efforts may also help reduce the cost and complexity of this type of research and identify the appropriate balance between the guarantee efficacy of products and their safety for consumers.

Consequently, the next step should be to conduct additional studies to adjust the densities used in the AIC test (WHO 2009) so that the resulting landing rates better represent what is observed in the field in Europe. One option could be to strive for a biting pressure range as individual laboratories could then adjust the mosquito density for a given colony or species to more accurately gauge a repellent's performance.

Furthermore, this study focused exclusively on *Ae. albopictus*, which displays certain species-specific traits, such as a tendency to feed repeatedly. Future work should therefore include other mosquito species with a view to more broadly improving current guidelines for mosquito repellent testing.

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