CHAPTER

6

Repelling mosquitoes with electric fields

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6.1 Electric fields

Electric fields are physical fields created by electric charges or time-varying magnetic fields. This chapter focuses on electric fields generated by electric charges between two electric conductors and how they can be used to repel mosquitoes.

An electric field is defined as the electric force per unit charge. It emanates from an electric charge and transmits its force on other charges in its vicinity. The electric field around a point charge can be visualized as radial lines of force (orienting away from a positive charge and toward a negative charge, Fig. 6.1) with the resulting field strength decreasing inversely in proportion to the square of the distance, i.e., the electrical field weakens with growing distance to the point charge (Brodie, 2000; Roche, 2016).

Constant, homogenous electric fields can be created between two parallel conductive surfaces with opposite charges, creating a potential difference (voltage) (Fig. 6.2). In this example, the field strength vector is perpendicular to the surfaces and orients from the positive to the negative charge. The electrical field strength is constant; it is proportional to the charge and inversely proportional to the distance between the surfaces (Marinescu, 2009). Thus, the magnitude of the electric field (E) is:

$$E = -\Delta V / d$$

ΔV = potential difference.
d = distance between the surface.

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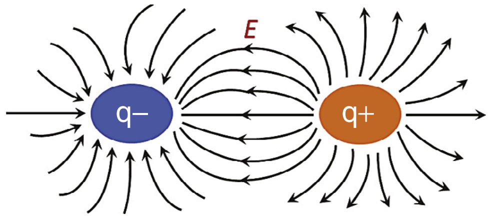

FIG. 6.1 Electric field (*E*) between two charges. By Farooq Tanveer, Biogents AG.

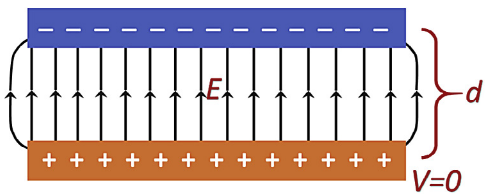

FIG. 6.2 Electric field between two parallel charged metal plates. By Farooq Tanveer, Biogents AG.

6.1.1 Natural occurrence and potential impact

In nature, electric fields are omnipresent. They occur natural or are man-made (anthropogenic). Natural electric fields are found above the surface of the earth, generated by a potential difference between the ground and the ionosphere (König et al., 1981). The earth field has a strength of about 100–300 V/m, depending on time of day and season, local temperature, and humidity, and decreases with height. Common man-made electric fields occur under power transmission lines, are generated by transportation (e.g., electric rail/bus systems), and visual display units such as TV screens, or are caused by charge separation as a result of friction (e.g., walking on nonconductive surfaces). While the short-term exposure to static magnetic fields can induce changes in blood pressure and heartbeat in humans, there is no evidence that the short-term exposure to static electric fields causes acute adverse effects in human health (World Health Organization, 2006). In animals, the exposure to anthropogenic electromagnetic fields may impede their orientation in the magnetic field of the earth, which is especially important for migratory birds and insects (Balmori, 2015).

6.1.2 Electroreception

A variety of animals respond to electric fields, especially those inhabiting the aquatic environment. Electroreception is very common in fish, teleost (bony) fish, amphibians, and dolphins (Von der Emde, 2013). Sharks and rays use weak electric fields that are emitted by other animals in their vicinity to locate their prey; gymnotiform fish, like eels, use lower voltage pulses for navigation, and detection of prey but are also able to generate high voltage electric shocks (Catania, 2015). Two electro sensory structures found in aquatic or semiaquatic organisms have evolved independently. Ampullae are small tubular cavities that detect differences in the electric potential between the inside of the animal and the aquatic environment and trigeminal electroreceptors respond to negative and positive charges (Von der Emde, 2013). Both structures depend on water as an electrically conductive medium. Up to the late 2000s, little was known about electroreception in terrestrial animals which must navigate in an electrically nonconductive environment (air). In humans, body hairs seem to be involved with the perception of electric fields, mainly through hair movement elicited by the electric field (Chapman et al., 2005). Comparable mechanisms involving certain body appendages, like wings or antennae, could also contribute to the electroreception in insects (Newland et al., 2008).

Recent research has shown that both honeybees and bumblebees are able to detect electric fields with their antennae (honeybees) (Clarke et al., 2013) and mechanosensory hairs (bumblebees) (Sutton et al., 2016) providing further fascinating insight into the pollination process and plant-pollinator communication. Entomophilic flowers use a variety of visual and chemical cues to attract pollinators and most flowers also exhibit a negative electric potential (Corbet et al., 1982) mainly on the edges of petal, stigma, and anthers (Clarke et al., 2017). Pollinators are usually positively charged, thus, during their interaction with the flower, the potential difference between the insect´s surface and the stigma promotes efficient pollen transfer and adhesion (Vaknin et al., 2000). As a consequence of the deposition of pollen, the electric potential of the flower changes and so does the pollination status. Bumblebees are able to discriminate between rewarding and non-rewarding electric fields, which greatly contributes to a rapid and dynamic communication between flowers and their pollinators (Sutton et al., 2016).

Antennae and mechanoreceptive hairs are not unique to bees and it is quite possible that other arthropods use the same structures to detect electric fields (Clarke et al., 2017). In a laboratory set-up, cockroaches (*Periplaneta americana*) avoided static electric fields of 8 kV/m and above. They were able to detect the electric field with their antennae and hair plates at the base of the scape. It was found that the random charge on the cockroaches' body changed in close proximity to a positively charged electrode, with negative charges being attracted to the long antennae. As a consequence, the antennae were drawn to the positively charged electrode and bent which caused an avoidance behavior (Newland et al., 2008). A similar mechanism might also apply to other insects making static electric fields an interesting tool for alternative pest control strategies. Research on other arthropods supports the hypothesis that, in general, insects are repelled by static electric fields (Hunt et al., 2005; Maw, 1961, 1962; Newland et al., 2008). A study from Japan investigated the avoidance behavior of a variety of insect species and spiders to an electric field screen that was generated between negatively charged conductor wires and a positively charged earthed metal net (Matsuda et al., 2015). All tested species (including the orders Coleoptera, Hemiptera, Diptera, Hymenoptera, Lepidoptera, and Blattodea) were deterred by the electric field, however, the Voltage (kV) required to cause the avoidance effect differed. The Asian tiger mosquito, *Ae. albopictus* was found to be repelled by 1.7 kV/cm while certain beetle species responded to voltages above 13 kV/cm. The authors also reported that insects contacted the negatively charged screen with their antennae and subsequently turned away; the voltage required to induce this behavior depended on the length of the antennae and body size.

6.2 Challenges in mosquito control

Mosquito-borne diseases remain a major threat to human health all around the globe. Mosquito control programs are constantly challenged by novel arboviruses, growing insecticide resistance, and the spread of invasive mosquito species (Benelli et al., 2017). When there is no (prophylactic) drug and/or vaccine available, control of the mosquito-borne disease relies on the control of the vector. Vector control is primarily carried out through intertwining measures such as (1) source reduction (elimination of breeding sites), (2) killing the vector in the larval or adult stage, and (3) creating physical or chemical barriers between the vector and its host (Norris and Coats, 2017). Killing the adult vector is still widely done using synthetic insecticides, however, the available toolbox is limited to a few classes of chemicals with comparable action and their success in controlling vectors like *Ae. aegypti*, *An. gambiae*, or *Cx. pipiens* is limited due to the emergence and rapid spread of insecticide resistance in these species (Fonseca-González et al., 2011; Marcombe et al., 2011, 2014; Weill et al., 2003). This is why research for alternative vector control strategies continues. Over the last 15 years, a variety of promising new approaches have been investigated to augment existing measures, including lethal traps (Barrera et al., 2014; Degener et al., 2014; Kröckel et al., 2006), genetically modified mosquitoes (Thomas et al., 2000); release of mosquitoes with Wolbachia (Rasgon et al., 2003; Sinkins and O´Neill, 2000), attractive targeted sugar baits (Fiorenzano et al., 2017; Lea, 1965); spatial repellents (Achee et al., 2012; Bibbs and Kaufmann, 2017; Norris and Coats, 2017; Ogoma et al., 2014); push–pull (Menger et al., 2015; Obermayr et al., 2015; Paz-Soldan et al., 2011) and investigating the potential of "green chemistry," including entomopathogenic fungi (Scholte et al., 2004) and plant terpenoids (Norris et al., 2018).

The use of electric fields to repel mosquitoes is enticing as it would offer a means of controlling mosquitoes physically thereby circumventing a potential adaptation or resistance in the target organism. Here, we introduce and summarize an approach that uses static electric fields to elicit avoidance behavior in *Ae. aegypti* and also investigate whether females´ exposure to electric fields has an impact on their reproductive fitness.

6.3 Assessing the repellency of electric fields in the laboratory

6.3.1 Test mosquitoes *Ae. aegypti*

Five to ten days old *Ae. aegypti* females were used for all laboratory tests. The colony was originally obtained from BAYER AG (Monheim, Germany) in 1998 and has been maintained in the Biogents facilities over the past 22 years. Mosquitoes were reared at a temperature of 27 ± 0.5 °C and 70 ± 5% relative humidity (RH) under a photoperiod of 12:12 (L:D). The light period (full spectrum LEDs, 450 Lux) was set from 8:00 to 20:00. After hatching of the eggs, larvae were kept in water basins (30 × 30 × 10 cm) filled with a 1:1 mixture of deoxygenized tap water and deionized water and fed with Tetramin fish food flakes (Tetra GmbH, Melle, Germany). Pupae were transferred to breeding cages (40 × 30 × 20 cm) for adult emergence. Adult mosquitoes were provided with a 10% sugar solution (dextrose) on filter paper. Nulliparous females were used for all tests, they were selected based on host-seeking behavior, as described by Obermayr et al. (2015). The breeding cage contained a circular opening covered by fine mosquito netting in the left wall, while the right wall was fitted with a port and rotating door, where a transport container could be attached. The transfer container consisted of a Perspex cylinder with a rotating door on one end and a cover made from fine mosquito netting at the other end. A fan running at 7.5 V was connected to the opening in the left wall of the breeding cage, while a human hand was held against the mosquito netting of the transfer container on the opposite side of the cage and rotating doors were opened. Female mosquitoes seeking a blood meal flew upwind into the transfer container, attracted to the skin odors, and were immediately used in the experiments.

6.3.2 Barrier assays in cage tests

The repelling potential of different static electric fields (generated between two parallel plate electrodes, the conductors) was evaluated in a specifically designed laboratory cage test set-up. All experiments were performed under standardized conditions in a climatized room without windows. The temperature and RH of the room air were set to 27.5 ± 0.5 °C and 75 ± 5%. The room was illuminated with full-spectrum LED light tubes (intensity 450 Lux).

Two cubic mosquito rearing cages with a volume of 27 L (BugDorm-1 Insect Rearing Cage, from Watkins & Doncaster, Herefordshire, United Kingdom) were connected by a glass tunnel that allowed mosquitoes to fly from one cage to the other. To produce uniform electric fields, five copper plate electrodes were placed parallel in the center of the tunnel (20 × 10 × 9.6 cm) and connected to an adjustable high-voltage

(HV) device (Spellman, Model: V6 DC 15 KV POS 2MA W/O RS232) in such a way that negatively charged plates and grounded copper plates were alternating. The distance between the plate electrodes was 2 cm and was chosen because it ensures the generation of strong electric fields and still allows mosquitoes to fly through (preliminary experiments, data not shown). The opposing walls of cages I and II contained circular openings covered by fine gauze for air entry/exit. Airflow between the cages was created by a commercial DC fan (12 V) that was placed in front of the gauze opening of cage II to gently suck the air from cages I to II. In this way, attracting volatiles emitted from the palm of the hand of the experimenter (male, 28 years) that was held to the gauze opening in cage I reached cage II and motivated mosquitoes to fly into the tunnel to reach the stimulus source (Fig. 6.3).

In each experiment, a total of 25 *Ae. aegypti* females, preselected for host-seeking behavior, were used to assess the efficacy of different static electric fields generated by voltages of 0.5–4.0 kV (with corresponding field intensities of 0.25–2 kV/cm). In control experiments, no electric field was applied (0.0 kV). Each voltage was tested in four repetitions following the same procedure, i.e., test mosquitoes were allowed to settle and adapt to the test environment for 5 min. In the absence of human odors, mosquitoes were found to remain in cage II and resting on the walls, not attempting to fly into the tunnel. After 5 min, the electric field was generated, the ventilator switched ON and the hand held to the gauze opening. The number of mosquitoes flying through the tunnel and into cage I (with attractive stimulus), hence passing the electrodes, was counted over a time period of 5 min. The electric field was then switched OFF and the number of mosquitoes now entering cage I was documented in the same way for another 5 min. Mosquito flight was also recorded by a GoPro camera (GoPro Hero3+ Black Edition, GoPro Inc., San Mateo, CA, United States), placed above the glass tunnel. Mosquitoes that did not respond to the attractive volatiles and instead remained in cage II were considered inactive. The repelling potential of the generated electrical field was expressed as an attraction reduction to the volatile stimuli emitted by the hand and calculated according to the following formula:

$$\%R = 100 - \left[\left(n_E \div n_0\right) \times 100\right]$$

R = Repellency.

n_E = number of mosquitoes passing the electrical field.

n_0 = number of mosquitoes passing in control tests (no electrical field).

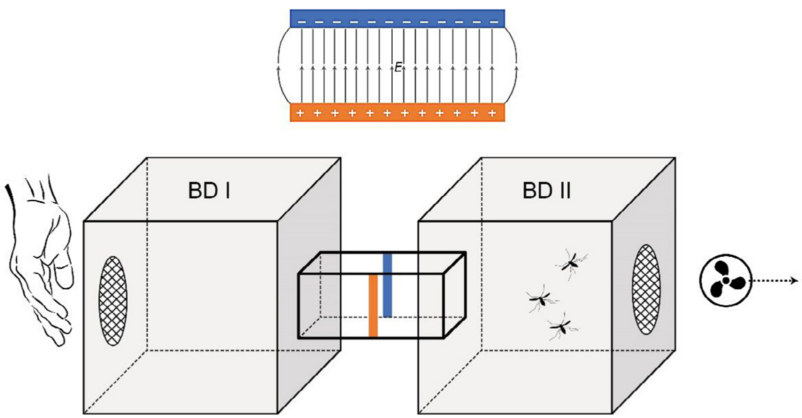

FIG. 6.3 Laboratory cage test set-up. Two BugDorm-1 cages were connected by a glass tunnel that held the plate electrodes. Mosquitoes were released in cage II, the positive stimuli emitted into cage I, by Dr. Ulla Gordon, Biogents AG.

During control experiments, mosquitoes quickly responded to the positive stimuli; an average of 75% entered cage I within the first 5 min. In the second half of the experiment (6–10 min) no further migration was observed in control experiments, thus an average of 25% of the tested mosquitoes was resting in cage II and considered inactive. The weakest electric field-tested had an intensity of 0.25 kV/cm. In these tests, mosquitoes passed the electrodes relatively easily, resulting in an average response rate of 69% during the first 5 min. Once the field was turned off (6–10 min), another 3% oriented toward the attracting stimuli. An incipient repelling effect was observed while testing an electric field with an intensity of 0.5 kV/cm: in these experiments, an average of 43% passed the electrical field (which corresponded to an attraction reduction of 42.7% compared to control experiments). Once the electric field was switched OFF, an additional 34% migrated from cages I to II, leading to an overall response rate of 77%. The repelling potential of electric fields with intensities of 0.75–2.0 kV/cm was noticeably stronger: compared to control tests, the repellency reached 84% (0.75 kV/cm) to 97.3% (2.0 kV/cm). Once the electric field was switched OFF, test mosquitoes were still strongly attracted to the human odors, indicating that the 5 min exposure to the electric field did not induce a behavioral change in *Ae. aegypti´s* response to the host stimuli. Response rates in the second half of each experiment (6–10 min) ranged between 68% and 74%, creating overall response rates (0–10 min) of 70%–80% (Fig. 6.4).

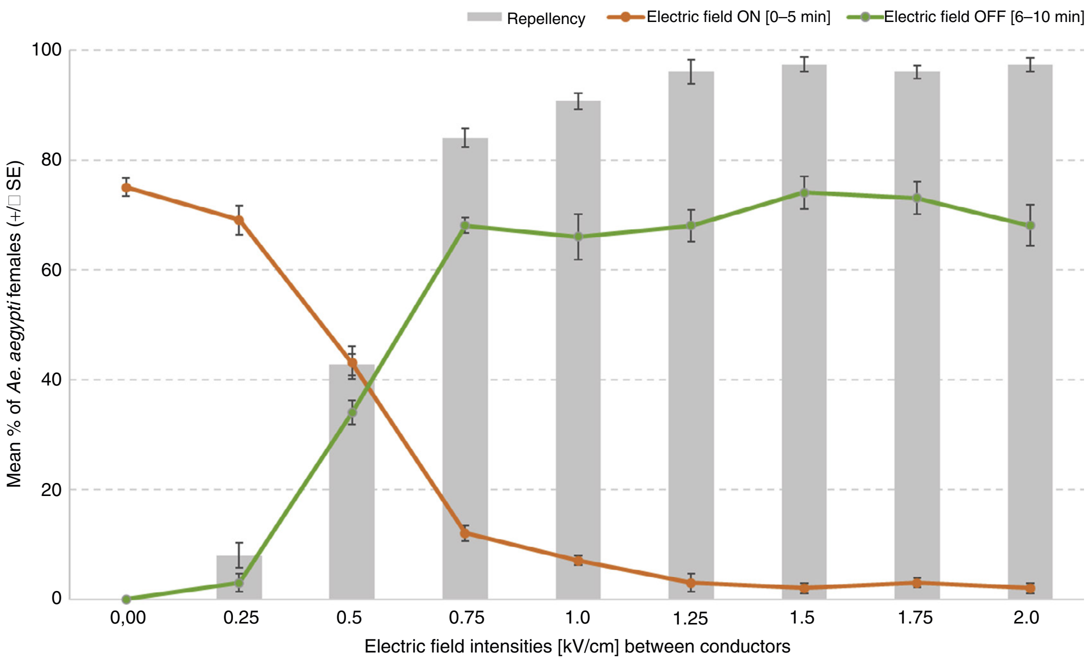

FIG. 6.4 Electric field barrier assays in cage tests. The x-axis shows the tested electric field intensities, the y-axis gives mean percentages of *Ae. aegypti* females passing the plate electrodes (*orange*: electric field ON; *green*: electric field OFF) and mean percentages of repelled individuals (*grey columns*) avoiding the electric field. The standard error (*SE*) is in bars (n = 4).

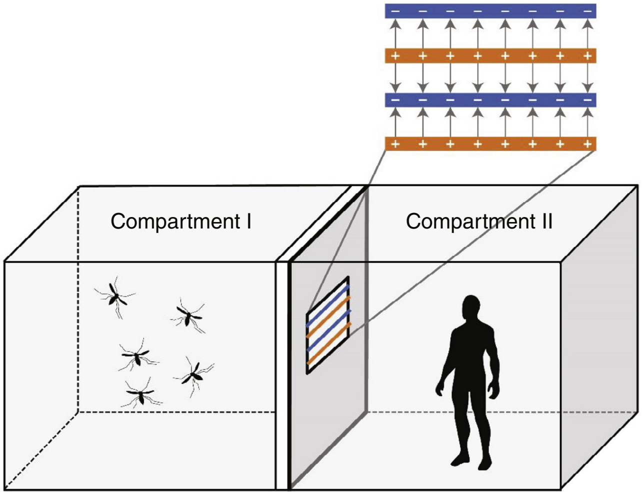

FIG. 6.5 Room test set-up. The two test compartments were separated by a portable wall. An opening in the portable wall was covered by window blinds which were positively and negatively charged. Mosquitoes were released into compartment I while the positive volatile stimuli (human volunteer) were emitted from compartment II, by Dr. Ulla Gordon, Biogents AG.

6.3.3 Barrier assays in room tests

Next, we tested if electric fields could prevent mosquitoes from reaching their host in a more practical set-up, which was tested in Biogents large free-flight rooms. The room is 37 m^3 and was divided into two compartments of 18.5 m^3 by a portable wall. The wall contained a window opening (35 × 35 cm) that allows mosquitoes to move from compartment I to II (Fig. 6.5). Both compartments were set to a temperature of 27.5 ± 0.5 °C and a RH of 75 ± 5%. The light intensity in both compartments was 450 Lux (full spectrum LED light tubes). All experiments were performed on host-seeking *Ae. aegypti* females, as described earlier.

Commercially available aluminum blinds (Jalousie Basic, Bauhaus AG, Regensburg, Germany) were placed in the window opening between the two compartments. Electric fields were created between the slats, which were spaced 2 cm. Slats were connected to an adjustable HV source (Spellman, Model: V6 DC 15 KV POS 2MA W/O RS232) in a way that positively and negatively charged slats were alternating, and voltages of 0.5–3.0 kV (with corresponding field intensities of 0.25–1.5 kV/cm) were tested. In each experiment, 50 host-seeking *Ae. aegypti* females were released into compartment I. In control experiments, no electric field was applied (0.0 kV). Each voltage was tested in five repetitions following the same procedure, i.e., test mosquitoes were allowed to settle and adapt to the test environment for 5 min, afterward the experimenter (male, 28 years) entered compartment II and switched ON the electric field. For 1 h, the number of mosquitoes flying through the window blinds and landing on the experimenter was counted. Mosquitoes that entered compartments II and landed on the experimenter were killed using a commercial electric insect swatter (Basetech eSwatter, from Conrad Electronics, Regensburg, Germany) to avoid counting the same mosquito twice and prevent the mosquitoes from biting the volunteer.

Mosquitoes that were found in compartment I at the end of an experiment were considered inactive. The repelling potential of the generated

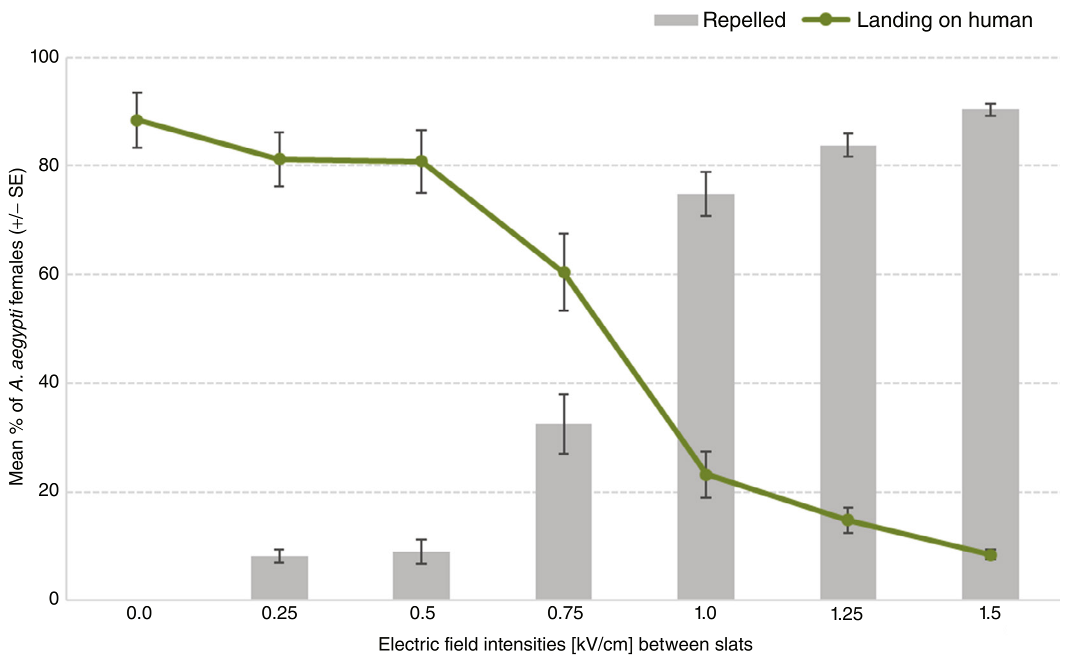

FIG. 6.6 Electric field barrier assays in room tests. The x-axis shows the tested electric field intensities, the y-axis gives mean percentages of *Ae. aegypti* females passing the windows blinds and landing on the volunteer (*green line*) as well as mean percentages of repelled individuals (*grey columns*). The standard error (*SE*) is in bars (n = 5).

electric fields was expressed as an attraction reduction to the volatile stimuli emitted by the volunteer and calculated according to the following formula:

$$\%R = 100 - \left[\left(n_E \div n_0\right) \times 100\right]$$

R = Repellency.

n_E = number of mosquitoes passing the electrical field.

n_0 = number of mosquitoes passing in control tests (no electrical field).

The response rate to the human odors in control tests was high, with an average of 88.4% of the test mosquitoes passing the blinds and landing on the volunteer within 1 h. In contrast to the barrier assays conducted in cages, avoidance behavior in room tests was not noticeable until electric fields with an intensity of 0.75 kV/cm and above were tested. Compared to control tests, the number of *Ae. aegypti* passing the window blinds was reduced by 32.4% and an average of 60.3% landed on the volunteer. At field intensities of 1.0 kV/cm and 1.25 kV/cm, the repellency was prominent. In these tests, the average number of mosquitoes passing through the electrically charged window blinds decreased from 23.2% to 14.8% as voltage increased. This led to an increase in repellency from 74.8% to 84.4% compared to control tests. The strongest effect was observed during tests of electric fields intensities of 1.5 kV/cm. In these tests, human landing rates were reduced by an average of 90.3% (Fig. 6.6).

6.3.4 Assessing the effect of electric fields on *Ae. aegypti* female reproductive rates

Results from initial laboratory cage tests indicated, that the short-term exposure to strong electric fields did not elicit changes in the host-seeking behavior of *Ae. aegypti* females, as once the electric field was switched OFF, test mosquitoes were still highly attracted to the human odors and showed regular flight maneuvers. Could the exposure to electric fields, however, have an impact on reproductive rates? In *Drosophila melanogaster*, the exposure to pulsed electromagnetic fields with an intensity of 4 kV/cm caused a slight increase in reproductive rates compared to control groups (Panagopoulos and Margaritis, 2003). When wheat aphids (*Sitbion avenae*) were exposed to static electric fields at intensities of up to 6 kV/cm, long-term adverse effects on the developmental duration and longevity were observed (He et al., 2014).

Potential effects of exposure to electric fields on the reproductive rates of *Ae. aegypti* were assessed by exposing batches of 20 females to static electric fields with an intensity of 1 kV/cm for 5 min. The procedure was based on the barrier assay presented earlier: 20 female mosquitoes preselected for host-seeking behavior were released into a BugDorm-1 cage that was connected to a second one via a glass tunnel that held four copper electrodes. Mosquitoes were allowed to settle for 5 min, after which the electric field was switched ON and a human palm was held next to the second cage to motivate mosquitoes to approach the copper electrodes. After the exposure, mosquitoes were gently collected from the cages with an aspirator and transferred into 0.5 L incubation cups. Control groups were treated in the same way, with the electric field switched OFF (0 kV/cm). A total of six experiments, each consisting of one exposed and one control batch, were conducted under standardized conditions at 27.5 ± 0.5 °C, 75 ± 5%rH, and a light intensity of 450 Lux (full spectrum LED light tubes). Within 1 h after the exposure, mosquitoes were offered an artificial blood meal using sterile bovine blood (Fiebig Nährstofftechnik GbR, Idstein-Niederauroff, Germany) and Hemotek feeding devices (Hemotek Ltd., Blackburn, United Kingdom). Females were allowed to engorge blood for 30 min, afterwards the feeding device was removed, and the number of blood-fed mosquitoes counted. The following 4 days, mosquitoes were incubated at 27 ± 1 °C and 80 ± 5%rH and had access to sugar water (10% dextrose). The incubation cup also provided an oviposition site, a plastic tube filled with tap water and lined with filter paper. At the conclusion of oviposition (day four after blood-meal), the filter paper with eggs was removed, dried, and stored in a sealed plastic container for at least 6 days. To induce larval hatching, a filter paper with eggs was submerged in a 0.5 glass jar filled with 375 mL of deoxygenized water. Larvae were fed with fish food flakes (TetraMin, Tetra GmbH, Melle, Germany), once they transformed into pupae, jars were placed inside BugDorm-1 cages for adult emergence. After adult emergence was completed, both cages were placed in a freezer at −20 °C for 1 h and adult mosquitoes were counted. The emergence rate was calculated according to the following formula:

$$\mathrm{ER} = n_M \div n_{\mathrm{BF}}$$

ER = Emergence rate.

n_M = number of adult mosquitoes emerged (F1 generation).

n_{BF} = number of blood-fed mosquitoes (F0 generation).

The number of blood-fed individuals in treatment and control groups was comparable after each experiment. In control experiments (0 kV/cm), an average of 16.7 ± 1.3 females had engorged blood while an average of 16.5 ± 1.0 females were found to be blood-fed in treatment groups (1 kV/cm). The number of

TABLE 6.1 Overview of reproductive experiments with *Ae. aegypti* (n = 6).

Experiment	Adults emerged from eggs collected from control group	Adults emerged from eggs collected from treatment group
1	228	260
2	47	73
3	481	517
4	185	157
5	363	54
6	129	248
Σ	1424	1309
Emergence rates (±SE)	14.3 ± 3.5	14.2 ± 4.5

Total number of adults emerged in exposed and control groups is given for each experiment as well as mean emergence rate (±standard error).

adult mosquitoes emerging from eggs collected from control and treatment groups varied between experiments, however, resulting emergence rates were comparable (Table 6.1). These results indicated that a short-term exposure to static electric field intensities of 1kV/cm had no adverse effects on *Ae. aegypti* reproductive rate.

6.4 Practical application of electric fields: an approach

Results from initial laboratory assays showed that strong electric fields with intensities of ≥1 kV/cm can cause an avoidance behavior in mosquitoes, hence preventing vector-host contact. How could the basic set-up used in these experiments be transformed into a practical, application-oriented solution, that considers (1) user safety, (2) cost efficiency, and (3) easy usability?

6.4.1 Development of a high-voltage prototype

Subsequent experiments focused on the development of a prototype device that generates an output voltage of at least 4kV but operates on 12 V DC input (Fig. 6.7). This is achieved through the implementation of a flyback converter, an isolated power converter that uses mutually coupled inductors to store energy when current passes through and releases the energy when power is removed. In a typical application, a switching device such as a transistor is turned on and off to control the direction of energy flow. In the on state, the energy is transferred from the input voltage source to the transformer. The

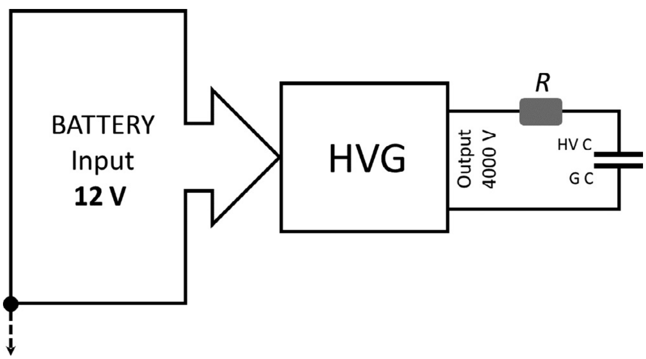

FIG. 6.7 Schematic drawing of the high-voltage (*HV*) prototype. The high-voltage generator (*HVG*) is supplied by 12 V DC power from a battery and generates an output of 4000 V. The voltage is used to create an electric field between the HV and grounded (*G*) conductor (*C*). The resistor (*R*) makes the system safe to the touch.

diode in the second winding is reverse-biased, thus current does not flow and instead the energy is stored in the transformer until a switching device, e.g., a metal-oxide-semiconductor-field-effect transistor, is turned off. Now, the stored energy produces a current that is forward biasing the diode which results in the production of an HV DC output. For safety and short circuit protection, a large resistor of ≥1 mega-ohm was integrated with a series of the output. According to Ohm´s law, the electric current I (A) is the quotient of the voltage (V) across a conductor and the resistance R (Ω) of the conductor. With an output voltage of 4 kV and a resistor of 22 mega-ohm, the electric current through the conductor is 0.1 mA. Currents below 10 mA are considered safe for humans, causing only mild sensations upon touch while 16 mA is the maximum current "an average man can grasp and let go" (Fish and Geddes, 2009).

The developed HV prototype can be supplied with 12 V DC power from a variety of power sources, including primary and secondary batteries (Lead Acid, Lithium-ion), plug-in power supplies using AC to DC converters or solar panels. Important factors that impact the decision on a specific power source are longevity and associated costs for both, acquisition and operating. So far, the technical development was based on the application of continuous electric fields. The implementation of pulsed electric fields could, however, be interesting as pulses offer certain advantages: reduction of operating costs, extension of battery life, and counteracting potential behavioral adaptations in exposed mosquitoes. Follow-up experiments therefore investigated whether pulsed electric fields still caused an avoidance behavior in *Ae. aegpyti*.

6.4.2 Repellency of pulsed electric fields

Laboratory barrier assays in cage tests were performed according to the protocol described earlier including the following modification: the HV device was connected to a function generator through a controller. The function generator produced pulses of desired shape, frequency, and duty cycle, and the controller sent these low voltage pulses to the input of the HV device. The HV device increased their amplitude to convert them into HV pulses (≈3.5 kV) that were monitored on a laptop screen using the software PC LAB 2000LT (Velleman Instruments, Gavere, Belgium). Through output terminals of the HV device, pulse durations of 0.25, 0.5, 1, 2, and 3 s with varying duty cycles (between 0% and 90%) were applied on the copper plate electrodes. A duty cycle of 10% means that the electric field was on for 10% and off for 90% of the pulse duration, i.e., it was on for 0.025 s and off for 0.225 s in trials with a pulse duration of 0.25 s. Each combination of pulse duration and duty cycle was tested in three repetitions with 25 host-seeking *Ae. aegypti* females per experiment.

At duty cycles of 60%–90%, repellency was high in all trials: an average of 84% (pulse duration 3.0 s) to 98% (pulse duration 0.25 s) of the mosquitoes did not pass the plate electrodes. At shorter duty cycles, between 10% and 50%, the repellency was less prominent in longer pulse duration experiments (1.0–3.0 s) compared to shorter pulse durations (0.5 and 0.25 s). When duty cycles were 10%, repellency reached an average of 38% and 29% at pulse durations of 2.0 and 3.0 s, respectively, while it was higher than 94% at pulse durations of 0.25 and 0.5 s. The response rate in control trials (duty cycle 0%, electric field off) was high with an average of 76.9% ± 1.59 of the test mosquitoes responding to the positive stimuli in the absence of electric fields (Fig. 6.8).

Results indicate that pulsed electric fields can repel *Ae. aegypti*, but the efficacy depends on the pulse duration and duty cycle. Best effects were obtained when pulse durations of 0.5 and 0.25 s were used, longer pulse periods (≥1 s) required duty cycles of at least 60 to reach 80% repellency.

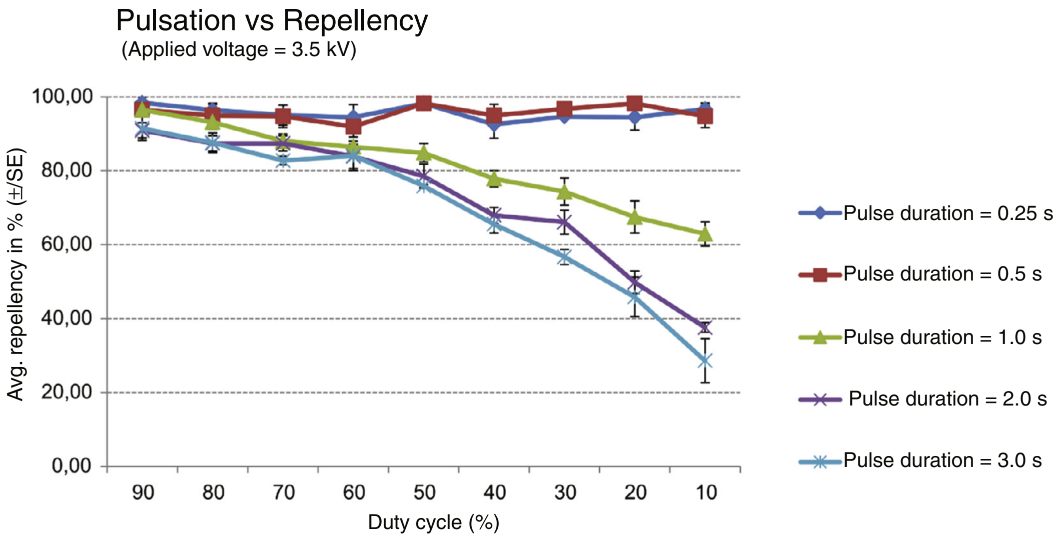

FIG. 6.8 Pulsation experiments in barrier assays with *Ae. aegypti* ($n = 3$). The x-axis shows the tested duty cycles, the y-axis gives mean percentages of test mosquitoes avoiding the electric field. The standard error (*SE*) is in bars.

6.4.3 Cost comparison and envisioned design

How do the previous findings relate to, e.g., battery life? The HV prototype has a maximum current consumption of 65 mA, this means that a battery with a capacity of 12 Ah would be discharged to 50% after approximately 4 days if continuous electric fields are applied.

Battery life is indirectly proportional to the duty cycle; thus, it increases with shorter pulse "on" periods. At duty cycles of 90%, battery life reached approximately 9 days but could be extended to 77 days using duty cycles of 10% (data not shown). In terms of longevity and cost-effectiveness, other power supply sources would also benefit from pulsation. Table 6.2 compares the estimated longevity and associated costs for

TABLE 6.2 Estimated longevity and purchase costs for different 12 V DC power supply sources for the high-voltage prototype.

Type	Purchase costs (US$)[a]	Continuous electric field (24 hours)		50% pulsed electric field (24 hours)	
		Longevity[b]	US$/day	Longevity[b]	US$/day
Batteries 8 × 1.5 V (AA)	7–12	1–2 days	3.5–12	3–4 days	1.75–4
Rechargeable batteries 12 V (Lead-Acid)	20–30	10–15 days	1.33–3	25–30 days	0.67–0.86
Solar power	75–100	15–20 years	0.01–0.02	15–20 years	0.01–0.02
Plug-in power supply (AC to DC transformer)	10–12	1–2 years	0.01–0.03	2–3 years	0.009–0.002

[a] *Estimated costs based on an internet search (https://www.amazon.com) conducted on October 20, 2020.*
[b] *The actual longevity depends on the size of the electrodes (e.g., window blinds).*

eligible power sources when used to generate continuous or pulsed electric fields.

While nonrechargeable batteries and solar panels do not create any additional costs after purchase, running costs for the plug-in solution still need to be considered and depend on the electricity costs at the operation site. When used continuously, the HV prototype device has a 24 h power consumption of 19 W. According to the US Energy Information Administration, the average price for electricity in the United States was 13.26 cents per kWh in July 2020 (https://www.eia.gov/electricity/monthly/epm_table_grapher.php?t=epmt_5_6_a, accessed October 20, 2020) in residential areas; thus, monthly running costs would reach approximately 7.8 cents under continuous use.

Based on the presented findings, an electric field should have an intensity ≥1 kV/cm to elicit avoidance behavior in mosquitoes and it can be applied continuously or pulsed. Potential applications of electric fields as barriers in a home setting could involve charged metal blinds to cover doors and/or windows to prevent mosquitoes from entering (Fig. 6.9). In an outdoor seated space, an electrically charged fence could create a comparable effect (Fig. 6.10). This new method of repelling or preventing mosquitoes from passing through a defined opening, that is largely permeable to ambient air, by the means of an electric field generated by at least two electrodes has been patented in 2017 (Rose et al., 2017) (European Patent Number 17208300.8).

6.5 Discussion

Our idea to use electric fields to control insect pests is not entirely new but had different objectives. Extensive research on the effects of high strength radio-frequency electric fields on stored grain insects started in the 1960s aiming at either killing or sterilizing the target pest (Ponomaryova et al., 2008). More recent research suggests high voltage electric field screens can be used as an air-shielding apparatus to capture airborne

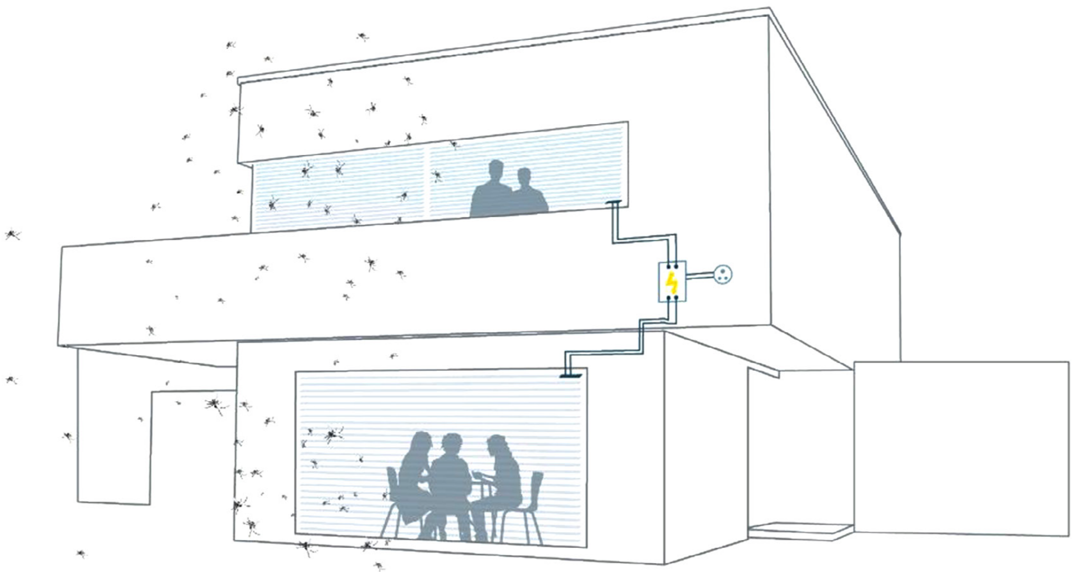

FIG. 6.9 Potential application of electric fields as barriers in a home setting. By Christian Müller, Biogents AG.

FIG. 6.10 Potential application of electric fields as barriers in an outdoor setting. By Christian Müller, Biogents AG.

spores, fungi, and flying greenhouse pests to reduce the use of fungicides and insecticides (Kusakari et al., 2020). A similar application using oppositely charged electric field screens was successful in capturing and trapping *D. melanogaster* and could be implemented in greenhouses or food storage facilities to exclude insect pests (Matsuda et al., 2012). In 2015, the same group presented electric field screens charged by ≥1.2 kV as physical barriers and a means to capture *Ae. albopictus* and *Cx. pipiens* before entering the house (Matsuda et al., 2015).

Our studies investigating the repelling effects of static electric fields on mosquitoes is novel. Our laboratory experiments support the hypothesis that *Ae. aegypti* is able to sense and avoid electric fields at intensities of 1 kV/cm and above. Emphasis was placed on comparing the effects of multiple voltages in laboratory tests. Due to the limited duration of the project, the number of replications in both, cage and room tests is too low for statistical analysis. However, standard errors in all experimental trials were small, indicating low variance within the data sets and allowing us to draw conclusions on the potential of electric fields to repel mosquitoes. The short-term exposure to electric field intensities of 1 kV/cm also did not negatively or positively impact *Ae. aegypti* reproductive rates, the total number of adults emerged from eggs laid by exposed and unexposed females was comparable. Forcing test batches into the electric field, as described by Panagopoulos and Margaritis (2003) in tests with *D. melanogaster*, might have resulted in a different outcome but in our opinion, the bioassay should allow mosquitoes to navigate in close proximity and respond to the electric field just as they would in a realistic setting. The promising outcome of initial laboratory tests needs to be verified, more research needs to be conducted involving different mosquito species, other arthropods of medical importance or food pests and the set-up has to be evaluated under realistic conditions in the field. These studies are currently being prepared to be conducted in Germany and the United States.

A potential benchmark to evaluate the efficacy of our system could be spatial arthropod repellents, chemicals that deter mosquitoes at a distance and inhibit their ability to locate a host (Gouck et al., 1967; Nolen et al., 2002), thereby reducing host- vector contacts. Spatial arthropod repellents are considered effective if they provide a minimum landing inhibition/reduction of 90% in semi-field or field trials (World Health Organization, 2013). In our room tests, such a landing inhibition could be achieved by electric field intensities of 1.5 kV/cm. Mosquitoes that did not pass the charged window blinds but remained in compartment I were still attracted to human odors at the end of a test. Thus, exposure to the electric field did not necessarily alter their host-seeking behavior. This observation is highly interesting and turns electric fields into a potential tool for push–pull strategies. Push–pull combines deterring and attracting stimuli to change the abundance of an insect pest and has been successfully implemented in crop pest management (Cook et al., 2007; Pyke et al., 1987). In mosquito control, suggested push–pull approaches involved the use of spatial repellents such as pyrethroids, transfluthrin, allethrin, or metofluthrin (Kitau et al., 2010; Mmbando et al., 2017; Wagman et al., 2015), catnip, *Nepeta cataria* (Menger et al., 2015; Obermayr et al., 2015; Paz-Soldan et al., 2011), or delta-undecalactone (Menger et al., 2015; Obermayr et al., 2015; Paz-Soldan et al., 2011) as push components in combination with attractive suction traps like the BG Sentinel (Salazar et al., 2012). The use of sublethal doses of volatile pyrethroids usually leads to a significant reduction in human vector contacts (Darbro et al., 2017; Ogoma et al., 2012). However, the neurotoxic action of these compounds might interfere with mosquito host-seeking behavior, therefore rendering the pull-component ineffective (Kitau et al., 2010; Salazar et al., 2013). Non-neurotoxic compounds like catnip on the other hand are less effective in reducing human landing rates when applied in a field setting (Obermayr et al., 2015).

Electric fields represent a physical barrier and provide certain benefits compared to chemical spatial repellents. They also have a high potential in repelling mosquitoes, are odorless, their efficacy does not fade over time, not likely to lead to behavioral adaptation (especially when electric fields are delivered in pulses), and depending on the power source, they are very cost-effective. The developed HV prototype can run on a variety of power sources and the most suitable one will be defined by location/area of application (availability of power supplies, area of coverage), the operating time per day (which will be linked to mosquito biting activity patterns) and personal preferences. While batteries offer a greater portability of the device they have to be recharged or replaced on a regular basis; solar panels represent the most sustainable and environmentally friendly solution but come at greater upfront costs, whereas the plug-in solution is cost-saving but immobile. Regarding safety, the safety resistor in the HV prototype limits the flow of current through the human body to 4 mA in case of accidental touching.

In a potential application, houses would be equipped with charged blinds in windows and even doors to prevent mosquitoes from entering. In this way, human-vector contacts are reduced while indoor spaces remain properly ventilated. In order to reduce the vector population, the system should be combined with attractive traps, e.g., the BG Sentinel, to lure and catch females that are deterred by the electric field. Such a set-up resembles a promising novel push–pull approach for the control of vector mosquitoes.

6.6 Conclusion

In this study, we investigated the repellent potential of strong electric fields on the yellow-fever mosquito, *Ae. aegypti*. In laboratory behavioral assays, host-seeking females had to pass an electric field in order to reach an attractive source (human odors). At field intensities of ≥1.5kV/cm, the response rate to the attractive odors was reduced by at least 90% in both, cage- and room-tests. Once the electric field was switched OFF, females showed regular host-seeking behavior indicating that the exposure to the electric field did not induce any short-term behavioral changes. In contrast to chemical repellents, electric fields are odorless and their efficacy does not fade over time, thus they could be an interesting tool for novel mosquito control approaches, such as Push–Pull. Future research needs to focus on the applicability of such a system in a realistic setting and investigate whether the promising repelling effects observed in the laboratory will persist in the field.

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