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Killing of Flies in Electrocuting Insect Traps Releases Bacteria and Viruses

James E. Urban,[1] A. Broce[2]

[1]Division of Biology, Ackert Hall, Kansas State University, Manhattan, KS 66506, USA
[2]Department of Entomology, Waters Hall, Kansas State University, Manhattan, KS 66506, USA

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Abstract. Electrocuting insect traps (EIT) are popular devices frequently used by homeowners and food handlers attempting to localize the control of flying insects, including the ubiquitous house fly (*Musca domestica* L.). The traps contain a visual attractant and a high-voltage metal grid. Upon contact with the grids, the insects are disintegrated by the high voltage. As part of a systematic evaluation of EITs and their role in infectious disease spread, we quantitated spread of bacteria and a bacterial virus during electrocution of house flies. We loaded flies with *Serratia marcescens* or with the *Escherichia coli* phage ΦX174 and placed sprayed or fed flies into a room containing an EIT. While flies were being electrocuted, liberated particles and bacteria were assayed via agar plates or via air filtration samplers. Sprayed flies released one of every 10,000 of the added bacteria or viruses, and fed flies released one of every 1,000,000 of the consumed bacteria or viruses. Results of our studies suggest EITs could play a role in the spread of infectious disease agents, but the potential is influenced by the insect's route of contamination.

Insect light traps have been used extensively since the middle of the last century for research and surveillance in disease prevention and control of indoor and outdoor insects in homes, agricultural and industrial operations, and hospitals. Over the years, the type of light source has varied from incandescent light bulbs to fluorescent lamps [7]. The most common method for arresting/eliminating insects that are attracted to the light sources has been the use of electrocuting grids. Grids are energized by 2,500–4,500 V (low current of 8–10 mA), and these electrocuting insect traps (EIT), commonly referred to as bug zappers, produce the characteristic crackling, zapping, and sputtering sounds as the insects are killed in the electrocuting grid.

The use of EITs around the home environment has been mainly for controlling bloodsucking and annoying insects (primarily dipterans), especially mosquitoes and gnats; yet it has been shown that these devices are ineffective in reducing these types of pest insects [9]. In fact, it is known that the probability of being bitten by mosquitoes increases in the vicinity of these traps [6]. Frick and Tallamy [5] found that the proportion of the insects killed by EITs in home backyards that were biting insects was minute (0.22%) and that at least 13% of the non-target insects killed were insect predators and parasites.

A major objection to the operation of EITs in premises where food is handled is the production of insect parts when insects are disintegrated by the high voltage [8]. Ananth et al. [1] demonstrated that the operation of EITs in killing house flies (*Musca domestica* L.) significantly increased the number of airborne (respirable) particles; however, they did not identify the source of these particles. Broce [2] investigated the production of airborne particles from flies and moths being killed by EITs by collecting these particles on microporous filters. Filters with the collected particles were then observed under a scanning electron microscope. Although numerous airborne insect particles, such as hairs and scales, were collected when the insects were released in the test rooms while the traps were off, the number of smaller-scale fragments significantly increased when the traps were used. He also found that the airborne particles consisted of elements from the insect bodies, but also

Correspondence to: J.E. Urban; *e-mail:* urban@ksu.edu

contained metals (Zn, Fe, and Cu) from the electrified grids. More recently, Tesch and Goodman [10] showed that microorganisms from the naturally occurring flora of house flies killed by EITs can be disseminated in large pieces of insect parts (which settle within 45 min) and in aerosolized (truly airborne) particles.

To better understand the fate of insect-borne microorganisms when insects are being attracted to and killed by EITs, we contaminated house flies with bacteria or bacterial viruses and determined the fate of each type of microorganism as flies were killed in a commercial trap. Preliminary aspects of the study have been presented [3, 4].

Materials and Methods

Test organisms. Our insect hosts were laboratory-raised populations of house flies (*Musca domestica* L.). The house fly was used because of its endophylic (habitually frequents indoor areas), synanthropic (associates with human activities) behavior and its reputation as a carrier of pathogens of humans and animals [11]. House flies were from a strain maintained for over 22 years in the Department of Entomology, Kansas State University. Rearing procedures are described elsewhere [2].

Our bacterial indicator was the Gram-negative bacterium *Serratia marcescens*, and our indicator virus was the *Escherichia coli* bacteriophage ΦX174. *S. marcescens* was considered typical of enteric bacteria with which filth flies might come into contact, and ΦX174 was chosen because it has a size and shape that is typical of the enteric human poliovirus.

Insect infection procedures. *Fed flies*: To conduct experiments with house flies that had been internally contaminated with bacteria or phages, 2- or 3-day-old flies were starved for 18–24 h and then were allowed to feed for 30 min on a 5% aqueous sucrose solution containing sodium fluorescein (as a dye) and 2.0×10^8 *S. marcescens*/ml or 2.0×10^9 ΦX174 viruses/ml. The fed flies were cooled, placed on a refrigerated tray under a black light (long wavelength UV light). Then, 210 phage-fed flies or 410 bacteria-fed flies whose abdomens fluoresced under the UV source were selected and sorted according to sex. The flies were allowed to recover from cooling for 30 min.

Five male flies and 5 female flies were individually crushed in 0.1% peptone dilution fluid (Bacto-Peptone, Difco, Detroit, MI), and the individual flies were used to quantitate bacterial or phage loads/fly. Bacterial loads were quantitated by spreading dilutions of the peptone dilution fluid to plate count agar; virus loads were quantitated by adding dilutions to soft agar overlays. Equal numbers of male and female flies were used for EIT assays to be described below. Male and female flies consumed averages of 1.7 μl and 4.6 μl of solution, respectively. Female flies usually consume more than males and feed more often than males, especially on protein sources, in order to develop their eggs. The average bacterial load in fed flies was 1.4×10^6 bacteria/fly, and the average phage load was 5.7×10^6 phages/fly.

Sprayed flies: Two hundred and five (in phage assays) or 405 (in bacterial assays) cold-anesthetized, 2- or 3-day-old flies were collected without regard to fly sex, and the flies were allowed to recover in a screened container. Using an atomizer, the confined flies were sprayed with about 2 ml of 0.1% peptone dilution fluid containing 2.0×10^8 *S. marcescens*/ml or 2.0×10^9 ΦX174 phage/ml. That level of spraying caused the flies to become damp, so the sprayed flies were allowed to dry and groom themselves for 30 min. Five flies from each lot were selected and washed in 0.1% peptone for 30 s. Bacterial loads were quantitated by spreading dilutions to plate count agar, and phage loads were quantitated by adding dilutions to soft agar overlays. The remaining flies were used for EIT assays to be described below. Sprayed flies harbored an average of 1.5×10^6 bacteria/fly, and 7.5×10^5 phages/fly.

Insect electrocution procedures. Contaminated flies were released into a windowless room containing an inactive electrocutor trap [the trap used (Insect-O-Cutor 6192DGA, Stone Mountain, GA) was chosen by lot from various traps available]. The room was 2.6 m × 2.1 m on each side and 2.4 m high. The trap was positioned in the center of one 2.6-m wall at a height of 1.37 m from the floor. Microporous air filters of 0.5-μm pore size (Millipore Filter Corp., Bedford, MA) were placed 1.52 and 0.61 m above the floor at a distance of 10 cm in front of the trap. Each filter was connected by metal tubing to an air sampling pump (model MRV-14C, HI-Q Environmental Products Co., La Jolla, CA) and collected particles for 10 min, at an air sampling rate of 37.75–42.50 L/min. Filter surfaces were protected from actual contact with flies by being covered with conventional screen wire (screen size = 7/cm). Open 10-cm Petri plates containing Plate Count Agar (Difco, Detroit, MI) or Nutrient Agar (Difco) were placed on the floor on a line that was perpendicular to the trap and in the center of the room. Petri dishes were positioned immediately under the EIT and at distances of 0.46, 0.91, 1.37, and 1.83 m from the trap. Single plates were used for assays of phage spread, and duplicate plates were used in assays of bacterial spread. Petri plates were exposed for the 10-min period just before the grid was electrified, and for 10-min periods at intervals after the trap was activated.

Experiments measuring virus spread were done with open Petri dishes, and in experiments measuring bacterial spread, dishes were covered with a hardware cloth screen (screen size = 1.6/cm) to prevent flies from contacting the plate surface.

Bacteria trapped on Petri dish surfaces were allowed to grow and to form colonies. Microporous membranes were placed on plate count agar surfaces, and cells on the membranes were allowed to grow and to form colonies. Bacteriophages trapped on Petri dish surfaces or impinged onto microporous membrane surfaces were revealed by overlaying the plate or the membrane with soft agar containing *E. coli* indicator and allowing plaques to form.

At the end of the electrocution period (40 min after activation of the EIT), five dead flies were collected. The dead flies were crushed and diluted in 0.1% peptone dilution fluid, and dilutions were plated and quantitated for bacteria or phage as described above.

Results and Discussion

Each virus experiment was replicated twice, whereas each bacterial test was replicated four times. The data were characterized by a high degree of variability among replicates, even though extreme care was exercised in using flies of known chronological and physiological ages, and in duplicating the experimental procedures. For instance, we observed that flies older than 3 days (as indicated in the Materials and Methods, we used 2- to 3-day-old flies) tended to have larger numbers of normal flora bacteria and, when such normal flora were present, the contaminant load that could be introduced on (or placed onto) their surfaces was proportionately smaller. In addition, flies tended to be highly variable with regard to how eagerly or how much they fed, or how readily or

Table 1. Dissemination patterns of bacteria and bacteriophages during fly electrocution

	Flies fed[a] with *Serratia marcescens*	Flies sprayed[b] with *Serratia marcescens*	Flies fed[a] with ΦX174 bacteriophage	Flies sprayed[b] with ΦX174 bacteriophage
1—Empty room	0[c]	0	0	0
2—10–0 min prior to EIT activation	2	38	2	181
3—0–10 min after EIT activation	16	79	11	218
4—10–20 min after EIT activation	6	159		
5—30–40 min after EIT activation	8	87	3	108
6—Air filters 0–10 min after EIT activation			1[d]	207
7—Air filters 30–40 min after EIT activation			2	112
8—Average no. of organisms/fly at release into room	4.8×10^6	1.6×10^4	4.9×10^6	1.0×10^6
9—Average no. of organisms/electrocuted fly[e]	4.2×10^6	6.8×10^3	3.3×10^6	
10--% of microorganisms/fly surviving fly electrocution	89	42	65	

[a] Flies which had been starved for 18–24 h were fed a sucrose solution containing *Serratia marcescens* bacteria or ΦX174 bacteriophage and fluorescein dye. Flies with fluorescent abdomens were collected, and 200 bacteriophage- or 400 bacteria-contaminated flies were released into a room with an EIT. Average contamination levels/fly at the time of release into the room containing the EIT are expressed in row 8 for each experiment.

[b] Flies were sprayed with a 0.1% peptone solution containing *Serratia marcescens* bacteria or ΦX174 bacteriophage. Flies were allowed to dry and groom for 30 min, then 200 bacteriophage- or 400 bacteria-contaminated flies were released into a room with an EIT. Average contamination levels/fly at the time of release into the room containing the EIT are expressed in row 8 for each experiment.

[c] The numerical value is the total number of microorganisms found on all Petri plates in the room with the EIT during the described sampling interval.

[d] The numerical value is the total number of microorganisms found trapped in the air samplers in the room with the EIT during the described sampling interval.

[e] Five dead flies were collected 40 min after activation of the EIT, and the flies were crushed and diluted in 0.1% peptone dilution fluid, and dilutions were plated and quantitated for bacteria or phage.

robustly they groomed after being sprayed with the solutions containing bacteria or phage. Also, it was impossible to predict how active flies would be when released into the room containing the trap, or how well flies would respond to the light attractant in the trap. Whatever the cause, fly loads between individual experiments varied considerably, and more or fewer flies were attracted to the trap in individual experiments; therefore, plate count and air filtration count values could vary by 1–2 logs from experiment to experiment. Dissemination patterns between experiments and within an individual experiment were consistent for the experiment type, but combining data of replicates was difficult, as was any statistical analysis.

The pattern of microorganism dissemination when flies were electrocuted is presented in Table 1, which shows the results of four individual experiments rather than averages of experiment replicates.

Examination of the data in Table 1 shows that bacteria and phages were detected on Petri dishes in the experimental chamber even before the trap was turned on. Far more microorganisms were released from surface-contaminated flies than from fed flies. Broce [2] showed that numerous airborne insect particles, such as hairs and scales, could be collected when insects were released into the test rooms while the traps were off, and we presume shedding is in part responsible for the microorganisms found in the samples taken during the 10 min before the trap was turned on. The shear action of wing beating could account for the production of some of the airborne particles and the release of some bacteria and phages prior to activation of the trap. Also, the experiments conducted with phage-contaminated flies were not done with wire grid screens covering the Petri plate, and some flies were observed walking on the plate surfaces. With internally contaminated flies, such walking did not materially affect microorganism numbers during that sample period, but with surface-contaminated flies actual contact of flies with the plate surface could clearly increase the number of phages detected.

Regardless of whether microorganisms came from shedding of fly surface appendages or from actual fly contact with the plate surface, the number of microorganisms detected on Petri plates increased after the trap was turned on and fly electrocution began. As was mentioned above, fly behavior from experiment to experiment was highly variable, but generally most flies were electrocuted during the first 10–15 min after the trap was turned on. Correspondingly, most microorganisms were detected during the sampling period or periods after the trap was turned on. However, in all experiments microorganisms were still detected during the 30- to 40-min

sampling period, suggesting that microorganisms introduced into the air by electrocution tended to require time to settle out of the air column.

Most microorganisms were detected in the Petri dishes immediately under the trap, and the dishes 0.46 m from the trap had the next highest number of bacteria or phages. All of the remaining dishes were contaminated with microorganisms in all experiments, but there was no longer a correlation between distance from the trap and the number of microorganisms detected.

A finding that we found surprising was that so many bacteria and phages survived in or on the corpses of electrocuted flies. As was the case with dissemination into the air, surface and internal contamination produced differences in survival of microorganism in killed flies. Bacteria on the fly's surface were more readily spread during electrocution, but either because the organisms were ejected from the fly surface or because they were more efficiently killed, fewer surface microorganisms were found in killed flies (about 40% of the original number) than were found in internally contaminated flies (about 75% of the original number). These differences could be explained if the electrical current were to travel along the fly exoskeleton rather than internally.

Our results clearly show that bacteria and viruses of the type likely to be found in human and animal excrement may be spread during the electrocution of contaminated house flies in an EIT. They agree with earlier findings by Tesch and Goodman [10] that EITs can be blamed for disseminating naturally occurring bacteria in house flies. Our study shows that the spread of microorganisms into the air by an EIT is greatest when flies are surface contaminated, but even internally contaminated flies can release some bacteria or phages. While internally contaminated flies are less likely to spread microorganisms into the air upon electrocution, the apparently relative protection afforded by the flies' alimentary tract apparently allows most of the bacteria or phages within a fly to survive when the fly is killed by electrocution. Because each live fly can carry more than a million organisms, the survival rates we measured mean that each dead fly is potentially nearly as contaminated as it was when it was alive.

We wish to emphasize that our study is not intended to argue against all uses of EITs, as these devices may have a valuable role in the control of pests in special situations, such as in warehouses, but rather that a potential for microorganism spread exists when EITs are used. Correspondingly, one should be careful when the devices are used in certain situations such as near open food, in areas of hospitals that must remain aseptic, and in certain other places such as day care centers or where infants and toddlers play.

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