



Article

A Simple and Safe Electrostatic Method for Managing Houseflies Emerging from Underground Pupae

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Abstract: A simple electrostatic apparatus that generates an arc discharge was devised to control adult houseflies emerging from a soil bed in a greenhouse. Adult houseflies emerging from a soil bed in a greenhouse are a potential vector of pathogenic *Escherichia coli* O157, carried by animal manure used for soil fertilization. A simple electrostatic apparatus that generates an arc discharge was devised to control these houseflies. The apparatus consisted of two identical metal nets; one was linked to a negative-voltage generator to create a negatively charged metal net (NC-MN), and the other was linked to a grounded line to create a grounded metal net (G-MN). A square insulator frame was placed between the two nets, separating them by 6 mm, and a plastic grating with multiple cells was placed beneath the G-MN to provide a climbing path (54 mm in height) to the arcing sites of the apparatus for adult houseflies emerging on the soil surface. Houseflies that climbed up the wall of the grating and reached the arcing zone were subjected to arc-discharge exposure from the NC-MN and thrown down onto the soil by the impact of the arcing. The impact was destructive enough to kill the houseflies. The structure of this apparatus is very safe and simple, enabling ordinary greenhouse workers to fabricate or improve it according to their own requirements. This study developed a simple and safe tool that provides a physical method to manage houseflies.

Keywords: arc-discharge exposer; electric field; expanded metal net; housefly; organic farming; pesticide-independent method; physical control; plastic grating



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1. Introduction

There is an increasing public concern regarding the use of chemicals for the management of all classes of pests (pathogens, insects, and weeds). Additionally, there is a serious risk of pesticide resistance developing in a wide range of weed species [1,2], pathogens [3,4], and insect pests [5,6]. This has led to the development of the organic farming of tomatoes in greenhouses. In organic farming, the introduction of food-waste compost, or green and animal manure, into soil beds in a greenhouse is a routine approach to for soil fertilization. Cattle manure is the major organic fertilizer in our greenhouse cultivation, and is typically applied once or twice each year. Unfortunately, the cattle manure often contains the larvae and pupae of the housefly *Musca domestica* (Linnaeus) (Diptera: Muscidae), resulting in the frequent emergence of adult houseflies from underground pupae during plant cultivation, because no synthetic insecticides are used in organic greenhouses.

The housefly problem presents a risk of transmitting pathogenic *Escherichia coli* O157 [7,8] posing a potential risk to public health. Food poisoning caused by *E. coli* O157 frequently occurs in people who have eaten fresh food contaminated by this pathogen. *E. coli* O157 in the intestines of cattle and sheep, where they do not cause disease, can spread to the human food chain through feces from these animals [8,9]. Housefly larvae develop in animal feces and very large populations accumulate, both on cattle farms and in other agricultural

facilities [7]. *E. coli* O157 ingested by houseflies remain viable in fly excreta; consequently, houseflies are able to carry and disseminate *E. coli* for several days [7]. Importantly, this bacterial pathogen is transferred from cattle manure used for soil fertilization [10]. Contamination of cultivated and postharvest crops with this pathogen is a serious problem that can endanger the food supply chain [11–13]. Insecticide substitution is therefore essential to control houseflies emerging from soil beds before they come into contact with crops in a greenhouse. The most basic and conventional method is to cover the soil surface with a weeding mulch film [14,15]. Unfortunately, mulch application is unsuitable for plant cultivation in the summer because of the undesirable increase in soil temperature. We therefore focused on electrostatic methods to manage houseflies at the soil bed surface.

Nononura et al. [16] devised an electrostatic soil cover to capture adult tomato leaf miner flies (syn. vegetable leaf miner), *Liriomyza sativae* Blanchard (Diptera: Agromyzidae), which emerged from underground pupae. This apparatus consisted of two sets of iron rods welded onto an iron frame. The iron rods and frame of one set of rods were coated with a soft polyvinyl chloride resin and linked to a negative-voltage generator, while the iron rods of the other set were not insulated and linked to a grounded line. Both sets of iron rods were arranged in an offset configuration to produce static electric fields between the oppositely charged iron rods. The charged insulated conductor wires of the apparatus exerted a strong force to capture the leaf miner adults that entered the electric field. However, the force of this apparatus was insufficient to capture larger insects such as adult houseflies.

Another electrostatic approach was the development of an arc (spark) discharge-exposing apparatus. The arc-discharge exposer (ADE) was originally devised to eradicate warehouse pests, such as rice weevil, *Sitophilus oryzae* (Linnaeus) (Coleoptera: Curculionidae), nesting in dried postharvest products [17,18], and Kakutani et al. [19] applied it to a pigsty window to kill the mosquitoes, *Culex tritaeniorhynchus* Giles (Diptera: Culicidae), that transmit Japanese encephalitis between pigs and humans. Matsuda et al. [20] utilized the arc discharge-exposing technique to simultaneously control weeds and houseflies emerging from the soil in a greenhouse. The proposed device consisted of two-storied ADEs. In each layer, identical iron plates were placed in parallel at a defined interval and fixed in an iron frame. Two layers of fixed iron plates were used, one (lower floor of the two-storied apparatus) for weed control and the other (upper) for fly control. For weed control, all of the iron plates were negatively charged, and the negative charges that accumulated on the plates were released to weed shoots through an arc discharge when the shoots entered the first floor. Houseflies were introduced into the space between the negatively charged and grounded plates on the second floor, and then subjected to an arc discharge from the charged plates. Both plant shoots and adult houseflies are electrically conductive; thus, they were killed by discharge exposure in the electric field between the charged iron plate and the ground soil, and between the charged and grounded plates. However, the complex configuration of this method discourages ordinary greenhouse workers from personally setting it up. The objective of this study was to propose a simple ADE that could be easily established by greenhouse workers.

Arcing is an electrical phenomenon caused by a high-voltage negative charge moving in the air from the charged conductor to the ground via grounded conductors [21]. The conductor is negatively charged by linking it to a negative-voltage generator. A negative charge accumulates on the surface of the conductor and positively polarizes the grounded conductor facing the charged conductor at a specified distance as a result of electrostatic induction [22]. An electric field is generated in the space between two metal nets [23]. The intensity of the arcing is determined by the voltage applied to the conductor and the distance between the opposite poles (charged and grounded conductors); larger voltages and shorter distances generate stronger arcing [21]. The stronger arcing can kill insect pests [17–20] and weeds [20,24] more effectively and in a shorter time after they enter the electric field. The insects that enter this electric field are exposed to an instantaneous exposure of high-voltage arc discharge from the charged conductor linked to the voltage generator [23]. In the present study, we used two identical expanded metal nets for the

oppositely charged conductors. The apparatus was constructed simply by pairing these nets in parallel at a given spacing; one was linked to a negative-voltage generator, and the other was linked to a grounded line. For practical applications, we used a pulse-charging-type voltage generator, which is commonly used with electric fences to repel wild animals. Electric fences are ubiquitous and essential in modern agriculture. Accidents associated with agricultural electric fences are very rare [25]. Although unintentional human contact with electric fences occurs regularly, it results in little more than temporary discomfort [25].

In this study, we aimed to determine: the optimal pole distance for arcing to houseflies without effects due to changes in greenhouse relative humidity (RH), the intensity of the sound generated by the pulsed arc-discharge exposure, and the number of pulsed arc discharges required to kill the houseflies. In addition, we clarified the two-step arcing system in the present apparatus, which enables the apparatus to cope with successive invasions by multiple houseflies. Based on the results obtained, this study is aimed to evaluate the feasibility of the present ADE for housefly control and provide an experimental basis for developing a promising physical method for managing adult houseflies emerging from underground pupae in the soil beds of greenhouses.

2. Materials and Methods

2.1. Insect Species

Adult houseflies (*M. domestica*) were purchased from Sumika Technoservice (Hyogo, Japan) and reared on a certified diet (MF; Oriental Yeast Co., Ltd., Tokyo, Japan) [26] in a closed 30 mL transparent acrylic vessel. Insect rearing was conducted in a growth chamber (25 ± 0.5 °C, 12 h photoperiod, 4000 lux) from the egg to adult stages. Pupae found on the medium were individually transferred onto fresh medium in a 20 mL vial for isolation, and the vial mouth was covered with gauze. The sex of adult flies emerging from the pupal stage was determined based on the sexual dimorphism of the external morphology of *M. domestica* [27]. The body sizes of adult male and female houseflies (length from head to wing edge) were measured using 30 randomly collected adult test insects: 6.3 ± 0.3 mm (male) and 8.8 ± 0.4 mm (female). Both pupae and adult houseflies were used in this study.

2.2. Experimental Instrument

2.2.1. Formation of an Electric Field by Two Oppositely Charged Metal Nets

Two identical metal nets (expanded steel nets) (30×30 cm²; thickness, 0.8 mm) (Okutani Ltd., Kobe, Japan) (Figure S1A) were horizontally held with polypropylene clamps (insulator). The upper net was linked to a direct current (DC) voltage generator (pulse-charge type; pulse interval, 1 sec; usable voltage, -10 kV; Suematsu Denshi, Kumamoto, Japan) (Figure S1B) to supply a negative charge to the metal net, and the lower net was linked to a grounded line. A negative-voltage generator, driven by a solar cell (Figure S1B), was used as a booster to enhance the initial voltage (12 V) to a desired voltage (in this case, -10 kV).

The role of a negative-voltage generator is to pick up negative charge from the ground using the enhanced voltage and supply it to a conductor linked to the voltage generator. A negative charge accumulates on the surface of the charged conductor and forms an electric field in the space around the charged conductor. If the grounded conductor is placed inside the electric field, the negative charge on the charged conductor pushes negative electricity (free electrons) out of the grounded conductor by electrostatic induction [22]. Eventually, the grounded conductor becomes positively charged. This positively charged grounded conductor acts as a recipient pole of negative charge released from the negatively charged conductor. In the present configuration, a single-charged dipolar electric field was formed in the space between the negatively charged metal net (NC-MN) and the grounded metal net (G-MN) (Figure 1). The occurrence of an arc discharge between two opposite poles is determined by the applied voltage and the pole distance (distance between the NC-MN and G-MN). In this study, the applied voltage was fixed to -10 kV, and the pole distance was adjusted for proper arcing.

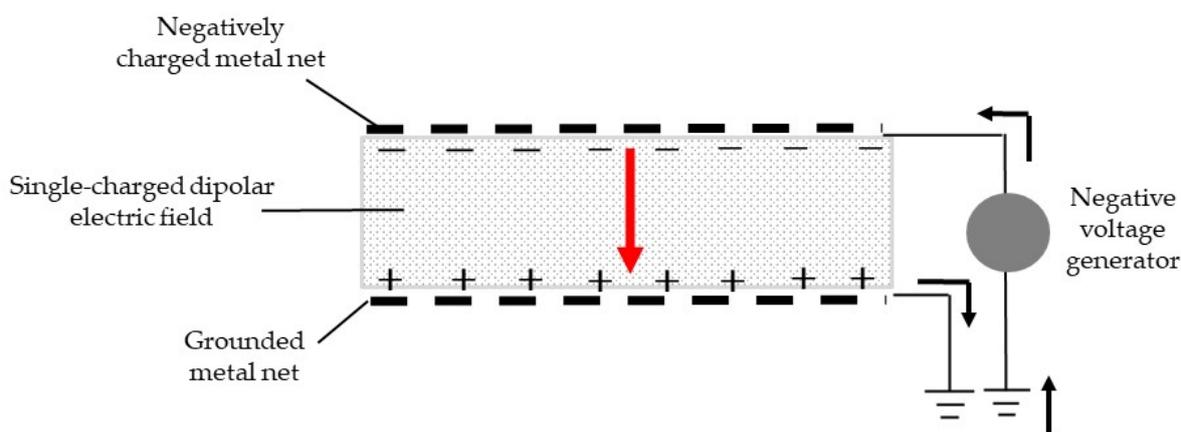


Figure 1. Schematic representation of a single-charged dipolar electric field formed between a negatively charged metal net and a grounded metal net. The black arrow represents the movement of the negative current, and the red arrow represents the movement of electricity through arcing.

2.2.2. Arcing between Two Oppositely Charged Metal Nets

In the preliminary experiment, the two metal nets (NC-MN and G-MN) were held horizontally using polypropylene (insulator) clamps in the cabinet; the RH was controlled at 25%, 50%, 75%, or 98%, and the distance between the nets was changed gradually until an arc discharge occurred between them to determine the distance that caused discharge at each RH. In this system, both metal nets generated the ground-to-ground circuit of electric current when a discharge occurred between them (Figure 1). Based on the results obtained, the following arcing experiments for houseflies were conducted at a pole distance of 6 mm, whereas no arc discharge occurs if the RH changes in a greenhouse.

2.3. Arcing to Adult Houseflies

2.3.1. Carbon Dioxide (CO₂) Anesthetization of Adult Houseflies

For immobilization, adult houseflies were anesthetized by CO₂ exposure according to a method described previously [28]. Briefly, vials containing an insect were placed in a non-vacuum glass desiccator (jar capacity, 5 L), and CO₂ gas (Air Water West Japan Inc., Osaka, Japan) was continuously introduced into the desiccator at 10 kg/cm² for 4–5 min. The air in the desiccator was simultaneously removed via the exhaust port of the desiccator lid. The introduction of CO₂ was stopped when all insects were anesthetized. In the present CO₂ treatment, all of the anesthetized houseflies awoke from anesthesia within 5 min.

2.3.2. Arc-Discharge Exposure Assay for Adult Houseflies

Arc-discharge exposure of houseflies was conducted using the experimental instrument shown in Figure 2A, in which three transparent acrylic cylinders of different lengths were placed vertically between the NC-MN and G-MN, beneath the G-MN and on a polypropylene plate, and beneath the polypropylene plate. The top cylinder (6 mm in length) was marked with lines every 2 mm to create three zones. An immobilized adult housefly was transferred into the bottom of the lowest cylinder (9 mm length) beneath the plate. After the housefly awoke from anesthesia, the plate was withdrawn to allow it to climb the cylinder wall (Figure 2B); it was then subjected to arc-discharge exposure from the NC-MN when it entered Zone 1 (Z1) (Figure 2C). The fly was thrown to the bottom of the lowest cylinder (Figure 2C) (cylinder-climbing assay). In this experiment, we confirmed the occurrence of arcing in both male and female houseflies when they reached the lowest zone (Z1). As the houseflies that were subjected to the arc-discharge exposure were thrown to the bottom of the lowest cylinder, we counted the number of repeated climbing trials by the discharged exposed houseflies until they died. Twenty adult houseflies of each sex were used, and separate experiments were repeated five times.

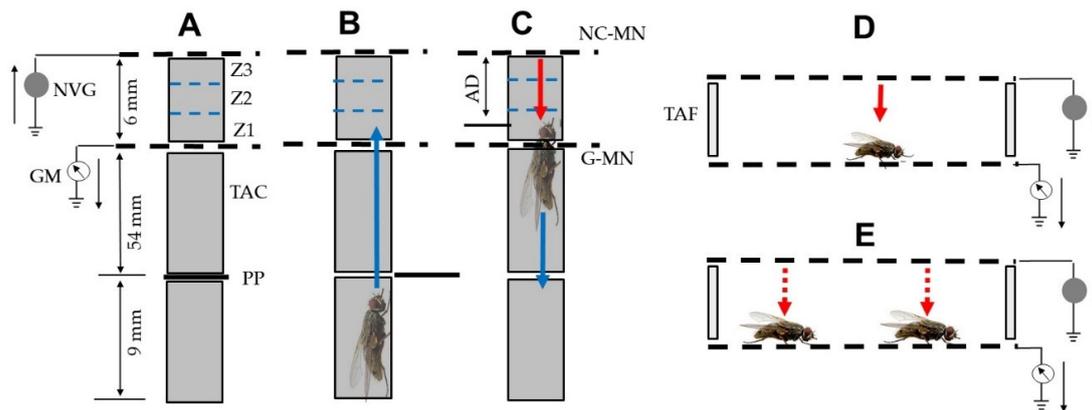


Figure 2. The experimental set-up for delivering an arc discharge to adult houseflies. (A) The apparatus consisted of two metal nets and three transparent acrylic cylinders (TACs) of different lengths. The upper net was linked to a pulse-type negative-voltage generator (NVG), and the lower net was linked to a grounded line. The top cylinder (6 mm in length) was placed vertically between the two metal nets, the middle cylinder (54 mm in length) was placed beneath the grounded metal net (G-MN) and on a polypropylene plate (PP), and the third cylinder (9 mm in length) was placed beneath the PP. The top cylinder was marked with lines every 2 mm to create three zones (Z1–3). (B,C) A climbing housefly (B) and its falling pathway following arc-discharge exposure (C). An adult housefly was placed in the lowest cylinder, and the PP was drawn out to allow the housefly to climb up the wall of the middle cylinder. The housefly was subjected to arc discharge from the negatively charged metal net (NC-MN) when it entered the upper cylinder on the G-MN and was within the arc distance (AD). (D,E) Another experimental set-up for exposing a housefly (or houseflies) transferred onto the G-MN. Two metal nets were separated by a transparent acrylic frame (TAF). Single (D) and double (E) CO₂-anesthetized houseflies were transferred onto the G-MN. The black arrow represents the movement of the negative current. The solid red arrow represents the movement of electricity through arcing, and the dotted red arrow represents selective arcing to one of the two houseflies. The blue arrow represents the pathway taken as the fly climbs and falls. A galvanometer (GM) was integrated into the grounded line to detect the electric current caused by the arc-discharge exposure.

In all experiments, we measured the magnitude of the electric current and the intensity of the sound, which reflected the arc-discharge exposure. The electric current was detected by a galvanometer (Sanwa Electric Instrument, Tokyo, Japan) integrated into the grounded lines. The sound produced by the arc discharge was measured in decibels using a sound-level meter (Sato Tech, Kanagawa, Japan). The sound profile was recorded with a spectrum analyzer integrated into the sound-level meter. Twenty adult houseflies of each sex were used, and each individual experiment was repeated five times.

2.4. Relationship between Autonomous Stoppage of Arc-Discharge Exposure and Loss of Body Water in Houseflies

2.4.1. Effect on Houseflies of Autonomous Stoppage of Arcing on the G-MN

In this experiment, we fabricated another instrument (Figure 2D) to ensure arc-discharge exposure to houseflies on the G-MN. The instrument consisted of the NC-MN and G-MN, which were separated by a square transparent acrylic frame (wall thickness, 1 mm; height, 6 mm). First, single CO₂-anesthetized male and female adult houseflies (body sizes 6, 7, 8, and 9 mm) were collected randomly, individually transferred onto the G-MN, and subjected to arcing by switching on the voltage generator after they awoke and began to crawl on the net (on-net-crawling assay). In this system, as pulsed arcing was continuously applied to the same fly at 1 s intervals, we switched off the generator immediately after the first arcing finished and examined the survival of the discharge-exposed fly. Similarly, we examined the survival of houseflies that were continuously exposed two to five times.

Second, we randomly collected single anesthetized houseflies of different sizes (without respect to sex), measured their body size and weight, transferred them individually

onto the G-MN, and switched on the voltage generator after they awoke. After the arcing stopped autonomously, we confirmed their death and re-measured their weight to determine the loss of body water. In each housefly, we determined the time taken for the arc to stop autonomously. Twenty houseflies were used to examine the relationship between the duration of the continuous arcing and body size. Additionally, we also determined the time taken for the arc to stop autonomously in cases when two houseflies were transferred onto the G-MN (Figure 2E).

2.4.2. Measurement of the Body Water Content of Houseflies

The body water content of a housefly was determined using the loss-on-drying (LOD) method [29]. Houseflies of different body sizes (6, 7, 8, and 9 mm) were weighed and placed in a thermostatic convection oven set to 30 °C to dehydrate. At intervals, insects were removed from the oven and weighed. This procedure was continued until the weight remained constant. Then, the difference between the initial and final weights was calculated to determine the moisture (body water) vaporized. Using a weight-loss calibration curve (Figure S1A), houseflies that lost different proportions of their body water were collected and transferred onto the G-MN to examine the occurrence or non-occurrence of arcing to their bodies (Figure S1B).

2.5. Construction of the Arc-Discharge Exposure

Figure 3A shows the structure of the arc-discharge exposure (ADE), which consisted of two identical expanded steel nets (100 × 90 cm², strand thickness, 0.5 mm). One was linked to a voltage generator and the other to a grounded line. These metal nets were adhered to both the upper and lower faces of a square polypropylene frame (wall width, 10 mm; wall height, 6 mm) by a silylated polyurethane adhesive (Figure 3B), creating a separation interval of 6 mm between the two nets. A fiberglass reinforced plastic (FRP) grating (100 × 90 cm²; 9 mm depth; 4095 cubic cells) (Figure S1C) (Chubu Corporation, Mie, Japan) was used to provide the flies with a climbing path. Six identical gratings (54 mm height) were stuck to each other and placed beneath the G-MN. Another single grating (54 mm height) was placed beneath the polypropylene plate (Figure 3A). The plate located between the two gratings was drawn out to provide a climbing path for flies in the cells of the lower grating at the start of the experiment.

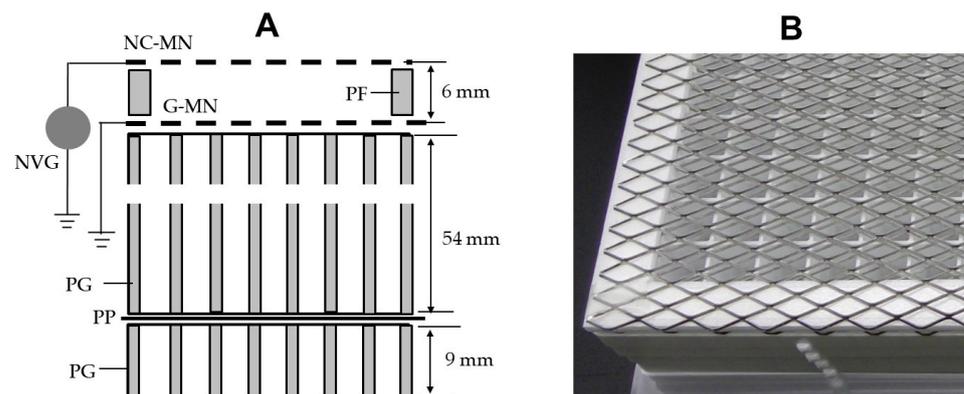


Figure 3. Schematic (A) and photograph (B) are representations of an arc-discharge exposure (ADE) consisting of two metal nets, two gratings, and a pulse-type negative-voltage generator (NVG) (cross-sectional view). A square polypropylene frame (PF) (6 mm in height) was placed between the negatively charged metal net (NC-MN) and the grounded metal net (G-MN). A plastic grating (PG) 54 mm in height was placed beneath the G-MN. Another grating (9 mm in height) was placed beneath the polypropylene plate (PP), which was positioned between the two gratings and withdrawn before the experiment. Pupae or CO₂-anesthetized adult houseflies were placed in separate cells of the lower grating.

2.6. Application of the ADE to Control Houseflies Emerging from Underground Pupae

In this experiment, we randomly collected pupae on the rearing medium (with no regard to their maturity) and embedded them into the soil in the cells of the lowest grating of an ADE that was placed in a greenhouse (July 2022; diurnal temperature range, 16–38 °C). We used 100, 200, 300, and 400 pupae in separate experiments. These pupae were individually transferred to separate cells (single pupa/cell). The experiments ran for 2 weeks. At the end of each experiment, we counted the number of dead adult houseflies on the bottom of the grating and on the G-MN. In addition, we dug pupae out of the soil to check the success or failure of adult emergence. In the first round of experiments, the number of pupae was increased stepwise from 100 to 300, and the experiment was conducted once for each number of pupae. In the second round, we used 400 pupae and repeated the same experiment five times.

2.7. Assessment of the Ability of the ADE to Control Adult Housefly Invasions

In this experiment, different numbers (25, 50, 100, and 150) of CO₂-anesthetized houseflies were individually transferred into the separate cells of the lowest grating of the ADE on the assumption that multiple adult houseflies would emerge from underground pupae, climb the cell wall and enter the arcing zone of the ADE. The experiments were continued for 3 days. We confirmed that all flies attempted to climb the grating wall and were exposed to the pulsed arc discharge. At the end of the experiment, we counted the number of dead houseflies on the bottom of the lowest grating and on the G-MN. In the first round of the experiments, we increased the number of adult houseflies stepwise from 50 to 100 in individual experiments, and the experiment was conducted once for each number of flies. In the second round, we used 150 adult houseflies and conducted the same experiment five times.

2.8. Statistical Analysis

All experiments were repeated five times; all data are presented as means with standard deviations. Analyses were performed using the EZR software version 1.54 (Jichi Medical University, Saitama, Japan) to identify significant differences among conditions and correlations among factors, as shown in the figures and tables.

3. Results and Discussion

3.1. Prevention of Target-Independent Arcing

The factors affecting arc discharge between the NC-MN and G-MN of the apparatus were the voltage applied to the NC-MN, the distance between the two nets (pole distance), and the change in vapor concentration in the air between the poles [21]. In the present apparatus, the applied voltage was fixed to −10 kV, and the pole distance and water-vapor concentration (RH) in the air were the parameters tested. The air conductivity between the two nets changed in response to changes in the RH of the air, with air conductivity increasing (i.e., higher amounts of electricity being transferred) under higher RH [30]. This implied that under higher RH, the arcing occurred at greater distances between the NC-MN and G-MN. We investigated the pole distances resulting in arc discharge between the nets under different RH conditions (Figure 4). As expected, the pole distance resulting in arc discharge increased as the RH increased. The change in the RH of the greenhouse over a year (recorded in 2021) was between 32% and 96%. Based on these data, the safe pole distance that did not result in arc discharge between the two nets was more than 6 mm, regardless of changes in the RH. In the following experiment, we examined possible arcing to houseflies at a pole distance of 6 mm. Incidentally, the temperature changes (between 5 and 50 °C) tested did not affect the generation of an arc discharge by the apparatus (data not shown).

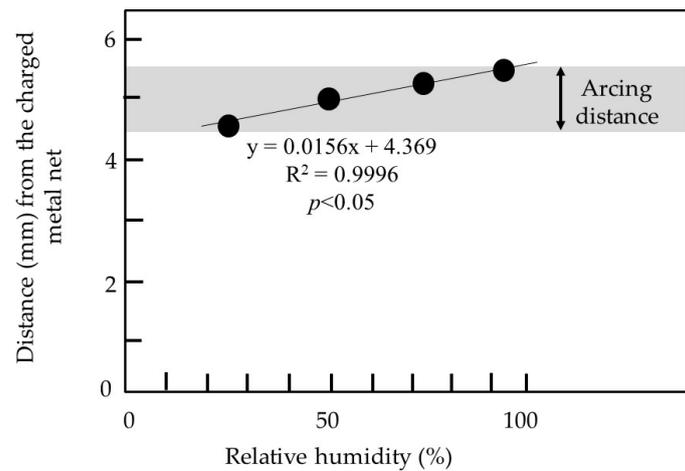


Figure 4. The distance from the pole required to cause arcing under different relative humidity (RH) conditions. Two identical expanded metal nets (negatively charged and grounded metal nets) were horizontally arranged, and the arcing distance (distance from the charged net) was determined by changing the distance between them at each RH.

3.2. Arcing to Kill Houseflies

Figure 4 indicates that the NC-MN was able to strike an arc discharge to a grounded conductor within positions at 4.5–5.5 mm from the NC-MN, depending on the RH. Houseflies received arc-discharge exposure [20,31] due to the conductive nature of their outer surface cuticle structure [32,33]. In the experiments, we examined whether the houseflies received discharge exposure from the NC-MN when they moved within this arcing distance. In the cylinder-climbing assay, we settled two cylinders on and beneath the G-MN, respectively, and marked the upper cylinder with lines to create zones. We then determined in which zone of the cylinder the housefly was subjected to an arc-discharge exposure from the NC-MN. In this experimental design, Z1 (4–6 mm from the NC-MN) was expected to be the first arcing site. We confirmed that all houseflies were subjected to arc-discharge exposure in this zone (Figure 2C).

The arcing is the movement of negative electricity in the air toward the grounded conductor by breaking down the resistivity of the air [21]. In the configuration of the instrument, negative electricity was picked up from the ground by a voltage generator and supplied to the metal net linked to the voltage generator. Using the applied voltage (−10 kV), the electricity on the metal net was released toward the conductor (housefly) in the electric field through the arc discharge. For successful arcing, it was essential for the electricity to flow back to the ground, and arcing did not occur if the grounded line of the G-MN was removed. Nevertheless, our attempt to detect the movement of electricity (i.e., the flow of electric current) from the G-MN to the ground was unsuccessful because of its low magnitude, i.e., below the detection limit (0.01 μA) of the current detector used. The sound was detectable and was shown to reliably indicate the occurrence of arc-discharge exposure to the houseflies, which allowed us to determine the numbers of arc-discharge exposures to houseflies based on the numbers and intensities of the sounds recorded. The intensity of the arc-discharge sound was an important parameter in the determination of the force required to push the fly down (Figure 2A–C). The arc-discharge sound was a sonic boom caused by the shock wave from the high-speed electrons moving in the electric field, and its intensity was an indicator of the impact strength of the shock wave produced by the arc-discharge exposure. Video S1 shows male and female houseflies that were violently thrown down to the bottom of the cylinder by a single arc-discharge exposure. Matsuda et al. [20] reported that the strength of the impact produced by the arc-discharge exposure was in direct proportion to the intensity of the arc-discharge sound; in fact, both male and female houseflies underwent arcing with the same sound intensity (Figure 5A). The housefly has an inherent habit of climbing upward [34]. In fact, they did so even

after they experienced arc-discharge exposure, as a result of which they were repeatedly exposed to harmful arc discharges. Eventually, both houseflies were killed by three to four arc-discharge exposures (Figure 5B).

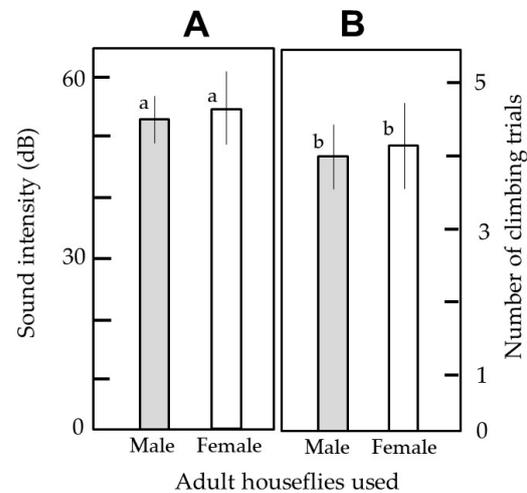


Figure 5. (A) The intensity of the sound produced by arc-discharge to a housefly climbing up the cylinder wall and reaching the arcing zone of the arc-discharge exposurer (ADE) in the cylinder-climbing assay. (B) Number of climbing trials by houseflies exposed repeatedly to arc-discharge exposure until they died. We used 20 insects of each sex. Means \pm standard deviation were calculated from five experimental replicates. The letters (a, b) on each column indicate no significant difference ($p < 0.05$) according to Tukey's test.

In the second experiment, we examined the lethal effects of arc-discharge exposure on the survival of the houseflies on the G-MN (on-net-crawling assay) (Figure 2D). Figure 6 shows the number of arc-discharge exposures required to kill the houseflies on the G-MN. The houseflies on the G-MN became motionless immediately after their first exposure to the arc discharge and were then subjected to subsequent arcing at the same position. The arc-discharge exposure was harmful, and the flies were killed by four pulsed arc-discharge exposures. There was no significant difference in survival rates between the male and female houseflies.

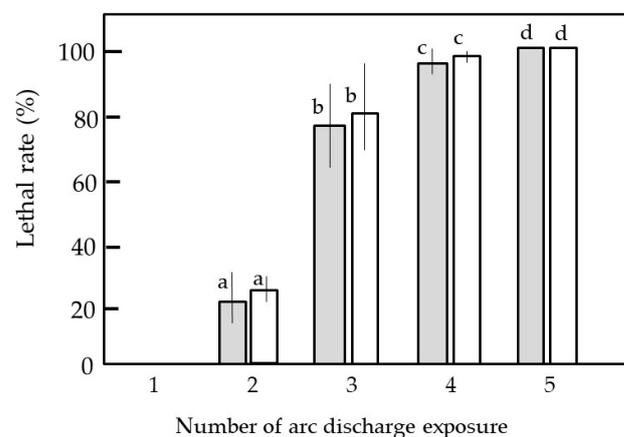


Figure 6. The mortality of the houseflies that were transferred onto the grounded metal net (G-MN) facing the negatively charged metal net (NC-MN) of the arc-discharge exposurer (ADE). Anesthetized male (gray) and female (open column) houseflies were individually transferred onto the G-MN and subjected to an arc discharge from the NC-MN. After 1–5 arc-discharge exposures, their survival was examined. We used 20 insects of each sex. Means \pm standard deviation were calculated from five experimental replicates. Different letters (a–d) on each column indicate significant differences ($p < 0.05$) according to Tukey's test.

For the same target, arc-discharge exposure continued regardless of whether it lived or died. In cases where single houseflies were introduced into the electric field, the arcing stopped autonomously after a lapse of 10–40 min. Figure 7 indicates that there was a linear relationship between the body size of the houseflies and the duration of the arcing, with larger houseflies requiring longer for arcing to finish.

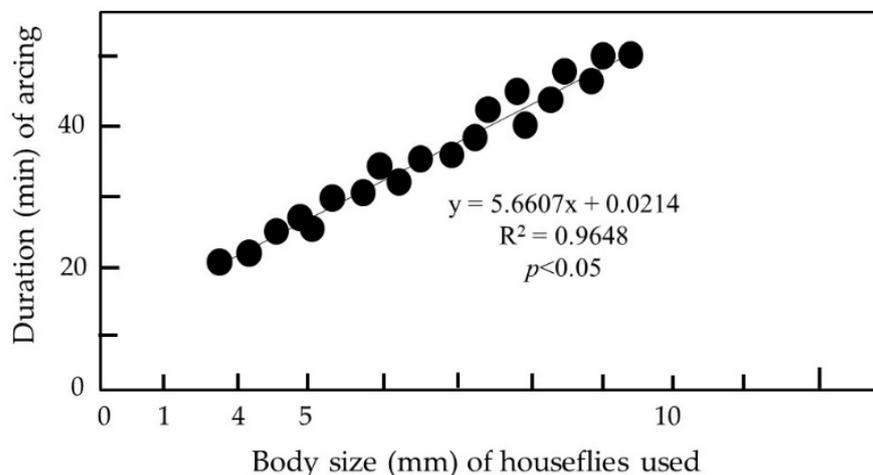


Figure 7. Relationship between the body size of houseflies and the length of time required for the arc-discharge exposure of houseflies to stop autonomously.

There was a need to determine why the arcing stopped autonomously. The most important outcome of this assay was that the weight of the houseflies decreased remarkably at the time arcing stopped (Table 1). The reduction in body weight implied a loss of water from the houseflies. Takikawa et al. [31] reported that the loss of body water resulted in a decline in the conductance of houseflies. Our supplementary experiment also indicated that the houseflies that approximately 50% of their body water by dehydration received no arcing due to reduced conductance, i.e., an increase in resistivity in the water-depleted houseflies due to the arc-discharge exposure (Figure S1B). Matsuda et al. [24] reported that continuous pulsed arc-discharge exposure to the growing stem tip of the kudzu plant (*Pueraria montana*) raised the temperature of the exposed region, implying that an increase in temperature caused the vaporization of body water.

Table 1. Change in the weight of houseflies before and after continuous pulsed arc-discharge exposure.

Size (mm) of Houseflies Used	Weight (mg)		Percentage of Body Water Lost	Duration (min) of Continuous Arcing
	Before Arcing	After Arcing		
6	13.5 ± 0.8 a	6.5 ± 0.5 a	48.6 ± 2.5 a	18.8 ± 1.8 a
7	17.1 ± 0.7 b	8.3 ± 0.6 b	48.4 ± 3.5 a	30.3 ± 2.0 b
8	23.5 ± 0.5 c	11.5 ± 0.7 c	49.1 ± 3.4 a	40.2 ± 2.7 c
9	29.2 ± 0.6 d	14.9 ± 0.7 d	51.1 ± 2.3 a	49.8 ± 1.3 d

Twenty houseflies were used for each body size category. The means and standard deviations were calculated from five repetitions of the experiments. The letters (a–d) on the means in each vertical column indicate significant differences ($p < 0.05$) according to Tukey's method.

Additionally, we simultaneously transferred two houseflies onto the G-MN and examined the duration of the continuous pulsed arc-discharge exposure of these two houseflies. In this case, arcing occurred to either of the two houseflies, whichever was closer to the NC-MN at the timing of arcing. Their antennae, wings, and legs were the sites that received the arcing from the NC-MN. Subtle changes in the positions of these organs affected the selective arcing from the NC-MN. Both houseflies died shortly after several arc-discharge exposures, whereas the arcing continued for some time. Eventually, the arcing stopped autonomously after a lapse of 20–80 min (data not shown).

3.3. Practical Application of the ADE to Control Houseflies Emerging from the Soil

3.3.1. Successful Grounding of the ADE

In this study, we fabricated an ADE operated by a pulse-type voltage generator. The prerequisite for normal functioning by the apparatus was to ensure successful grounding, to pick up the negative charge from the earth's ground by the voltage generator and send it back to the ground. In the laboratory experiments, we inserted the ground-line plug of the voltage generator into the ground-contact outlet of a wall socket, which was equipped with a conductive pipe or rod physically driven into the earth to a minimum depth of 8 feet (about 2.5 m) to protect buildings against fire resulting from leakage of electricity [35]. In the greenhouse experiments, it was necessary to create an effective ground for the voltage generator because the dry surface layer of the field could increase the ground resistance (earth resistance), thereby impeding the current flow to the ground [36]. For the pulse-type voltage generator used in this study, the manufacturer recommended a 50 cm steel rod driven into the ground completely. As a result of this grounding procedure, the apparatus was able to produce pulsed arc discharges at all 200 points tested inside and outside the greenhouse.

3.3.2. Construction of the ADE and Its Two-Step Arcing System to Control Houseflies

The two-net system was able to deliver an arc discharge to adult houseflies that were about to enter the space (electric field) between the two nets and that had already entered the electric field. Because the discharge-exposed houseflies are killed, this system can be used to manage houseflies. Based on this feature, we devised the ADE (Figure 3A) as a simple physical tool to effectively kill adult houseflies emerging from underground pupae in a greenhouse soil bed. The combination of a multicell grating with the two nets provided a climbing path for the houseflies on the soil to guide them to the killing site in the apparatus. The impact generated by the arc-discharge exposure was so strong that the houseflies that climbed up to the arcing zone were thrown down to the soil surface. Importantly, the ADE was able to control the houseflies that slipped through the first arcing. The second arcing was strong enough to make houseflies on the net motionless with a single hit. For practical use of the ADE, we attempted to clarify the population size limit of adult houseflies that the ADE could control in the first stage of defense.

The NC-MN generated a pulsed arc discharge to the nearest housefly in the electric field at 1 s intervals. In our preliminary measurement, the pace of climbing by the houseflies was 1.9 ± 0.3 mm/sec (average of 50 flies). The probability of passing through the first arcing was determined by the relationship between the interval of pulsed arcing and the climbing pace achieved by multiple houseflies climbing synchronously. Figure 8A1 shows the hypothetical case in which three houseflies simultaneously entered the electric field, where the housefly on the left side (nearest to the NC-MN) was first subjected to the arcing and thrown down. Other houseflies could move forward for 1 sec, and the second arcing occurred toward the second housefly that was nearest to the NC-MN (Figure 8A2). The investigation then turned to the third fly. In this case, before the third arcing, the houseflies that climbed over the G-MN were then subjected to an arc-discharge exposure (Figure 8A3). This implied that the third housefly could pass through the first defense mechanism of the apparatus. As mentioned earlier, the housefly that was subjected to the arc-discharge exposure was rendered motionless by the first single arcing of the second defense mechanism and was then continuously exposed to the pulsed arcing.

The houseflies on the G-MN underwent pulsed arc-discharge exposure continuously (Figure 8B1) (Video S1B). This arc-discharge exposure continued until the arc stopped autonomously. Figure 8B2 shows an uncommon case in which two houseflies entered the arcing zone simultaneously before the arcing for the preceding fly had stopped autonomously. Of these two flies, the earlier one was subjected to the arcing and thrown down before entering, whereas the second fly was able to climb over the net before being subjected to the arcing (Figure 8B3). Thus, a series of coincidences led to the existence of two houseflies on the G-MN (in the electric field). These two houseflies were motionless,

and whichever one was closer to the NC-MN was subjected to the arcing each time during the continuous arc-discharge exposure. This situation continued until the arcing stopped autonomously. Although the probability of these events was expected to be extremely low, understanding their possibility was essential for the successful management of houseflies by the apparatus.

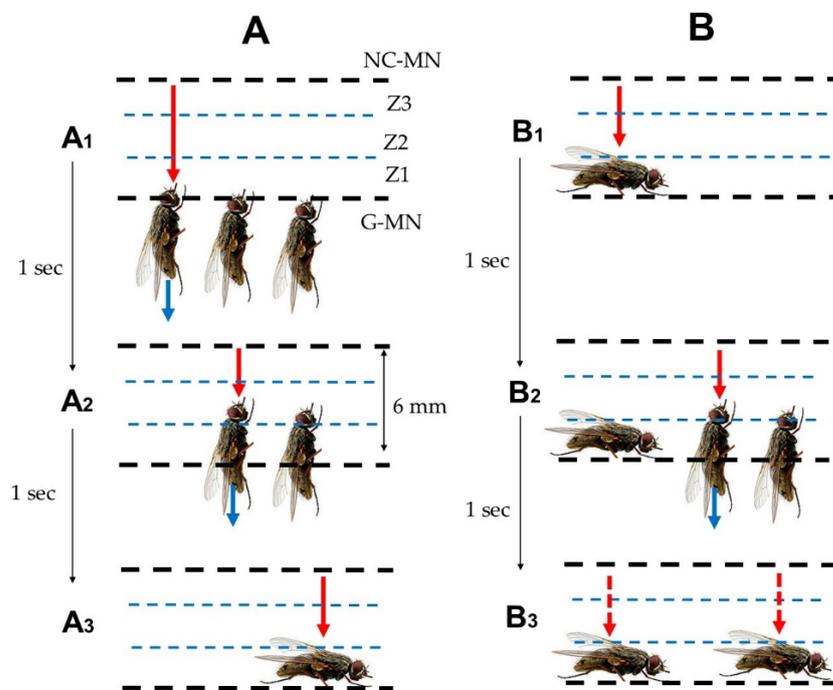


Figure 8. Schematic representation of the standby and follow-up arcing to multiple houseflies that entered the arcing zone of the arc-discharge exposurer (ADE) simultaneously. **(A)** Simultaneous entry to the first arcing zone (Z1) by three houseflies. The housefly on the left, which was nearest to the negatively charged metal net (NC-MN), was the first to be subjected to arc discharge from the NC-MN and thrown down (A1). The other flies moved into Zone 2 (Z2) for 1 sec, and a second fly was subjected to the arcing (A2). Before the third arcing, the last fly climbed over the grounded metal net (G-MN) and was subjected to arc discharge (A3). **(B)** Simultaneous entry by two houseflies to the arcing zone in which the first fly had been present. The housefly that remained in the electric field was subjected to a pulsed arc-discharge exposure (B1) continuously until the next fly entered and moved close to the NC-MN. Of the two flies that entered the arcing zone simultaneously, the one that was closest to the NC-MN was subjected to the arcing and thrown down (B2). The second fly was subjected to arcing only after it had climbed over the G-MN (B3). The solid red arrow represents arcing to the fly from the NC-MN. The dotted red arrow represents selective arcing to one of the two flies, i.e., whichever fly was closest to the NC-MN at the moment of arcing. The blue arrow represents the pathway taken as the fly falls.

3.3.3. Application of the ADE to Adult Houseflies Emerging from Underground Pupae

According to our records for the past three years, in which flypapers (sticky paper ribbons) were hung over the soil beds, and the numbers of trapped houseflies were counted, their occurrence (during the 3-month summer season) was between 8 and 62 per m². However, this approach provided no information about how many houseflies escaped from the trap and, more importantly, how many houseflies emerged simultaneously from the soil bed. In the experiment, we established the ADE in a greenhouse, embedded 100–400 pupae into the soil of separate cells of the lowest grating of the ADE (single pupa/cell), and surveyed the working of the ADE during the period of the experiment (2 weeks). The access of the houseflies that emerged from pupae to the electric field of the ADE was estimated based on the total number of arc-discharge sounds. If no adult housefly was detected on the G-MN, it was considered that all of the houseflies that reached the arcing zone were

thrown down to the soil surface by the arcing of the first dense mechanism (Table 2). In the experiment, more than 90% of the embedded pupae produced adult houseflies within 5–10 days. However, emergence was highly irregular, and therefore there was no chance that multiple houseflies (more than three houseflies) entered the arcing zone. This uneven emergence of adult houseflies probably depended on the different maturities of the pupae used. Eventually, we detected no adult houseflies on the G-MN, neither in the stepwise applications of 100–300 pupae nor in the repeated applications of the highest number of pupae (400 pupae). In conclusion, the study revealed that at <400 pupae/m², there was no opportunity for the ADE to undertake the second-step measures to control houseflies.

Table 2. Evaluation of the ability of the arc-discharge exposer (ADE) to control adult houseflies emerging from underground pupae by continuous pulsed arc-discharge exposures.

No. of Pupae Used ^a	No. of Disable Pupae Bearing No Adults	No. of Dead Adult Houseflies on BLG ^b	No. of Dead Adult Houseflies on G-MN
100	6	94	0
200	12	188	0
300	4	296	0
400	11	389	0
	7	393	0
	25	375	0
	11	389	0
	10	390	0

^a Pupae at different stages of maturity were collected randomly and embedded into the soil of separate cells of the lowest grating (L-grating). Adult houseflies that emerged from pupae climbed the cell of the upper grating (U-grating), which was set on the L-grating, and were subjected to arcing from the negatively charged metal net (NC-MN) when they reached the arcing zone above the grounded metal net (G-MN), which covered the U-grating.

^b Bottom of the L-grating.

3.3.4. Capability of the ADE to Control Adult Houseflies Invading Successively

In the second experiment in a greenhouse, we directly applied adult houseflies to the ADE to set up an acute situation in which adult houseflies (in this case, 25–150 adults) simultaneously emerged from underground pupae and successively invaded the ADE. The experiment was designed to actualize the events hypothesized in Figure 7. However, in the preliminary application of the 25–150 houseflies, no fly was detected on the G-MN. Eventually, in one of five repeated applications of 150 adult houseflies, we detected one housefly on the G-MN (Table 3). Conversely, numerous dead houseflies were confirmed on the bottom of the lowest grating, which had been thrown down by the arcing during the first defense of the ADE. The study indicated that if the number of synchronously emerging adult houseflies did not exceed 200, the ADE was able to effectively cope with successive invasions by multiple houseflies using the first defense measures. The second arcing was a fallback measure to respond to the few houseflies that overcame the first defense.

Table 3. Evaluation of the ability of the arc-discharge exposer (ADE) to control successive invasions by adult houseflies.

No. of Adult Houseflies Used ^a	No. of Dead Houseflies on BLG ^b	No. of Dead Houseflies on G-MN
25	25	0
50	50	0
100	100	0
150	150	0
	149	1
	150	0
	150	0
	150	0

^a Anesthetized adult houseflies were placed in separate cells of the lowest grating (L-grating). These flies climbed up the wall of the upper grating (U-grating), reached the L-grating, and entered the arcing zone above the grounded metal net (G-MN), where they were subjected to arcing from the negatively charged metal net (NC-MN) and then thrown down to the bottom of the L-grating. ^b Bottom of the lower grating.

The impact of the arc-discharge exposure was strong enough to kill adult houseflies. However, this arcing technique has not proven applicable to all fly species that emerge from underground pupae. The apparatus was found to be unsuitable for tomato leaf miner flies because of their small body size. If the metal net was not completely flat, the point that protruded most from the net surface became the site that received the arcing from the charged metal net. If the size of the fly was smaller than the vertical drop (i.e., the distance between the highest and lowest sites on the G-MN), it remained on the metal net without undergoing arcing from the NC-MN. Theoretically, only flies larger than the vertical drop were specifically targeted for arcing. Alternatively, another type of electrostatic tool, which consisted of a pair of insulated and non-insulated metal nets, was successfully applied to capture the smaller flies emerging from underground pupae [16].

The most useful feature of the ADE is its simple structure, which enables ordinary workers to fabricate and improve the apparatus cheaply using common materials based on their own requirements. The most important feature of the ADE is that it provides pulsed arc-discharge exposure to the nearest target from the NC-MN, and therefore it is possible to scale up the present system simply by linking additional ADEs together with an electric line. The arcing system of the ADE is operated safely using a pulse-type voltage generator, which is widely used with electric fences to deter wild animals without harming people [25]. Thus, the present study provided the experimental basis for the development of a new physical method for managing houseflies emerging from soil beds in greenhouses.

4. Conclusions

The electrostatic apparatus described here is a newly developed device that can be applied to net the surface of soil beds in a greenhouse to manage the emergence of adult houseflies from the ground by exposing them to an arc discharge. The convex array over the whole surface of an expanded metal net is the site from which a spark can be discharged to targets at any location. The target-responsible arc-discharge exposure treatment was extremely effective at pushing invading houseflies down into the soil surface through the destructive force generated by the arc-discharge exposure. The apparatus was developed by pairing commercially available expanded metal nets and connecting one to a negative-voltage generator and the other to a ground line. The study developed a simple physical method for an insecticide-independent pest-management approach that can be integrated into sustainable crop-production systems in greenhouses.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy13020310/s1>, Figure S1: (A) An expanded steel net with diamond-shaped meshes. (B) A pulse-type voltage generator operated by a solar panel. (C) A fiberglass reinforced plastic (FRP) grating with square cells; Video S1: (A) Arc-discharge exposure from the negatively charged metal net (NC-MN) to adult male (left) and female (right) houseflies that reached the arcing zone above the grounded metal net (G-MN) of the arc-discharge exposer (ADE). (B) Arc-discharge exposure of an adult housefly (female) transferred onto the G-MN.

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