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An electric field screen prevents captured insects from escaping by depriving bioelectricity generated through insect movements

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ABSTRACT

An insulated conductor wire (ICW) paralleled with an earthed net was used to observe movements by vinegar flies in relation to their electricity release. ICW was negatively charged to create a positive charge on the net. At particular voltages, flies were attracted to ICW. This attraction was triggered by the deprivation of the insect negative charge with the net. Eventually the insects became net positive and were drawn to the ICW negative charge. The attracted insects generated bioelectricity through skeletal muscular movements. However, the electricity produced was depleted by the net without neutralizing their positive charge in the insect body.

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1. Introduction

Agricultural crops suffer from pathogen infection and/or insect attacks during pre- and post-harvest stages. We have developed electrostatic methods for disinfecting bacterial and fungal plant pathogens [1,2] and for preventing airborne pathogens and flying insect pests from entering greenhouses [3,4]. These methods are aimed at reducing the use of agrochemicals such as fungicides and insecticides. An electric field screen is a practical and environmentally friendly tool for excluding pathogens and pests from plant cultivation [5] and storage [6] spaces.

The electric field screen consists of three parts: 1) insulated iron wires (ICWs) arrayed in parallel with a definite interval, 2) earthed stainless nets placed on both sides of the ICW layer and 3 mm from the ICW layer, and 3) a direct current (DC) voltage generator. The ICWs are linked to each other and to a voltage generator to receive a negative charge. The negative surface charge of the ICWs induces an electrostatic charge on both sides of the earthed nets (conductor), creating an opposite charge on the ICW side surface of the nets. An electric field forms between these opposite charges.

Insects that enter the electric field are deprived of their negative charge and turn net positive [6].

According to our interpretation, the positive electrification of insects is vital to create an electrostatic force against the negative charge of the ICW. At the same time, this working hypothesis implies that the attraction force could be nullified if the positive charge of the attracted insect is neutralized by some bioelectric measures. Bioelectric power generation is a potential method for supplying the negative charge. In fact, some insects create electricity through muscular movement [7–11] and/or neural excitation [7,12]. We speculated that this biological neutralization leads to a loss or weakening of the attraction force of the electric field screen. Hence, we were interested in knowing whether the insects restrained in the electric field can generate electric power, and whether the electricity produced could compensate for the negative charge deprived by the electric field screen.

In the present study, we analyzed the dynamic relationship between the physical force of the electric field screen and the biological power exerted by the insects struggling to escape from the electrostatic attraction. For this purpose, we constructed a simple version of the electric field screen and video-recorded insect movements that were synchronized with measuring the electric current generated by the insects.

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2. Materials and methods

2.1. Simplified electric field screen

We constructed a pair of electrodes; one was an iron wire (20 cm length, 2 mm diameter) linked to a DC voltage generator (Kansai Denshi, Tokyo, Japan), and the other was an earthed stainless net (1.5 mm mesh size, $5 \times 20 \text{ cm}^2$). An iron conductor wire was passed through a vinyl chloride sleeve (1 mm thickness) to make an insulated conductor wire (ICW). Both electrodes were arrayed in parallel at 3 mm intervals, and a galvanometer PC520M (Sanwa Electric Instrument, Tokyo, Japan) was integrated into the electric line of the earthed net (Fig. 1A). The ICW was negatively charged to dielectrically polarize a cover insulator; positively on the iron wire-side surface and negatively on the outer surface of the insulator sleeve [6]. The negative surface charge of the ICW polarized the earthed net to create a positive charge on the ICW side surface, and an electric field formed between the opposite charges of the ICW and the earthed net (Fig. 1B). In the present study, the ICW was negatively charged with voltages of 1–10 kV. The potential difference in the ICW surface was measured continuously with an FMX-

003 field meter (Simco, Kobe, Japan) placed at 2.5 cm from the ICW during the entire period of charging and until the potential difference vanished after stopping voltage to the ICW (Fig. 1B).

2.2. Assay for insect discharge

Adult vinegar flies, *Drosophila melanogaster* (Drosophilidae), were purchased from Sumika Technoservice (Hyogo, Japan) and reared on blue medium (Wako Pure Chemical, Osaka, Japan) in plastic containers (20 cm in diameter, 20 cm high) in a growth chamber ($25 \pm 0.5 \text{ }^\circ\text{C}$, 14 h photoperiod with 4000 lux). Newly emerging adults (15–24 h after eclosion) were used as active flies for the following experiments. Flies were transferred using an insect aspirator (Fig. 1C), a polypropylene tube (10 mm diameter) with a pointed tip (tip diameter, 1 mm) and whose opposite open end was linked to an aspirator (aspiration pressure, 1.2 kg/cm^2). All transferred flies walked and flew normally and appeared to be unhurt by the collection operation.

Vinegar fly adults were singly placed at a particular site of the earthed net to determine the range of voltages causing attraction to the ICW. Movements by the attracted flies were video-recorded with a charge coupled device (CCD) camera equipped with a dissecting microscope while applying voltage for 60 s. Movements were observed continuously until the insects were removed from the ICW after voltage to the ICW was stopped.

2.3. Current measurement

The electric current generated during a mechanical discharge (transfer of electricity from the charged ICW to a ground) and an insect discharge (transfer of electricity from the insect to a ground) was measured at different voltages with a current detector (detectable limit, $0.1 \mu\text{A}$) integrated into the galvanometer in the earthed line. The magnitude and duration of the current were recorded in both discharges.

3. Results

First, we examined the occurrence of a mechanical discharge from the ICW under different voltage conditions. Current from the ICW was detected at $>7.1 \text{ kV}$ (Fig. 2). At 7.1–10.0 kV, the current magnitude rose from 0.1 to $10.5 \mu\text{A}$ as voltage increased. Current magnitude was continuous and constant at each voltage. In the following experiments, the insects were examined for their discharge at voltages of $<7.0 \text{ kV}$ to avoid concealing the insect discharge by larger electric currents derived from the ICW mechanical discharge.

Fig. 2 also shows the voltage ranges that resulted in insect attraction and insect discharge. An electrostatic force was initially detectable at 0.5 kV, because the flies seemed to erect their wings and brace against the ICW attraction force. Attraction of flies to the ICW was detected at voltages $> 0.9 \text{ kV}$. However, at 0.9–2.8 kV (range A), the force was not sufficient to capture the insects with the ICW. In fact, the flies were drawn upwards but flew away from the electric field without being captured. For voltages $>2.9 \text{ kV}$, the flies were attracted to the ICW, regardless of the voltage applied. At 2.9–4.0 kV (range B), the attracted flies twisted their bodies with vigorous strokes of their legs and then rolled over to remove themselves from the charged ICW. The time duration for removal was 2–20 s. These times were directly proportional to an increase in the voltage applied; eventually, all flies escaped the ICW within this voltage range. At 4.1–7.0 kV (range C), the attracted flies were completely prevented from removing themselves from the ICW during the period when voltage was applied. They struggled to remove themselves from the ICW for a short period but then became motionless.

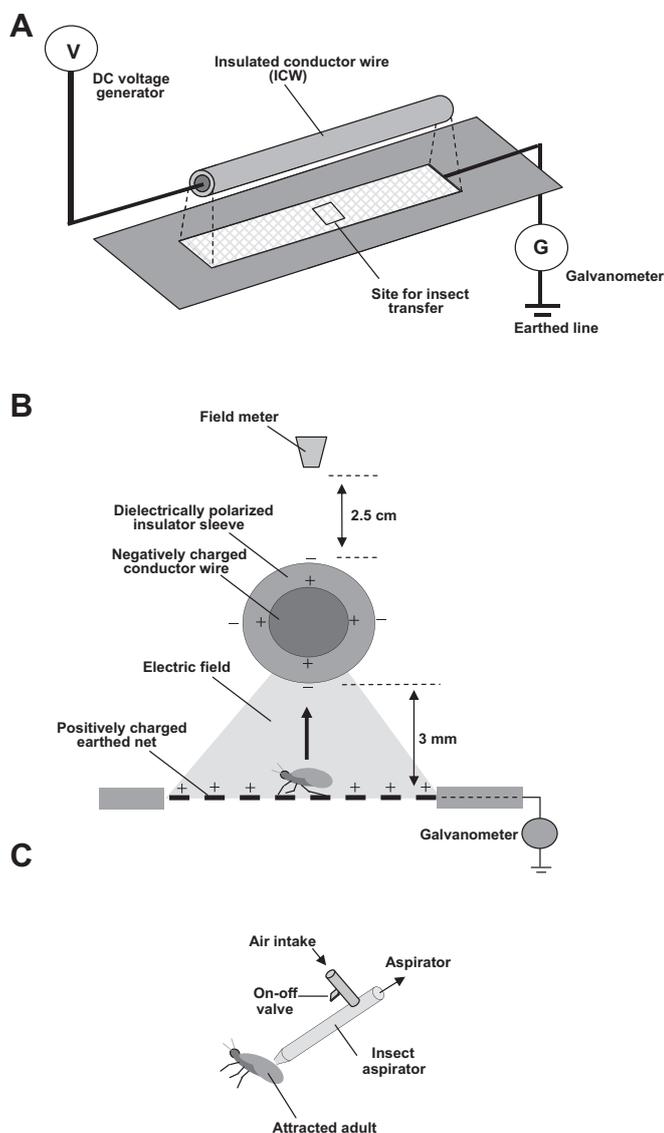


Fig. 1. Diagrams of a simplified electric field screen (A), the electrostatic details in the formation of an electric field (B), and the insect aspirator to transfer the flies (C).

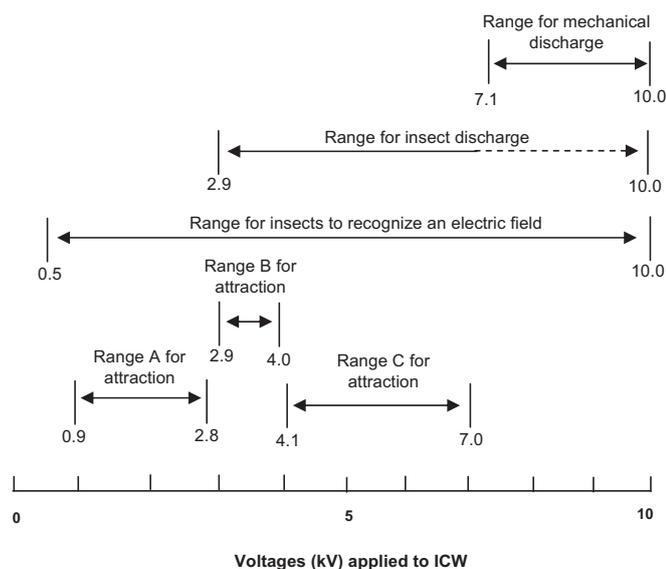


Fig. 2. Voltage ranges for insect attraction and mechanical and insect discharge. Numbers in the figure show the voltages (kV) applied to the insulated conductor iron wire (ICW).

Insect discharge was detected at >2.9 kV. We selected three voltages (2.9, 4.1, and 7.0 kV) to analyze the relationship between the movements and electric current generated by the flies. The lowest voltage permitted the insects to remove themselves from the ICW, and the two higher voltages prevented the insects from escaping from the ICW. In Fig. 3, we show the electric current profiles from the flies before and after their attraction to the ICW that was negatively charged with 7.0 (Fig. 3A), 4.1 (Fig. 3B), or 2.9 kV (Fig. 3C). The first peak at each voltage was detected immediately after a fly was placed on the net but prior to attracting the insect to the ICW. The peak magnitudes became higher as the voltage increased (Table 1). Subsequent peaks (secondary peaks) were the electricity release associated with the movements of the attracted flies, which included bending of the head and/or tail upward and twisting of the body as the flies were lying on their backs. These movements were accompanied by rapid or slow leg strokes. Wing flapping was not observed because of the tight attraction of the wings to the ICW. All secondary peaks corresponded to these individual movements. At the two higher voltages (Fig. 3A and B), the movements were first successive and then intermittent. Eventually, the flies became motionless without removing themselves from the ICW. The magnitudes of these peaks were larger in direct proportion to the increase in the voltages applied. This tendency was clear, particularly when the separate and intermittent peaks among different voltages were compared (Table 1). However, at the lowest voltage (Fig. 3C), the flies successfully lifted their attracted wings by first twisting their body and then rolling over to escape the ICW, without generating secondary electric current peaks.

After the voltage application was stopped, the potential difference in the ICW vanished within 5–8 s, depending on the strength of the voltages that had been used to charge the ICW. During this period, the flies remained motionless (Fig. 3A and B). Apparently, the insects needed time to resume movement and this duration seemed to depend on the decreased potential difference in the ICW. In fact, the flies tried to lift their heads and tails and then twist their bodies with active strokes of the legs when the potential difference decreased to approximately 0.5 kV. After being removed from the ICW, all flies appeared to be unharmed; they walked and flew normally and showed similar longevity to untested flies (data not shown).

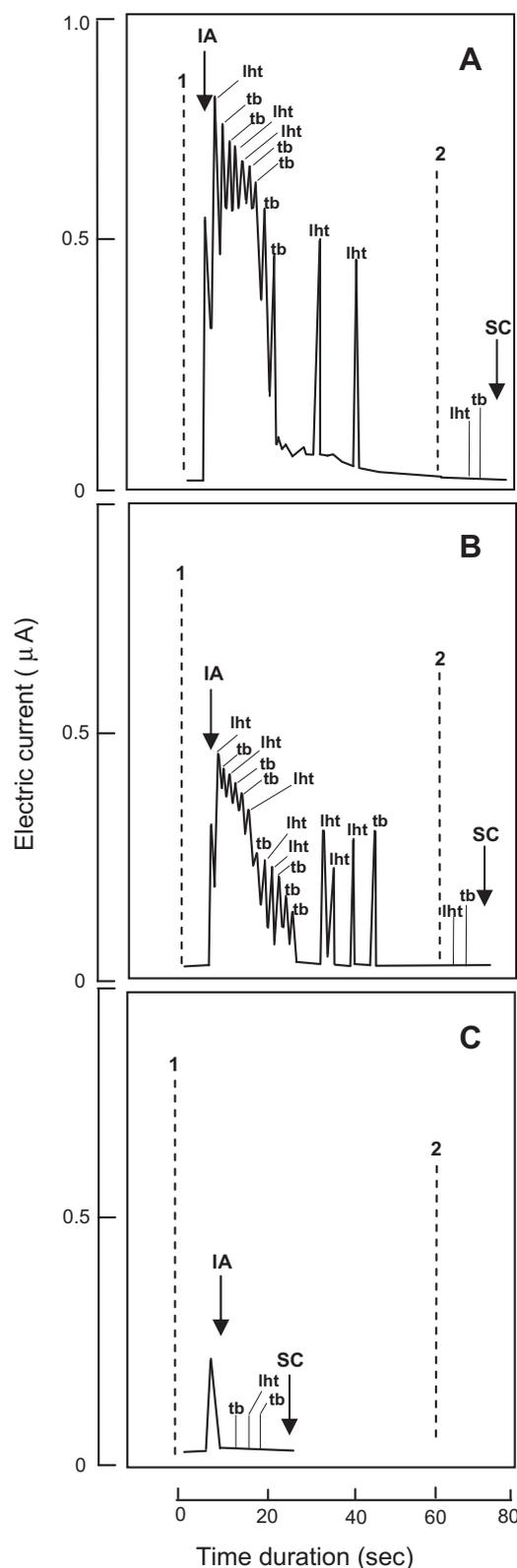


Fig. 3. Profiles of electric current generation and movements by vinegar fly adults attracted to the insulated conductor iron wire (ICW) negatively charged with 7.0 (A), 4.1 (B), or 2.9 kV (C). Dashed lines 1 and 2 represent the timing of placing a fly on the earthed net and stopping the voltage application, respectively. Arrows IA and SC represent the timing of attracting the fly to the ICW and escaping from the ICW, respectively. Fly movements observed were lifting of the head and/or tail (lht) and twisting of the body (tb). Abbreviations for these movements are given on the peaks or vertical solid lines (in case of no current generation).

Table 1
Electric current from adult vinegar flies in an electric field.

Voltage range ^a	Voltage applied (kV)	Magnitude (μ A) of electric current at	
		The first peak before attraction	Separate secondary peaks after attraction ^b
A	0.9	n.d. ^c	n.d.
	2.8	n.d.	n.d.
B	2.9	0.12 \pm 0.02 a	n.d.
	3.5	0.12 \pm 0.01 a	n.d.
	4.0	0.13 \pm 0.02 a	n.d.
C	4.1	0.14 \pm 0.02 a	0.11 \pm 0.01 a
	4.5	0.16 \pm 0.03 a	0.12 \pm 0.02 a
	5.0	0.18 \pm 0.02 a	0.14 \pm 0.02 a
	5.5	0.21 \pm 0.03 ab	0.16 \pm 0.02 ab
	5.5	0.29 \pm 0.05 b	0.19 \pm 0.02 b
	6.0	0.38 \pm 0.05 c	0.24 \pm 0.02 c
	7.0	0.51 \pm 0.06 d	0.31 \pm 0.03 d

Data are given as the mean and standard deviation of five replications. Different letters with mean values indicate a significant difference ($p < 0.05$) according to Tukey's method.

^a Refer to Fig. 2.

^b Of the secondary peaks; separately generated peaks were selected to facilitate exact measurement.

^c Not detected.

4. Discussion

The first aim of the present study was to clarify the voltage ranges that would cause a continuous electric current from the ICW to the earthed net, because this current made it difficult to detect smaller currents from the insects. An electric current from an insulated conductor depends on the insulation resistance at a given voltage, which determines the level of insulator conductivity [13]. Under the present conditions, the electric current occurred at voltages > 7.1 kV, so we conducted the experiments at 0.1–7.0 kV, at which the electric current from the ICWs was not detected.

The second aim was to assess whether a negative charge in the charged conductor wire could be transferred to the insect on the earthed net over the insulation resistance of a vinyl chloride sleeve used for covering, because insects are a type of conductor [14,15]. If so, the distance between two oppositely charged conductor poles becomes shorter. The pole distance is an additional factor that determines the discharge between opposite poles [16]. If electricity transfer occurs, the negative charge can be accumulated in the insect or flow to the earthed net via the insect on the net. If accumulation occurs, the negative charge in the insect creates a force to repel against the ICW of the same charge, preventing the insect from being drawn toward the ICW. Alternatively, the flow of the charge to the earthed net implies that the insect on the net acts as part of the earthed conductor. This implies continuous flow of current from the ICW to the insect connected to the earthed net. However, the present results contradicted these possibilities; the insects were attracted to the ICW immediately after they reached the net, and the electric current generated was transient under the present voltage conditions.

The primary focus of this study was to analyze the dynamic relationship between the physical force of the ICW and biological power exerted by the insects struggling to escape from the ICW. For this purpose, it was essential to clarify the insect attraction mechanism. From the present results, we can postulate that the attraction was the consequence of three successive events in the insect: 1) polarization on the earthed net, 2) positive-charging, and 3) being drawn toward the ICW. The first problem was specifying a polarization site in the adults. Many studies [8,10,17,18] have reported that the cuticle, an outer protective layer that covers the body of many invertebrates, is efficiently electrified because of its highly conductive nature. Vinegar fly adults also develop this cuticle structure [19]. Based on this electrostatic characteristic of

the cuticle, we assumed that the cuticle structure was a potential site for polarization in vinegar fly adults. The negative charge of the cuticle moved toward the earthed-net side of the insect, because the ICW side surface of the earthed net was oppositely charged [6]. Eventually, the insects polarized positively on the ICW side and negatively on the net side of the cuticle. Subsequently, the negative charge on the earth-side of the cuticle was transferred to the earthed net, and the adults became net positive. An additional important result was that insect electricity flowed prior to insect attraction (see the first peaks in Fig. 3A, B, and C). These results strongly suggest that the insect discharge (electricity released) was a trigger for subsequent insect attraction during the final step. Force was generated between opposite charges of the insect (positive) and the ICW (negative charge). Importantly, grounding the conductor (net) was essential to receive a charge; in fact, cutting off the earthed line resulted in the loss of insect discharge, and a failure of the insect to be attracted to the ICW (data not shown). Judging from these results, we concluded that the electricity released from the insect was essential to attract the insect.

Success or failure to continuously restrain the attracted flies depended on the voltages applied. The results indicated that higher voltage applications pushed larger amounts of electricity out of the insect (Table 1). Apparently, the higher positive electrification of the insect created a higher electrostatic force against the opposite charge of the ICW. If our interpretation is correct, then insects can remove themselves from the ICW by dispelling this attraction force; that is, by neutralizing the positive charge in their body. We did not detect a flow of negative charge to the adults from the earthed net, so self-production of electrons by the attracted insect was postulated as an alternative mechanism for this purpose. Muscular movement-mediated electric power generation has been reported in some insect species such as the cockroach [7], flour moth [11], and mealworm beetle [9], and our results also showed possible power production by vinegar fly adults in an electric field. In range C, in which the flies were prevented from escaping the ICW, we detected electric currents that were associated with skeletal muscular movements. All movements observed were muscular exertion by which the insects tried to regain their balance and fly away from the ICW. Although the mechanisms for the generation of bioelectric power remain obscure, it was obvious that the physical action of skeletal muscles generated bioelectricity, and that the generation efficiency of the muscular actions varied among the different voltage conditions. In particular, compared to the peaks in the electric currents between 4.1 and 7.0 kV, we found a prominent difference in the peaks for the movements that were intermittently performed by the attracted flies (the last two peaks in Fig. 3A, four peaks in Fig. 3B, and Table 1).

Electricity produced biologically can be transmitted to a superficial cuticle conductor [10,17]. Also, in the present case, the produced electricity could have been transferred to the cuticle but then quickly drawn to the earthed net. Our results indicated that the range C voltages were sufficient to push the produced electricity out of the flies, leading to a failure to neutralize the positive charge. In our opinion, this is the major reason why the attracted flies could not escape from the force of the ICW. In contrast, the range B voltages were apparently insufficient to push out the electricity produced by the flies (see insect movements without electric current in Fig. 3C), so the electricity produced through movement was utilized for neutralization, which resulted in the release of the flies from the ICW.

In the electric field, the flies were always exposed to the attraction force driven toward the ICW. This force was larger with application of larger voltages. Obviously, muscles appeared to be loaded with the force to hinder their actions, so the movements of the flies were very slow and heavy. Under this condition, muscle fatigue was quick, and

the flies became motionless. Nevertheless, this situation did not harm the insects. In fact, the tested flies walked and flew normally, and could lay eggs (data not shown) after they were released from electrostatic restraint (after a 1 min restraint).

The charge of an electrified insulator vanishes gradually after the voltage application is removed [20]. In our study, the levels of ICW surface charge decreased gradually after the voltage application was stopped. The attracted, motionless flies tried to move again in response to the attenuated voltage (potential difference) on the ICW. Interestingly, the flies resumed their movement when the potential difference dropped to 0.5 kV. Assuming that bioelectricity was also generated during this period, this voltage was insufficient to push the produced electricity out of the insect. In fact, we detected no flow of electric current accompanied by individual movements. In our understanding, the electricity produced could be used to neutralize the positive charge in the insect body, because the remaining positive charge in the insect may have the potential to create additional force. It is known that the charge of a conductor causes polarization (dielectric polarization) of an insulator [21,22]. The positive charge of the insect caused polarization in the insulator sleeve at the site of insect attraction; negatively on the insect-side surface and positively on the conductor-side surface of the sleeve. An electrostatic attraction force was generated between opposite surface charges of the ICW and the insect. It was necessary to nullify the positive charges in the insects for their release.

5. Conclusion

We succeeded in clarifying the mechanism for attraction and the relationship between muscular movements and electric power generation by the trapped insects, using a simple version of an electric field screen. By applying appropriate voltages, the earthed net deprived the negative charge localized on the net side of the insect cuticle layer. These insects then became net positive and were drawn toward the negative charge of the ICW. This attraction force increased in direct proportion to the increase in voltage applied to the ICW. The attracted insects generated electricity during skeletal muscular movements, but the generated electricity flowed to the earthed net and was not used to neutralize their positive charge, which was expected to be a possible countermeasure to nullify the ICW attraction force. Thus, the results provide a reliable explanation for the ability of an electric field screen to continuously restrain attracted insects.

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