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RESEARCH ARTICLE

Insects are electrified in an electric field by deprivation of their negative charge

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Abstract

An electric field screen (EF-screen) is a physical device for excluding pest insects from greenhouses and warehouses to protect crops during their production and storage periods. In this study, a simple version of the EF-screen, an insulated conductor iron wire (ICW) paralleled to an earthed net, was constructed to effectively observe the attraction of test insects in relation to their electricity release. The ICW was negatively charged to dielectrically polarise the insulator sleeve of the ICW: negatively on the outer surface and positively on the inner conductor wire surface of the sleeve. The negative surface charge of the ICW caused an electrostatic induction in the earthed net and a resultant positive charge at the ICW-side surface of the net. An electric field formed between the ICW (negative pole) and earthed net (positive pole). Insects were attracted to the ICW when they were placed onto the earthed net. A vital step for the attraction was the creation of a transient bioelectric discharge from an insect. During this discharge, an electric charge of the insect was transferred to the earthed net. Eventually, the insect became net positive and was then attracted to the ICW. The magnitude of the current increased in direct proportion to the increase in voltage applied to the ICW, and the attraction force was directly proportional to the increase in the electric current. Larger voltages were necessary to attract much larger insects because larger insects were stronger and therefore more able to escape from the ICW attraction. Similar results were obtained for a wide range of pest insects belonging to different taxonomic groups (8 orders and 15 families). This study demonstrated that transient bioelectric discharge is common in insects and can be utilised to create an electrostatic force capable of moving insects in a generated electric field.

Introduction

Agricultural crop plants suffer from pathogen infection and/or insect attack during their pre- and post-harvest stages. Chemical, biological and physical methods have been developed to prevent these attacks. However, excessive application of agrochemicals such as fungicides and insecticides often causes chemical resistance or less sensitive mutants of the pathogens and pest insects and/or environmental pollution (Ma & Michailides, 2005; Nauen & Denholm, 2005), and biological controls using antagonistic microbes or natural enemies are not always effective and stable in their suppressive effects (Helyer *et al.*, 2004). Insect-excluding woven screens with a fine mesh size have been a conventional physical method to minimise insect entry to glasshouses, but the disadvantage of screening is a reduction in ventilation that can cause overheating and increase relative humidity (Weintrub & Berlinger, 2004). In the interest of protecting crops during production and storage, we have developed electrostatic methods to disinfect bacterial and fungal plant pathogens

(Shimizu *et al.*, 2007; Nonomura *et al.*, 2008) or to prevent airborne pathogens and flying insect pests from entering greenhouses (Matsuda *et al.*, 2006; Tanaka *et al.*, 2008), with the aim of reducing the use of fungicides and insecticides. An electric field screen (EF-screen) has been practically used as an environment-friendly tool to exclude pathogens and pests from spaces of plant cultivation (Kakutani *et al.*, 2012) and storage (Matsuda *et al.*, 2011) with better air penetration.

The EF-screen consists of three components: insulated conductor iron wires (ICWs) arrayed in parallel and at 5-mm intervals, earthed stainless nets placed on both sides of the ICW layer and 3.0 mm from the ICW layer, and an electrostatic voltage generator to supply charge to the ICWs. Iron wires were passed through vinyl chloride sleeves for insulation; these wires were then linked to each other and to a voltage generator. The ICWs were negatively charged to dielectrically polarise the insulator sleeve of the ICW: negatively on the outer surface and positively on the inner conductor wire surface. The negative surface charge of the ICWs causes an electrostatic induction in the earthed nets (conductor), creating the opposite surface charge on the ICW side surface of the nets. These opposite charges act as dielectric poles to form an electric field between the ICW layer and the earthed nets. Insects coming into the electric field were attracted to the ICW.

Optimisation of the voltage applied to the ICWs is crucial for economical and safe use of the screen. Especially with >5.1 kV, the screen constantly generates needless direct current from the ICWs to the earthed nets. The experiment was ideally conducted with application of lower voltages (Matsuda *et al.*, 2011). At between 4.0 and 5.0 kV, without causing mechanical discharge from the ICWs, test insects (cigarette beetle and vinegar fly, pests of warehouse and food processing factories) were tightly captured with the ICWs so that they could not escape from the trap. In this voltage range, the release of negative charge from the insects to the earthed net was immediately detected and lasted for a short period (within 2 min) after they came into the electric field of the screen. This short-period discharge from the insect was designated as a transient bioelectric discharge. In the lowest range (1.0–3.9 kV), we observed weak and insufficient attraction of insects without this transient discharge. From these results, we presumed that the tight attraction of the insects to the ICW was a result of their release of negative charge.

In our recent survey, however, this transient bioelectric discharge was not detected in insects, such as whiteflies, thrips and aphids, even when these insects were successfully trapped with the screen. Hence, it was essential to clarify an insect-attraction mechanism that is generally applicable to many insects. In this study, we examined a wide range of ubiquitous insect pests classified into different taxonomic groups covering eight orders (including 15 families): leafminer flies, green peach aphids, whiteflies, western flower thrips, green rice leafhoppers and greenhouse shore flies, all of which are pests of greenhouses and/or field crops (Foote, 1995; Helyer *et al.*, 2004); rice weevils, red flour beetles and adzuki bean weevils are pests that attack post-harvest crops in warehouses, and book lice cause damage to manuscript stacks in museums (Hill, 1990); German cockroaches, oriental termites, bathroom flies and common cloth moths are pests resident in homes and offices (Hill, 1990); and the Asian tiger mosquito is a human pest (Foster & Walker, 2002). In this study, we constructed a simple version of the EF-screen to effectively examine electricity release from insects in relation to their attraction to the ICW to generalise this bioelectric phenomenon among the insects.

Materials and methods

Insects

Test insects are listed in Table 1. Four insects (whitefly, green peach aphid, western flower thrips and greenhouse shore fly) constituted our laboratory stocks and were maintained in the growth chamber (25.0 ± 0.5°C, 12-h photoperiod of 4000 lux) according to a method described previously (Kakutani *et al.*, 2012). Larvae or pupae of other insects were purchased from Sumika Technoservice (Hyogo, Japan) and incubated for eclosion in the growth chamber under the same conditions mentioned above. Adults of test insects were collected with an insect aspirator (Fig. $1A_1$); a polypropylene tube (diameter, 10 mm) with a pointed tip (tip diameter, 0.1 mm for whiteflies, aphids, western flower thrips and book lice; 1 mm for other larger insects), and the opposite open end of the tube was linked to the aspirator (aspiration pressure, 1.2 kg cm^{-2}). All collected insects walked and flew normally and appeared to be unhurt by the collection. Body sizes of adults were expressed as length from head to wing edge. Length was measured using 30 adult test insects collected at random.

A simplified EF-screen and assay for insect attraction

We constructed a pair of electrodes: an iron wire (length, 20 cm; diameter, 2 mm) linked to a DC voltage generator (Max Electronics, Tokyo, Japan) and an earthed stainless net (mesh size, 1.5 mm, 5 × 20 cm) as a simplified EF-screen. An iron conductor wire was passed through a vinyl chloride sleeve (thickness, 1 mm; resistance, 1.5×10^9 Ω) to make an ICW. Both electrodes were arrayed

Table 1 Insect pests used in this study

Order	Family	Genus and Species	Common Name	Body Length (mm)
Diptera	Agromyzidae	*Liriomyza sativae* (Blanchard)	Tomato leafminer fly	1.7 ± 0.1
	Ephydridae	*Scatella stagnalis* (Fallen)	Greenhouse shore fly	4.1 ± 0.2
	Psychodidae	*Clogmia albipunctatus* (Williston)	Bathroom fly	1.9 ± 0.1
	Culicidae	*Aedes albopictus* (Skuse)	Asian tiger mosquito	2.8 ± 0.2
Hemiptera	Aphididae	*Myzus persicae* (Sulzer)	Green peach aphid	2.1 ± 0.1
	Aleyrodidae	*Bemisia tabaci* (Gennadius)	Whitefly	0.8 ± 0.1
	Cicadellidae	*Nephotettix cincticeps* (Uhler)	Green rice leafhopper	4.8 ± 0.4
Coleoptera	Rhynchophoridae	*Sitophilus oryzae* (Linnaeus)	Rice weevil	4.4 ± 0.2
	Tenebrionidae	*Tribolium castaneum* (Herbst)	Red flour beetle	3.4 ± 0.2
	Bruchidae	*Callosobruchus chinensis* (Linné)	Adzuki bean weevil	3.5 ± 0.1
Isoptera	Rhinotermitidae	*Coptotermes formosanus* (Shiraki)	Oriental termite	2.9 ± 0.1
Psocoptera	Liposceilidae	*Liposcelis bostrychophilus* (Badonnel)	Book louse	1.1 ± 0.1
Blattodea	Blattellidae	*Blattella germanica* (Linnaeus)	German cockroach	5.1 ± 0.3
Lepidoptera	Tineidae	*Tineola bisselliella* (Hummel)	Common clothes moth	3.0 ± 0.1
Thysanoptera	Thripidae	*Frankliniella occidentalis* (Pergande)	Western flower thrips	1.8 ± 0.1

in parallel at a 5-mm interval (Fig. 1B). The ICW was negatively charged to dielectrically polarise a cover insulator: positively on the iron wire side surface and negatively on the outer surface of the insulator sleeve (Matsuda *et al.*, 2011). The negative surface charge of the ICW polarised 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. 1C).

Adult test insects were placed singly on a particular site on the earthed net (Fig. 1C) to determine the range of voltages that caused attraction of the insects to the ICW. Twenty adults were used per insect and per voltage. Three separate experiments were conducted for each insect species. Experiments were conducted in a temperature-controlled laboratory (25.0 ± 1.0°C) at relative humidity of 40% to 55%.

Measurement of electric current from mechanical and bioelectric discharges

A galvanometer (PC520M; Sanwa Electric Instrument Co., Tokyo, Japan) was integrated into the electric line of the earthed net (Fig. 1B) to detect the transfer of electricity to the ground through an earthed line linked to the net, as negative charging pushes negative electricity (free electrons) in an insulator to ground via an earthed opposite pole (Jonassen, 2002). In this study, different voltages (1–15 kV) were applied to the ICW to determine the voltage range that caused a mechanical discharge (transfer of electricity from the charged ICW to the earthed net). The electric current generated during the discharge was monitored with a current detector integrated into the galvanometer (detectable limit, 0.1 μA).

At voltage ranges causing no mechanical discharge, insect discharge (release of electricity from the insect to the earthed net) was similarly monitored with the galvanometer, as insect electricity could also be transferred to ground in an electric field caused by negative voltage. Twenty adults were used per insect and per voltage, and data are given as mean values and SD of three replications. In addition, different numbers of adults were simultaneously placed on the earthed net using a multiple-channel aspirator (Fig. $1A_2$) composed of tipped polypropylene tubes (diameter, 2 mm; tip diameter, 0.1 mm) linked to each other and to an aspirator. In these cases, the highest electric current magnitude in the insect discharge was recorded (multiple insect assay). For each insect species, three separate experiments were conducted per voltage. Experiments were conducted under the same conditions as mentioned above.

Results

In this study, we set the distance between the ICW and earthed net to 5 mm because the distance (3 mm) of the original screen was so narrow that the antennae of larger insects touched the ICW when they were placed on the net, and because the 5-mm distance kept the wings distant from the ICW, even when insects opened their wings on the net.

First, we examined the occurrence of mechanical discharge from the ICW under different voltage conditions. Discharge was detected at >13.2 kV (Fig. 2). At 13.2–15.0 kV, the electric current magnitude rose from 0.1 to 10.5 μA as voltage increased. The electric current magnitude was continuous and constant at each voltage. In the following experiments, the insects were examined for

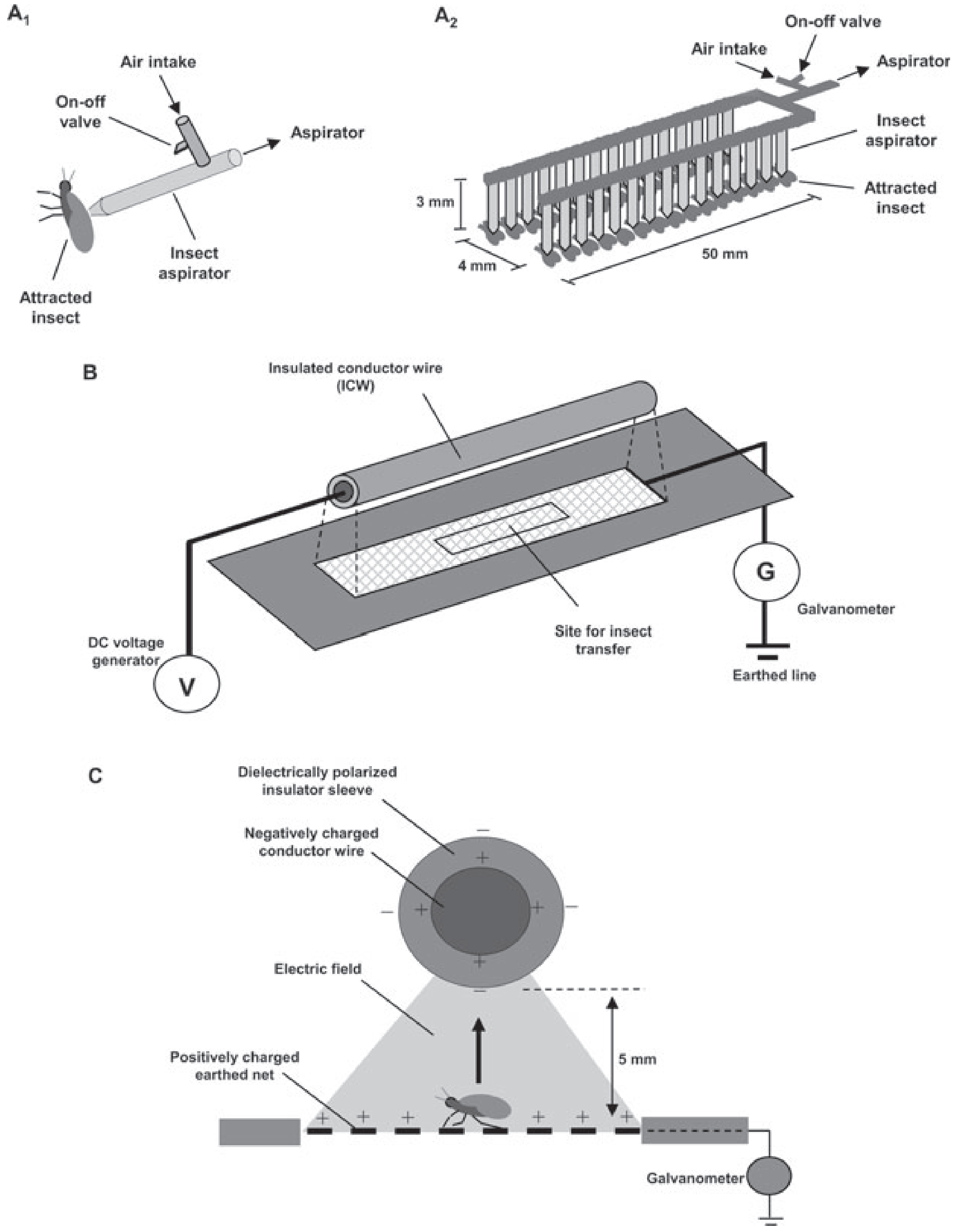

Figure 1 Diagrams of the two insect aspirators (A_1 and A_2), the simplified electric field screen (B), and the electrostatic details in the formation of an electric field (C).

their discharge at voltages of <13.1 kV because larger electric currents derived from the ICW mechanical discharge concealed smaller currents from the insect discharge at larger voltage ranges.

We examined the range of voltages showing a 100% capture rate for each insect species (Fig. 2). Although the lowest voltage in the range varied among the insects tested, all insects were attracted to the ICW immediately after they were placed on the earthed net. The attraction force of the ICW increased in direct proportion to the increase in the voltage applied to the ICW. At these voltages, all insects were tightly captured so that they could not move away from the ICW; they struggled vigorously by lifting their heads and/or tails or twisting their bodies for 20–30 min, and then became motionless. Although the insects remained motionless until the end

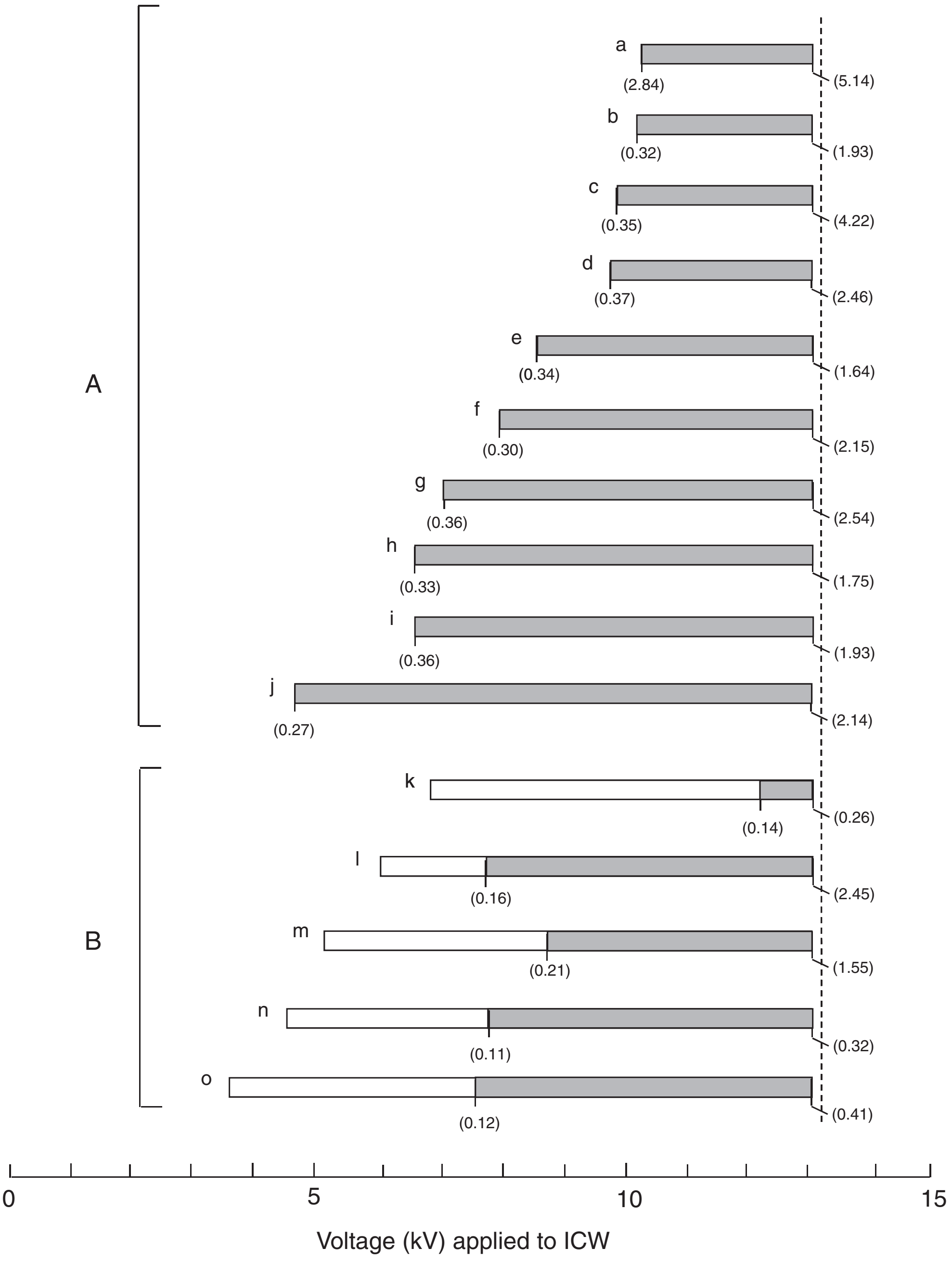

Figure 2 Voltage ranges showing the 100% capture rate (open column) and a transient bioelectric discharge (grey column) in test insects. The dotted line represents the voltage causing a mechanical discharge. Figures in parentheses show the current magnitudes of the transient bioelectric discharge. Test insects were classified into two groups (A and B) on the basis of appearance of the transient bioelectric discharge. Insects used were German cockroach (a), rice weevil (b), green rice leafhopper (c), greenhouse shore fly (d), adzuki bean weevil (e), red flour beetle (f), Asian tiger mosquito (g), green peach aphid (h), common clothes moth (i), bathroom fly (j), western flower thrip (k), oriental termite (l), tomato leafminer fly (m), book louse (n) and whitefly (o).

of the experiment, they initiated movement after the voltage impression to the ICW was stopped, and then they flew away from the ICW. In the following range of lower voltages, however, the force was not strong enough to prevent the captured adults from escaping the ICW, and eventually the insects struggled to move away from the ICW (data not shown). In the lowest voltage range, attraction did not occur in all test insects. In Fig. 3, we examined the relationship between body size and the lowest voltages of the 100% capture rate among the

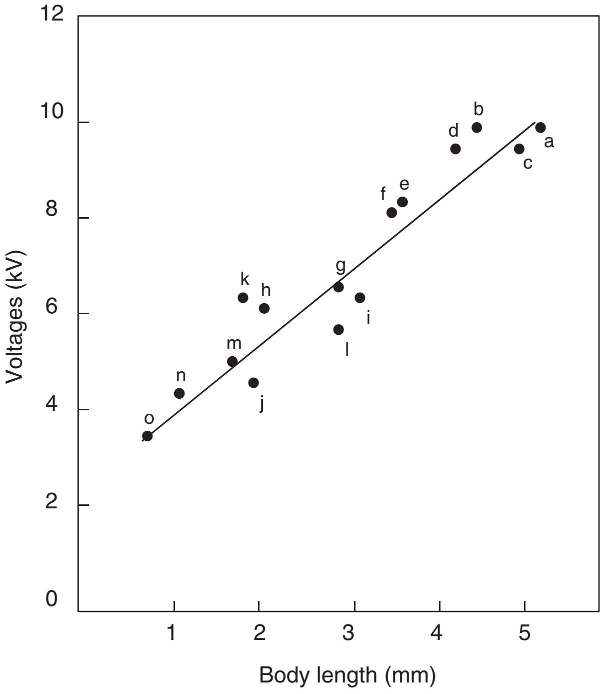

Figure 3 Relationship between body sizes of test insects and the lowest voltages showing the 100% capture rate. Small letters assigned to dots represent the insects tested (refer to the legend of Fig. 2).

test insects. Obviously, larger voltages were necessary to capture larger insects because larger insects were stronger and therefore more able to escape from the ICW attraction (linear regression $y = 1.4751x + 2.7121$; $R^2 = 0.9035$, $P < 0.0001$).

Fig. 2 also shows the range of voltages that caused transient discharge from the test insects. In this experiment, we classified the insects into two groups (Groups A and B). The insects of Group A caused discharge in all voltages of the 100% capture range, whereas the insects of Group B showed discharge at voltages larger than the lowest voltage of the 100% capture range. In both groups, the electric current magnitudes were larger as the voltages increased.

Fig. 4 shows the profile of the electric current flow from a German cockroach adult when the ICW was negatively charged with the highest (A), middle (B) and lowest (C) voltages of its 100% capture range. The first release of insect electricity (pre-attraction peak) occurred immediately after the adult was placed on the net and just before the adult was attracted to the ICW. The subsequent release (post-attraction peaks) was detected when the attracted insect struggled by repeatedly lifting its head and tail and twisting its body. The post-attraction peaks disappeared when the insect became motionless (Fig. 4A and Fig. 4B).

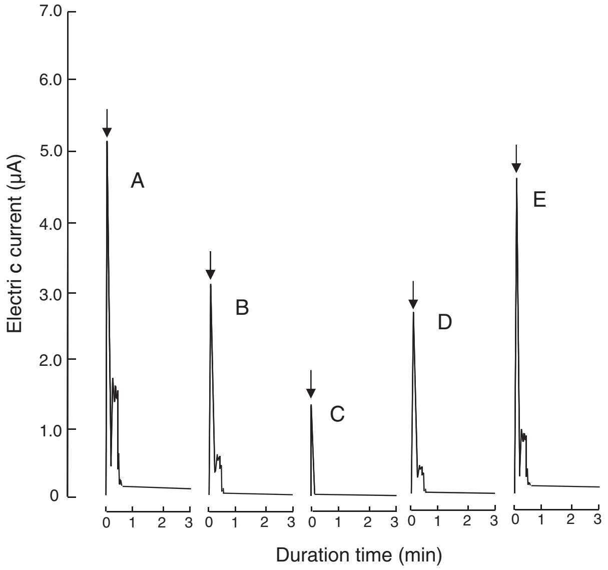

Figure 4 Electric current profiles in a transient bioelectric discharge of German cockroach adults. The ICW was negatively charged with 13.1 (A), 12.0 (B), and 9.8 kV (C), and single adults were placed on the earthed net. The applied voltages were the highest, middle, and lowest voltages of the 100% capture range, respectively. Two (D) and three adults (E) were simultaneously placed on the net at 9.8 kV. Arrowed single peaks indicate the first current from the insect prior to the attraction to the ICW, followed by the multiple smaller peaks of the current after the attraction (A, B, D, and E).

The magnitudes of both pre- and post-attraction peaks were lower as the applied voltage decreased (Fig. 4A and Fig. 4B), and the post-attraction peaks disappeared with application of the lowest voltage (Fig. 4C). To confirm that this disappearance was as a result of the decrease in the magnitude below the limit of a galvanometer, we conducted a multiple insect assay. The electric current magnitudes at the first peak increased additively when two (Fig. 4D) and three adults (Fig. 4E) were simultaneously placed on the net, and the second peaks appeared again in the application of two and three adults (Fig. 4D and Fig. 4E). Similar results were obtained in all insects of Group A.

In addition, the multiple insect assay was conducted to confirm the occurrence of discharge at the lowest voltage of the 100% capture range in all insect species (Table 2). The electric current became detectable when multiple adults were simultaneously placed on the net, and the increase in the current magnitude was additive in all tested insects. From these results, we estimated the current magnitude per adult. It was obvious that these small insects also released their electricity to the earthed net at all voltages of the 100% capture range.

Table 2 Electric current from test insects simultaneously placed on the earthed net

Test Insects	Number of Adults Used	Current Magnitudes (μA) at the First Peak	Estimated Current Magnitudes (μA) per Adult
German cockroach	1	2.82 ± 0.14	2.82 ± 0.14
	2	5.25 ± 0.22	2.62 ± 0.11
	3	8.52 ± 1.12	2.84 ± 0.37
Rice weevil	1	0.32 ± 0.01	0.32 ± 0.01
	3	0.86 ± 0.09	0.29 ± 0.03
	5	1.41 ± 0.23	0.28 ± 0.05
Green rice leafhopper	1	0.35 ± 0.04	0.35 ± 0.04
	3	1.06 ± 0.15	0.35 ± 0.05
	5	1.75 ± 0.36	0.35 ± 0.07
Greenhouse shore fly	1	0.37 ± 0.09	0.37 ± 0.09
	3	1.18 ± 0.28	0.39 ± 0.09
	5	1.90 ± 0.28	0.38 ± 0.05
Adzuki bean weevil	1	0.34 ± 0.03	0.34 ± 0.03
	3	0.97 ± 0.29	0.32 ± 0.10
	5	1.60 ± 0.39	0.33 ± 0.08
Red flour beetle	1	0.30 ± 0.04	0.30 ± 0.04
	3	0.90 ± 0.07	0.30 ± 0.02
	5	1.47 ± 0.21	0.29 ± 0.04
Asian tiger mosquito	1	0.36 ± 0.02	0.36 ± 0.02
	3	1.12 ± 0.32	0.37 ± 0.11
	5	1.88 ± 0.17	0.38 ±0.03
Green peach aphid	1	0.33 ± 0.23	0.33 ± 0.23
	3	0.96 ± 0.25	0.32 ± 0.08
	5	1.60 ± 0.15	0.32 ± 0.03
Common clothes moth	1	0.36 ± 0.02	0.36 ± 0.02
	3	1.09 ± 0.35	0.36 ± 0.12
	5	1.90 ± 0.21	0.38 ± 0.04
Bathroom fly	1	0.27 ± 0.02	0.27 ± 0.02
	3	0.87 ± 0.08	0.29 ± 0.03
	5	1.38 ± 0.19	0.28 ± 0.04
Western flower thrips	15	0.13 ± 0.03	0.009 ± 0.002
	20	0.15 ± 0.05	0.008 ± 0.003
	30	0.23 ± 0.07	0.009 ± 0.003
Oriental termite	3	0.24 ± 0.02	0.08 ± 0.006
	5	0.44 ± 0.03	0.09 ± 0.007
	7	0.61 ± 0.03	0.09 ± 0.005
Tomato leafminer fly	5	0.15 ± 0.02	0.03 ± 0.005
	7	0.21 ± 0.02	0.03 ± 0.003
	10	0.31 ± 0.03	0.03 ± 0.003
Whitefly	10	0.13 ± 0.03	0.013 ± 0.004
	20	0.28 ± 0.08	0.014 ± 0.005
	30	0.41 ± 0.06	0.014 ± 0.002
Book lice	15	0.11 ± 0.02	0.007 ± 0.002
	20	0.12 ± 0.02	0.006 ± 0.001
	30	0.19 ± 0.03	0.006 ± 0.001

In the last experiment, we examined the relationship between body size and magnitude of released electricity in all test insects. In this experiment, the highest voltage (13.1 kV) of the present work was used to obtain the highest electric current magnitude in the test insects. At this voltage, all insects of Group A and two insects (tomato leafminer flies and oriental termite) of Group B showed both pre- and post-attraction peaks, whereas the remaining three insects (book lice, whiteflies and western flower thrips) of Group B showed only the pre-attraction peak (because the magnitudes of their post-attraction peaks were below the detection limit). From these results, we compared the magnitude data of the pre-attraction peaks commonly detected in all test insects (Fig. 5). The data showed a considerably high linear regression slope (linear regression $y = 0.8184x + 0.3479$; $R^2 = 0.8035$, $P < 0.0001$), suggesting that larger insects released larger amounts of electricity in the present electric field.

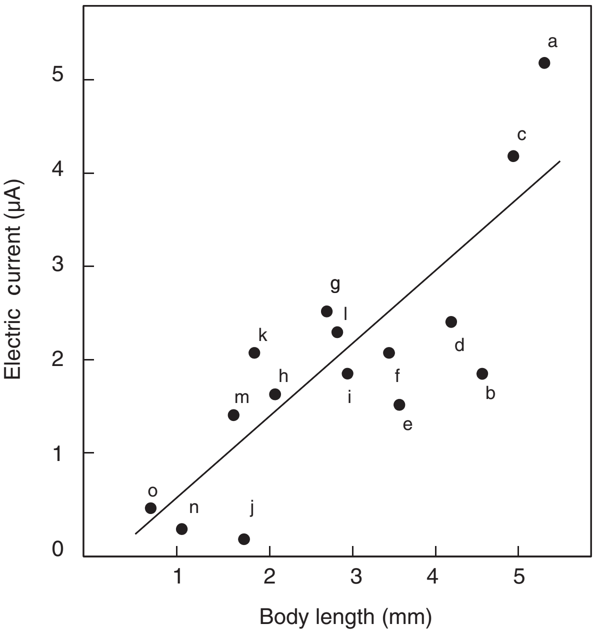

Figure 5 Relationship between body sizes of test insects and the magnitudes of the insect electric current at the highest voltage (13.1 kV). Small letters assigned to dots represent the insects tested (refer to the legend of Fig. 2).

Discussion

The first aim of this 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 (Halliday *et al.*, 2005). Under the present conditions, the electric current occurred at voltages >13.2 kV. Considering this, we conducted the experiments at a voltage range of 1.0 to 13.1 kV, at which the electric current from the ICWs was not detected.

In the present simple version of an EF-screen, the distance between the ICW and earthed net (5 mm) was longer than that in the original version (3 mm) (Matsuda *et al.*, 2011). This longer distance enabled application of a wider range of voltages to the ICW because the mechanical discharge occurred at a higher voltage than in the original version (5.2 kV). Actually, the high voltage impression was useful to amplify hidden peaks of the bioelectric current because higher voltages can push larger amounts of electricity out of an insect to the earthed net.

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 (Jackson & McGonigle, 2005; Chaoui & Keener, 2008). 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 (Jonassen, 2002). 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 that repels 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 were placed on the net, and the electric current generated was transient under the present voltage conditions.

The primary focus of this study was to prove that transient discharge is a common bioelectric phenomenon of insects in a high-voltage-mediated electric field. In this study, we found that the electric current magnitude of the transient discharge increased additively with the increase in the number of insects that were simultaneously placed on the net. This finding made it possible to estimate an electric current from single small insects whose current was not detectable in an application of single adults. By this approach, we proved that the screen caused transient bioelectric discharge in all insects in direct proportion to the increase in the voltage applied and with no relation to their different sizes, shapes, or constructions. Thus, we confirmed our working hypothesis that transient discharge is common in insects.

The second priority of this work was 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 insects: (a) polarisation on the earthed net, (b) positive charging and (c) being drawn toward the ICW. The first problem was specifying a polarisation site in the insects. Many studies (Ishay *et al.*, 1992; McGonigle & Jackson, 2002; McGonigle *et al.*, 2002; Honna *et al.*, 2008; Moussian, 2010) 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. Considering this electrostatic characteristic of the cuticle, we assumed that the cuticle structure was a potential site for polarisation in the adults of the present insect species. 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 (Matsuda *et al.*, 2011). Eventually, the insects polarised positively on the ICW side and negatively on the net side of the cuticle. The negative charge on the earth-side of the cuticle was subsequently 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. 4). 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 failure of the insect to be attracted to the ICW (data not shown). Judging from these results, we concluded that deprivation of insect electricity by the earthed net was essential to attract the insect.

The ability to continuously restrain the attracted insects depended on the voltages applied. The results indicated that higher voltage applications pushed larger amounts of electricity out of the insect. 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 neutralising the positive charge in their body. We did not detect a flow of negative charge to the adults from the earthed net, and thus 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 (Belton & Brown, 1969), flour moth (Deitmer, 1977), and mealworm beetle (Markou & Theophilidis, 2000). In the 100% capture range, in which the adults were prevented from escaping the ICW, we detected electric currents that were associated with skeletal muscular movements. All movements observed involved 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.

Electricity produced biologically can be transmitted to a superficial cuticle conductor (McGonigle & Jackson, 2002; McGonigle *et al.*, 2002). Also, in the present case, the produced electricity could have been transferred to the cuticle, but then quickly drawn to the earthed net. Our result indicated that the 100% capture range voltages were sufficient to push the produced electricity out of the insects, leading to a failure to neutralise the positive charge. In our opinion, this is the major reason for the inability of the attracted adults to escape from the force of the ICW. In contrast, the lower voltages were apparently insufficient to push out the electricity produced by the insects, so the electricity produced through movement was utilised for neutralisation, which resulted in the release of the adults from the ICW.

In the electric field, the insects were always exposed to the attraction force driven toward the ICW. This force was larger with the application of larger voltages. Muscles appeared to be loaded with the force to hinder their actions, and the movements of the insects were very slow and heavy. Under this condition, muscle fatigue was quick, and the insects became motionless. Nevertheless, this situation did not harm the insects. In fact, the tested insects walked and flew normally and could lay eggs (data not shown) after they were released from electrostatic restraint (after a 3-min restraint).

In this study, we selected some ubiquitous pests as test insects. The present collection of the test insects covers eight orders, including 15 families of the insect taxon. We considered this collection to be adequate for examining the generality of the transient bioelectric discharge among the insects placed in the electric field. This study provides an experimental basis for wide application of the EF-screen to various facilities, including greenhouses and warehouses, as a physical tool to control insect pests.

References

Belton P., Brown B.E. (1969) The electrical activity of cockroach visceral muscle fibers. *Comparative Biochemistry and Physiology*, **28**, 853–863.

Chaoui H., Keener H.M. (2008) Separating earthworms from organic media using an electric field. *Biosystems Engineering*, **100**, 409–421.

Deitmer J.W. (1977) Electrical properties of skeletal muscle fibers of the flour moth larva *Ephestia kuehniella*. *Journal of Insect Physiology*, **23**, 33–38.

Foote B.A. (1995) Biology of shore flies. *Annual Review of Entomology*, **40**, 417–442.

Foster W.A., Walker E.D. (2002) Mosquitoes (*Culicidae*). In *Medical and Veterinary Entomology*, pp. 203–262. Eds G. Mullen and L. Durden. San Diego, CA, USA: Elsevier Science.

Halliday D., Resnick R., Walker J. (2005) Electric fields. In *Fundamentals of Physics*, pp. 580–604. Eds S. Johnson and E. Ford. New York, NY, USA: John Wiley & Sons.

Helyer N., Brown K., Cattlin N.D. (2004) Pest profiles. In *A colour handbook of biological control in plant protection*, pp. 21–41. Ed. J. Northcott. London, UK: Manson Publishing.

Hill D.S. (1990) *Pests of Stored Products and Their Control*. London, UK: Belhaven Press.

Honna T., Akiyama Y., Morishima K. (2008) Demonstration of insect-based power generation using a piezoelectric fiber. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, **151**, 460.

Ishay J.S., Shimony T.B., Shalom A.B., Kristianpoller N. (1992) Photovoltaic effects in the oriental hornet, *Vespa orientalis*. *Journal of Insect Physiology*, **38**, 37–48.

Jackson C., McGonigle D. (2005) Direct monitoring of the electrostatic charge of house-flies (*Musca domestica* L.) as they walk on a dielectric surface. *Journal of Electrostatics*, **63**, 803–808.

Jonassen, N. (2002) Electrical breakdown. In *Electrostatics*, pp. 6–73. Boston, MA, USA: Kluwer Academic Publishers.

Kakutani K., Matsuda Y., Nonomura T., Kimbara J., Kusakari S., Toyoda H. (2012) An electric field screen can make conidium-free and pest-free space with better air penetration in window-opened greenhouses. *Acta Horticulturae*, in press.

Ma Z., Michailides T.G. (2005) Advances in understanding molecular mechanisms of fungicide resistance and molecular detection of resistant genotypes in phytopathogenic fungi. *Crop Protection*, **24**, 853–863.

Markou T., Theophilidis G. (2000) The pacemaker activity generating the intrinsic myogenic contraction of the dorsal vessel of *Tenebrio molitor* (Coleoptera). *Journal of Experimental Biology*, **203**, 3471–3483.

Matsuda Y., Ikeda H., Moriura N., Tanaka N., Shimizu K., Oichi W., Nonomura T., Kakutani K., Kusakari S., Higashi K., Toyoda H. (2006) A new spore precipitator with polarized dielectric insulators for physical control of tomato powdery mildew. *Phytopathology*, **96**, 967–974.

Matsuda Y., Nonomura T., Kakutani K., Takikawa Y., Kimbara J., Kasaishi Y., Kusakari S., Toyoda H. (2011) A newly devised electric field screen for avoidance and capture of cigarette beetles and vinegar flies. *Crop Protection*, **30**, 155–162.

McGonigle D.G., Jackson C.W. (2002) Effect of surface material on electrostatic charging of houseflies (*Musca domestica* L). *Pest Management Science*, **58**, 374–380.

McGonigle D.G., Jackson C.W., Davidson J.L. (2002) Triboelectrification of houseflies (*Musca domestica* L.) walking on synthetic dielectric surfaces. *Journal of Electrostatics*, **54**, 167–177.

Moussian B. (2010) Recent advances in understanding mechanisms of insect cuticle differentiation. *Insect Biochemistry and Molecular Biology*, **40**, 363–375.

Nauen R., Denholm I. (2005) Resistance of insect pests to neonicotinoid insecticides: current status and future prospects. *Advances of Insects Biochemistry and Physiology*, **58**, 200–215.

Nonomura T., Matsuda Y., Kakutani K., Takikawa Y., Toyoda H. (2008) Physical control of powdery mildew (*Oidium neolycopersici*) on tomato leaves by exposure to corona discharge. *Canadian Journal of Plant Pathology*, **30**, 517–524.

Shimizu K., Matsuda Y., Nonomura T., Ikeda H., Tamura N., Kusakari S., Kimbara J., Toyoda H. (2007) Dual protection of hydroponic tomatoes from rhizosphere pathogens *Ralstonia solanacearum* and *Fusarium oxysporum* f. sp. *radicis-lycopersici* and airborne conidia of *Oidium neolycopersici* with an ozone-generative electrostatic spore precipitator. *Plant Pathology*, **56**, 987–997.

Tanaka N., Matsuda Y., Kato E., Kokabe K., Furukawa T., Nonomura T., Honda K., Kusakari S., Imura T., Kimbara J., Toyoda H. (2008) An electric dipolar screen with oppositely polarized insulators for excluding whiteflies from greenhouses. *Crop Protection*, **27**, 215–221.

Weintrub P.G., Berlinger M.J. (2004) Physical control in greenhouses and field crops. In *Insect Pest Management*, pp. 301–318. Eds A.R. Horowitz and I. Ishaaya. Berlin, Germany: Springer-Verlag.