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Prevention of Whitefly Entry from a Greenhouse Entrance by Furnishing an Airflow-Oriented Pre-Entrance Room Guarded with Electric Field Screens

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Abstract

Two types of an electric field screen were used to exclude whiteflies from a greenhouse. Singly charged dipolar electric field screen had insulated conductor iron wires arrayed in parallel (ICW-layer), two earthed metal nets on both sides of the ICW-layer, and a direct current voltage generator. Screens were attached to the lateral windows of the greenhouse to repel whiteflies that approached the nets. To electrostatically guard the greenhouse entrance, doubly charged dipolar electric field screens (DD-screens) were used to capture whiteflies entering through the door. The ICWs, oppositely charged with equal voltages, were arrayed one after the other, and an insulator board or net was placed on one side of the ICW-layer. ICWs captured whiteflies entering an electric field of DD-screens. A small pre-entrance room was constructed at the entrance area, and three DD-screens (with yellow and gray board or gray net) were installed in the pre-entrance room taking into consideration the airflow inside the room, as most whiteflies were brought in by the air when the door was opened. Two DD-screens with the board were useful for directing the wind toward the wall into which the netted DD-screen was integrated. Insects were eliminated by this screen, and the pest-free air was circulated inside the greenhouse. The yellow-boarded DD-screen was highly attractive because of the photo-selective movement of the whiteflies and was effective for trapping the whiteflies when there was no wind. Our electrostatic method is effective at keeping the greenhouse pest-free throughout the entire period of tomato cultivation.

Keywords: electric field screen, pest management, photo-selective movement, whiteflies

1. Introduction

The whitefly *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) is a major pest in tomato cultivation (Perring, 2001). Its greatest economic threat involves the transmission of damaging plant viruses, primarily the Geminiviruses (Cohen & Berlinger, 1986; Oliveira et al., 2001). Whiteflies are difficult to control with insecticides because they feed and oviposit mainly on the abaxial surfaces of leaves (Sharaf, 1986) and because they are resistant to most classes of insecticides (Prabhaker et al., 1985; Palumbo et al., 2001; Horowitz et al., 2004; Nauen & Denholm, 2005). Physical methods could be used to manage the pest because they are compatible with other components of integrated pest management, have little impact on the environment, and do not rely on the use of pesticide, thereby, slowing the development of insecticide resistance (Weintraub & Berlinger, 2004).

In Japan, a viral disease caused by tomato yellow leaf curl virus (TYLCV) is vectored by *B. tabaci* and is a major cause of the loss of tomato crops grown in greenhouses (Ueda & Brown, 2006). Woven screens with a fine mesh have been used extensively to prevent whiteflies from entering greenhouses, but these screens reduce ventilation, causing overheating and increasing relative humidity (Teitel et al., 1999). A physical method of excluding airborne fungal pathogens and flying pests from greenhouses with good air penetration involves forming an electrostatic barrier; this method reduces the use of agrochemicals such as fungicides and insecticides. The electrostatic method was initially devised to collect mature conidia on powdery mildew conidiophores (Moriura et al., 2006a, 2006b; Nonomura et al., 2009), and the first electrostatic spore precipitator was a screen that created a nonuniform electric field around insulated copper conductor wires arranged in parallel (Matsuda et al., 2006; Shimizu et al., 2007). This field generated an electrostatic force that attracted fungal conidia. Unfortunately, the spore precipitator was ineffective at trapping most flying insects in greenhouses. A more practical, successful method involved the use of a singly charged dipolar electric field screen (SD-screen) in which grounded metal nets were placed on both sides of the original spore precipitator to create dielectric poles (Matsuda et al., 2011). This screen was able to capture insects ranging in size from adult whiteflies (body width, 0.1–0.3 mm) to cigarette beetles and vinegar flies (0.8–1.2 mm), the latter of which are common in warehouses and processing factories.

In addition to capturing insects, the SD-screen repelled pests that reached the screen net (Matsuda et al., 2011). Insects on the net probed the inside of the screen with their antennae and then flew away without entering the screen. Obviously, the insects could sense the electric field and avoided entering it. This avoidance behaviour is common among greenhouse pests such as whiteflies, western flower thrips, green peach aphids, and shore flies (Kakutani et al., 2012). The SD-screen was also effective at capturing insects that were forcibly pushed inside by the wind as they walked on the net.

A limitation of our method is that whiteflies were still able to enter the greenhouse through the entrance door, because workers went back and forth through this door several times per day. In fact, the complete exclusion of whiteflies from the greenhouse was achieved only when the windows were guarded with SD-screens and the entrance door was locked (Nonomura et al., 2012). Yet, even if just a few whiteflies enter the greenhouse, the effect can be devastating because of the threat of secondary infestation caused by whiteflies that multiply on the tomato plants. Viruliferous whiteflies that multiply on diseased tomato plants spread virus and, consequently, disease. From these results, it appeared that the best way to prevent whiteflies from entering through the entrance door was to develop an electrostatic guarding method for the entrance area. A doubly charged dipolar electric field screen (DD-screen; Matsuda et al., 2012) was used for this purpose. In the current study, a small pre-entrance room is constructed at the entrance area, and three DD-screens (with yellow and gray board or gray net) are installed in three parts of the pre-entrance room taking into consideration the wind flow inside the room, as most whiteflies are brought in by the air when the door is opened. Three types of modified DD-screens are used to trap all whiteflies at the pre-entrance room of the greenhouse. The present paper describes the complete exclusion of whiteflies from the greenhouse by electrostatic guarding of the entrance area with DD-screens and lateral windows with SD-screens.

2. Materials and Methods

2.1 The Insect

Whitefly adults (*Bemisia tabaci* Gennadius, type B, virus-free) were originally collected from greenhouse-grown tomatoes in Chiba Prefecture and maintained at the National Institute of Vegetable and Tea Science in Mie, Japan. The whiteflies were reared on tomato plants in a temperature-controlled greenhouse (26 ± 2 °C, 35–55% relative humidity) at Kinki University (Nonomura et al., 2012). Male and female adults that multiplied on tomato plants were collected for experiments using an insect aspirator (Wildlife Supply, Binghamton, NY).

2.2 The Electric Field Screens

Two types of an electric field screen (an SD- and DD-screen) were constructed according to the previous reports (Matsuda et al., 2011, 2012). The SD-screen (Figure 1a) had three components: insulated conductor iron wires (ICWs) in parallel arrays, two earthed stainless steel nets (1.6-mm mesh) on both sides of the ICW layer, and an electrostatic direct current (DC) voltage generator (Max Electronics, Tokyo, Japan) that supplied a charge to the ICWs. To prepare ICWs, an iron conductor wire (2 mm diameter, 0.9 m length) was insulated by passing it through a transparent insulator vinyl sleeve (1 mm thickness, $1 \times 10^9\ \Omega$). The ICWs were connected to each other and a voltage generator and were negatively charged, and the negative surface charges of the ICWs polarized the grounded nets, creating a positive charge on the surface of the ICW side of the nets (Matsuda et al., 2011). The opposite charges acted as dipoles that formed an electric field between the ICW layer and the grounded nets (Figure 1b). The structure of the DD-screen was basically identical to that of the SD-screen, but the ICWs of the DD-screen were oppositely charged with equal voltages using two voltage generators (Matsuda et al., 2012). In the modified DD-screen (Figure 1c) used here, the earthed net on one side (insect-trapping side) was omitted to capture all insects approaching the screen, because the insects reaching the earthed net were deterred from getting inside the screen (Nonomura et al., 2012). The metal net on the opposite side was replaced with an insulator (polypropylene) net or board. The DD-screens with a yellow board, a gray board, and gray net were designated as yellow-boarded, gray-boarded, and gray-netted DD-screens, respectively. The DD-screen formed gap-free electric fields with no spaces to allow the insects to escape (Figure 1d). Both SD- and DD-screens were fitted in an aluminium frame (90 × 180 cm), and the wire-connecting part of the frame was sealed with silicon resin.

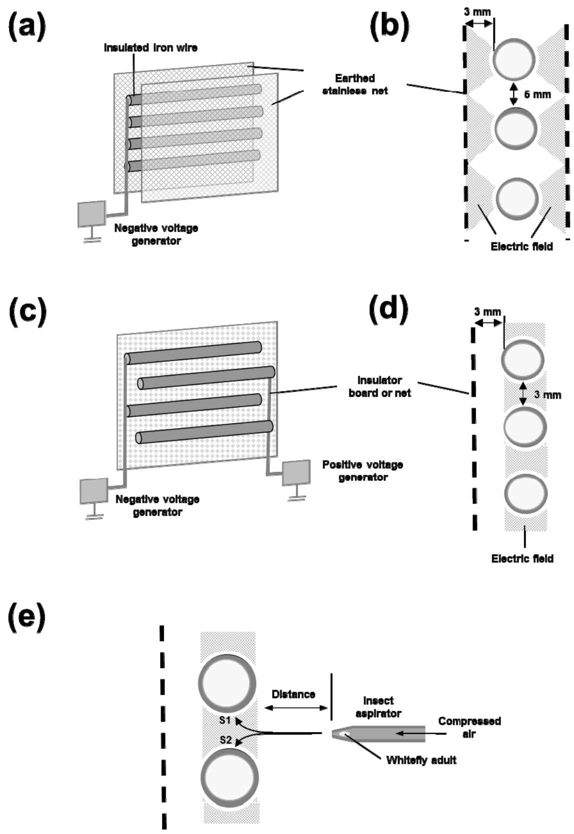

Figure 1. Diagrams and cross-sectional views of singly (**a** and **b**) and doubly (**c** to **e**) charged dipolar screens. Solid arrows in **e** represent the directions of attraction of the whitefly blown inside an electric field of a DD-screen by compressed air from an insect aspirator. S1 and S2 indicate the sites of insect attraction on the ICWs.

2.3 Construction of a Pre-Entrance Room and Application of Electric Field Screens

An A-shaped greenhouse (20 m length, 8 m width, 5 m height at the highest part) (Figure 3a left) was divided into two parts (rooms A and B) by a wall partition (Figure 2). A small pre-entrance room (room C, insect-trapping room) was constructed inside room A next to the second entrance door. The yellow- and gray-boarded DD-screens were fitted in the central wall side and third entrance door of room C, respectively. The gray-netted DD-screen was integrated into the wall opposite the third entrance door. SD-screens were attached to the window frames (90 × 180 cm) on both sides of room A (Figure 3a right) to repel the insects reaching the screen net, and were negatively charged with 1.5 kV as described previously (Nonomura et al., 2012).

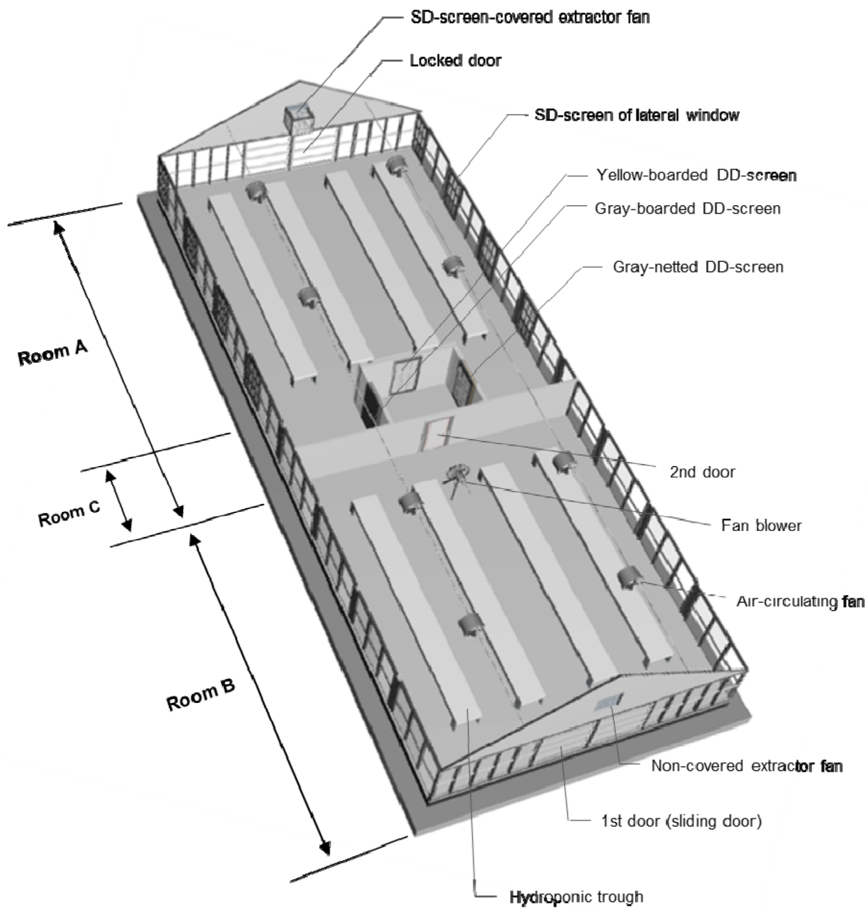

Figure 2. Three-dimensional diagram of the greenhouse divided into three rooms by wall partitions. SD-screens were attached to the window frames of lateral windows on both sides of the left room (room A), and DD-screens were installed in the door and walls in the pre-entrance room (room C) constructed inside room A. The right room (room B) of the divided greenhouse was not guarded, and whiteflies were released here to propagate on hydroponic tomato plants

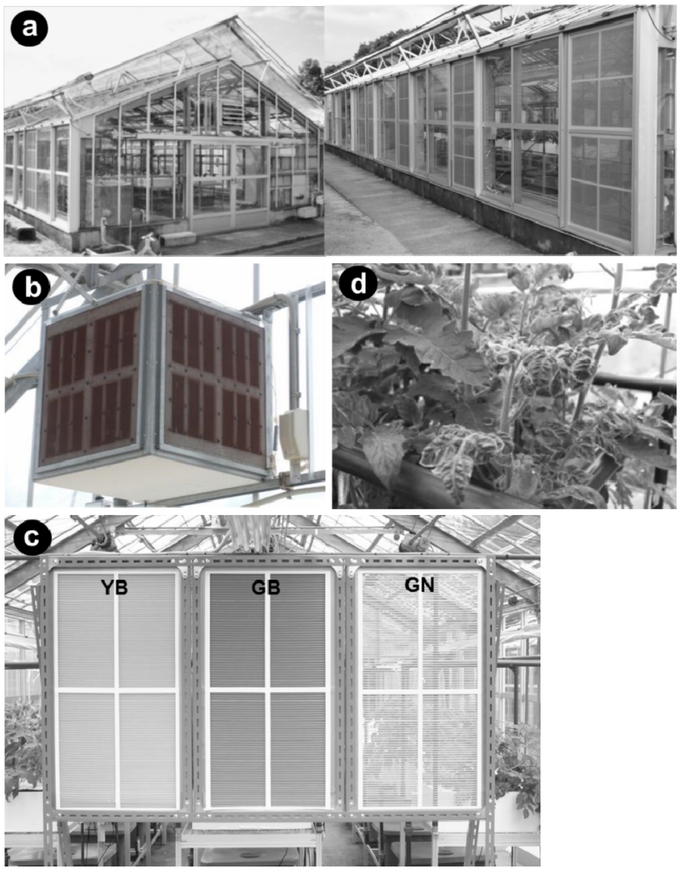

Figure 3. SD- and DD-screens installed in a greenhouse to prevent whiteflies from entering. (a) A-shaped greenhouse (left) and SD-screens (right) attached to the lateral-window frames of room A of the divided greenhouse shown in Figure 2. (b) A box of SD-screens to cover an extractor fan in room A. (c) Yellow-boarded (YB), gray-boarded (GB) and gray-netted (GN) DD-screens placed on the floor of room B (not guarded) of the greenhouse. (d) Typical symptoms of TYLCV carried by biotype-Q whiteflies in tomato plants in a hydroponic trough of room B

The door of room A was locked throughout the experiment to prevent the entry of whiteflies as researchers entered the greenhouse, whereas the entrance door of room B (the first entrance door) and the inner entrance door of the central partition (the second entrance door of room A) were opened and closed several times a day for ordinary plant care. Roof windows were not furnished with the screens because of the structural difficulty of doing so, and these remained closed during the experiment. Extractor fans were fitted on the front wall of each half (A and B) of the greenhouse. The extractor fan of room A was covered with a box of SD-screens (Figure 3b) to prevent the entry of whiteflies through the gap of the fan. An air-circulating fan was attached to a crossbeam in each room, and all fans began operating automatically when the inside temperature reached 30 °C. All side windows were automatically closed when the outside air speed reached 3 m s^{-1}. The windows were opened again when the wind decreased to 1.5 m s^{-1}.

2.4 Determination of the Optimal Voltage for the DD-Screen

Three types of DD-screens attached to the pre-entrance room were charged with the same negative and positive voltages (0.4–1.4 kV) to determine the voltage range that captured all test insects. Adult whiteflies were blown into the space between the ICWs by sending compressed air (1.5 kg cm^{-2}) through the tip of an insect aspirator (Figure 1e). The distance between the tip of the aspirator and the surface of the ICW was altered to create different wind speeds (1–3 m s^{-1}). Wind speed was measured at the surface of the ICW using a sensitive anemometer (Climomaster 6533; Kanomax, Tokyo, Japan). Seven to ten adults were used for each blowing and for each voltage tested. To confirm the successful capture of whiteflies with the ICW, we directed a blower (max. wind speed: 7 m s^{-1} at the ICW) at the captured insects for 10 min. Experiments were repeated five times, and the data are presented as the mean ± SD. Significant difference among the data was statistically analysed using

Tukey's method (see the legend of Table 1).

Table 1. Percentage of whitefly adults captured by ICWs of doubly charged electric field screens (DD-screens)

Wind speed ($m\ s^{-1}$)	DD-screens used	Negative and positive voltages (kV) applied to ICWs									
		0	0.4	0.6		0.8		1		1.2	1.4
1	Yellow-colored board	0	0	42.7±6.3	a	100	a	100	a	100	100
	Gray-colored board	0	0	44.4±5.3	a	100	a	100	a	100	100
	Gray-colored net	0	0	46.0±3.9	a	100	a	100	a	100	100
2	Yellow-colored board	0	0	26.5±2.1	b	77.4±5.7	b	100	a	100	100
	Gray-colored board	0	0	25.1±3.6	b	76.9±6.4	b	100	a	100	100
	Gray-colored net	0	0	32.5±5.5	b	81.2±5.1	b	100	a	100	100
3	Yellow-colored board	0	0	0	c	46.2±5.6	c	80.5±4.1	b	100	100
	Gray-colored board	0	0	0	c	49.5±7.1	c	78.7±5.4	b	100	100
	Gray-colored net	0	0	0	c	41.3±6.4	c	82.1±3.9	b	100	100

Seven to ten adults were used for each voltage and wind speed, and the means and standard deviations were calculated from five replicates. The different letters on the mean values in each vertical column indicate significant differences ($p < 0.05$) according to Tukey's method.

2.5 Assays for Wind Delivery of Whiteflies

A fan blower was placed in room B in front of the second entrance door to examine the direction of airflow produced in room C when the door was opened. The wind speed of the blower at the second entrance door was adjusted to 1.0, 2.0, and 3.0 $m\ s^{-1}$, and the wind speed at each DD-screen was measured. To examine the effect of the wind on the whiteflies, we placed a 50-ml transparent polypropylene vessel containing 200 whiteflies at the second entrance door and then tapped the vessel so that the whiteflies would fly up in the air stream of a blower (see Figure 5a). In a subsequent experiment, we tapped the vessel after the second door was closed and then the third door opened (see Figure 5b). The number of whiteflies captured by the three DD-screens was counted to determine the points the whiteflies reached. Experiments were repeated five times at each blower wind speed, and data are reported as the mean ± SD. In the last experiment, the insect-containing vessel was tapped after both doors were closed to trace the movement of the whiteflies when there was no wind (Figure 5c).

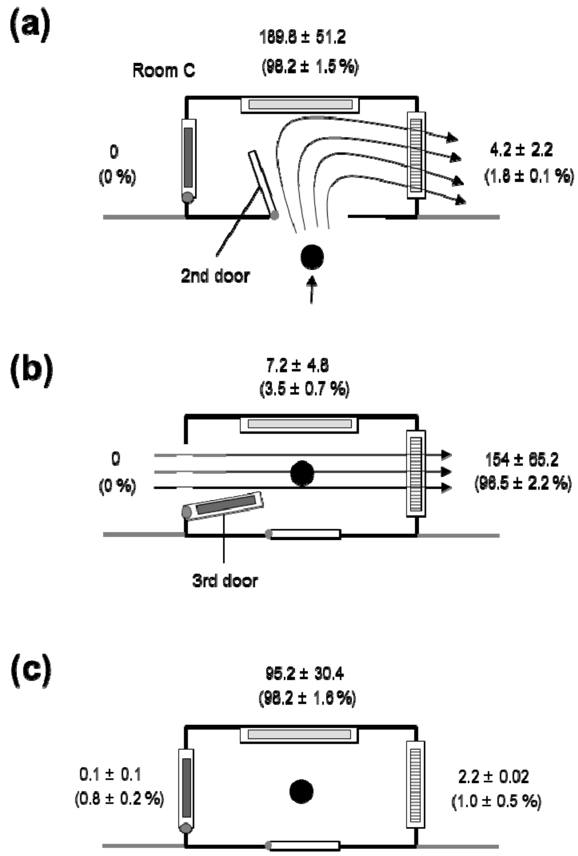

Figure 5. Direction (arrows) of the wind blowing inside the pre-entrance room and trapping of whiteflies with DD-screens. Yellow-boarded and gray-netted DD-screens were attached to the central wall and side walls of the room, respectively, and a gray-boarded DD-screen was attached to the third entrance door. **a** and **b** show the door opening, and **c** shows the door closing. The number of trapped whiteflies is shown near the screen. Figures in parentheses are the percentage of whiteflies trapped by the three DD-screens. Data are mean ± SD of five replicates. Closed circles indicate the sites from which totally 200 whiteflies in a 50-ml transparent polypropylene vessel were released by tapping

2.6 Assay for the Photo-Selective Movement of Whiteflies

Tomato plants (30-day-old seedlings) were transplanted to four hydroponic troughs (200 seedlings per trough) in room B, and adult whiteflies were released on the plants to propagate. One month later, the three types of DD-screens were placed in room B (Figure 3c) and in front of the second entrance door to trace the photo-selective movement of the whiteflies to the DD-screens. The number of whiteflies trapped by the screens was counted daily for 1 month. Separate experiments were repeated three times. Significant difference among the data was statistically analysed using Tukey's method (see the legend of Figure 4). The screens were removed at the end of this experiment, but we maintained a large population of whiteflies in room B throughout the entire experimental period (6 months) by adding new tomato plants to the hydroponic troughs in that room.

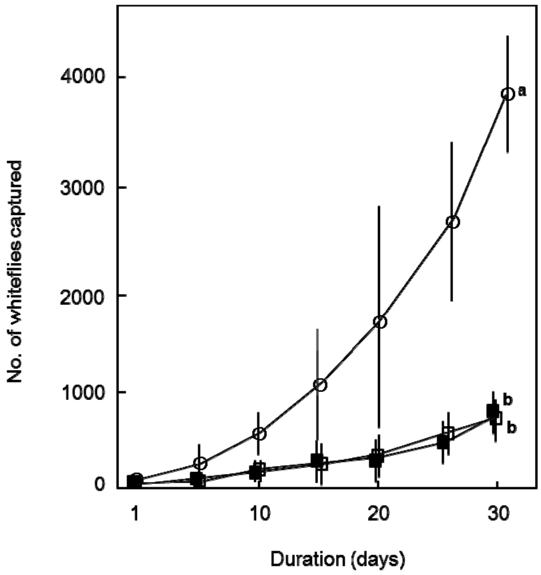

Figure 4. Increase in the number of whiteflies trapped by DD-screens placed in a greenhouse filled with whiteflies. Open circles and open and closed squares represent the number of whiteflies trapped by yellow-boarded (YB), gray-boarded (GB), and gray-netted (GN) DD-screens, respectively. Each mean ± SD was calculated from three replicates. The different letters along the final plots of each screen indicate significant differences ($p < 0.05$) according to Tukey's method

2.7 Confirmation of Successful Repelling of Whiteflies by Window-Installed SD-Screens

A total of 400 insect-free, healthy, 40-day-old tomato plants were transplanted to hydroponic culture troughs in room A. On each trough, 10 yellow sticky plates (Y-plates; Arysta Life Science, Tokyo, Japan) were hung from a crossbeam at 1-m intervals to determine the number of whiteflies entering the room. In this experiment, the second and third entrance doors were closed throughout the experimental period to confirm the insect repelling by the lateral window-installed SD-screens. Three experiments were conducted, one every 2 weeks from July (when vigorous whitefly infestation was confirmed in other tomato greenhouses) to September 2012. At the end of each experiment, we counted the number of whiteflies trapped by the Y-plates.

2.8 Evaluation of DD-Screens in a Greenhouse Assay

The DD-screens were examined for their ability to prevent whiteflies from entering room C and then invading room A when researchers went to room A through rooms B and C. The researchers entered room A 20 times per day throughout the experimental periods. A fan blower on the floor operated at 3 m s^{-1}, while the second door was open. As a control, a similar experiment was conducted without applying any voltage to the DD-screens of room C. Three experiments were conducted, one every 2 weeks from July to September 2012. At the end of each experiment, we counted the number of whiteflies trapped by the Y-plates or by the DD-screens.

2.9 Biotype Determination of Trapped Whiteflies and Virus Detection in Whiteflies

To identify the biotypes of the whiteflies trapped by the Y-plates, we collected single whiteflies from the plates and mixed their homogenates with the reaction mixture from a commercial biotype detection kit (Nippon Gene, Tokyo, Japan) for a loop-mediated isothermal amplification (LAMP) of specific genome sequences of the whiteflies (Hsieh et al., 2012). To detect TYLCV in the trapped whiteflies, we pierced the whiteflies with sterilized toothpicks and then dipped the toothpicks in solution from a commercial TYLCV-detection kit (Nippon Gene) for LAMP of viral DNA (Fukuta et al., 2003). Both experiments were conducted according to the manufacturers' protocols. A total of 20 whiteflies per Y-plate were collected at random from three plates in each experiment to determine the ratio of biotype-B and -Q whiteflies and to identify viruses they may have carried.

2.10 Assay for Artificial Delivery of Whiteflies

To consider the possibility of unintentional entry of whiteflies into the facility, we tested whether whiteflies on plants grown hydroponically would move to researchers walking near the plants or actually touching them. Researchers wore yellow or white clothing and walked between the hydroponic troughs for 5 min. In addition, they stopped and touched infested plants for 5 min before entering room C. The whiteflies on their clothing were counted, and then a hand blower was directed at the whiteflies at 2.5 m s^{-1} (maximum speed of a blower) for 1 min to evaluate the removal of whiteflies.

3. Results and Discussion

The main goal of the current study was to use two types of electric field screens (SD- and DD-screens) to electrostatically guard the openings of a greenhouse. Previously we demonstrated that SD-screens attached to the window of a greenhouse repelled insects, as the insects avoided the electric field (Kakutani et al., 2012; Nonomura et al., 2012). Also in the current experiments, we designed a greenhouse assay to confirm the repelling function of SD-screens attached to windows. For this assay, it was useful to monitor the appearance of typical symptoms of TYLCV (yellowing and curling of tomato leaves) in greenhouse tomatoes, because we had released only biotype-B and virus-free whiteflies in room B, and because TYLCV-carrying biotype-Q whiteflies had prevailed in our district (Matsuda et al., 2013). In fact, the present PCR-based detection assay showed that the ratio of biotype-Q whiteflies on the Y-plates in room B increased gradually during the 3-month experimental period: values of 0.8, 2.3, 7.6, 8.8, 11.3, and 21.1 per cent were observed over 2-week intervals. Moreover, the appearance of typical symptoms of TYLCV in the greenhouse tomatoes was another sign of invasion by whiteflies carrying the virus. Symptoms of TYLCV (Figure 3d) were detected in 40 tomato plants in room B within 2 months after transplanting. These results indicate the entry of outside virus-carrying whiteflies into room B from the lateral windows, suggesting that room A suffered similarly from invasion by these whiteflies. Nevertheless, room A (in which both entrance doors were locked throughout the experimental period) remained pest free. Obviously, the SD-screens on the windows were successful at preventing outside whiteflies from entering the greenhouse through the windows.

The main purpose of the current study was to trap all whiteflies at the pre-entrance of the greenhouse. The DD-screen was used for this purpose. The structure of the original DD-screen was basically identical to that of the SD-screen (Matsuda et al., 2012); the ICWs possessed the earthed metal nets on both sides. Also in the DD-screen, whiteflies were able to perch on the nets and sense the electric field inside the screen. The use of the net-less DD-screen was effective at directing whiteflies to the ICWs. In the modified DD-screen, the capture sites were in the electric field formed between the opposite poles (negatively and positively charged ICWs), and the whiteflies in the electric field were attracted to the closer pole. The mechanism for capturing insects in this field was described previously (Matsuda et al., 2012). In the present study, we examined three types of DD-screens for their ability to capture whiteflies blown into an electric field at different wind speeds. Table 1 lists the percentage of whiteflies captured by the screens at different voltages (0.4–1.4 kV) for each wind speed. Stronger forces of ICWs were necessary to capture whiteflies carried at higher wind speeds, and the force became stronger with increasing voltage applied to the ICWs. In addition, there was no significant difference in the capture rate among the three DD-screens for any combination of wind speed and voltage. At >1.2 kV the force was strong enough that the ICWs captured all whiteflies regardless of the wind speed. The whiteflies were attracted near the ICWs (S1 or S2 site; Figure 1e) (Video Supplements 1-3), and the force at both sites was strong enough to keep capturing the whiteflies despite a wind speed of 7 m s^{-1}. At lower voltages, however, the force was insufficient to permanently capture the whiteflies; the whiteflies captured fluttered their legs, twisted their bodies, and then flew away from the ICWs; otherwise, they were blown away from the ICW by a blower. Based on these observations, in the subsequent experiments the DD-screens were charged with 1.2 kV to ensure successful capture. From these results, we obtained some important conclusions; 1) at the present voltage charge (1.2 kV in each negative and positive ICW), the ICWs of the three types of modified DD-screens exerted a force sufficient to capture whiteflies blown inside the electric field at 3 m s^{-1} (the maximum speed of wind inside a greenhouse), 2) the whiteflies captured could not escape the ICW even when they were blown at 7 m s^{-1}, and 3) the attractive force of the ICWs was not affected by replacing the earthed metal net in the original version with a coloured or non-coloured insulator board.

Nevertheless, the attractive force of the ICWs was restricted to the whiteflies that entered the electric field; the ICWs could not attract whiteflies flying outside of this field. The photo-attracted movement of whiteflies (Helyer et al., 2004) was the key to provide a splendid resolution to this problem. For this approach, we tested the attracted movement of whiteflies to the yellow-boarded DD-screen. Figure 4 shows an increase in the number of trapped whiteflies with each of the three types of DD-screen. There was a significant difference in the number of whiteflies trapped between the yellow-boarded and gray-boarded or gray-netted DD-screens, although there was no difference between the latter two DD-screens. The yellow-boarded DD-screen was effective at attracting flying whiteflies to the electric field of the screen. In fact, the yellow board placed close behind the ICWs was highly attractive, bringing almost all flying whiteflies in close range of the ICW. In our opinion, the placement of this type of DD-screen in a narrow space such as the present pre-entrance room (room C) enabled the whiteflies to visually catch the yellow board behind the ICWs.

The vital point of the present work was the production of air stream that could direct flying whiteflies in the desired direction. Specifically, we tried to regulate the direction of the wind in room C by arranging three different types of modified DD-screens on the walls and entrance door of the pre-entrance room. Namely, the central wall of room C was furnished with the yellow-boarded DD-screen because of its strong photo-selective attraction, and the side wall and third entrance door were furnished with the gray-netted and gray-boarded DD-screens, respectively, considering fluid dynamic characteristics. In this experiment, we counted the number of whiteflies trapped by the screens in room C under conditions of wind (Figures 5a and 5b) and no wind (Figure 5c). Figure 5a shows the direction of the wind coming from a fan blower placed on the floor of room B. Wind speeds were 3 m s^{-1} at the second entrance door and decreased to 1.9, 0.6, and 0 m s^{-1} at the central wall, side wall, and third entrance door screens, respectively. Under these conditions, the majority of whiteflies (98.2 ± 1.5 per cent) released into the wind stream were captured by the central wall screen, and few whiteflies (1.8 ± 0.1 per cent) were captured by the side wall screen. No whiteflies were trapped by the screen on the entrance door. Figure 5b illustrates the scenario in which the third entrance door was opened after the second door was closed. The wind from the third entrance door was directed to the side wall of room C. Wind speeds at the side wall screen varied from 0.1 to 0.4 m s^{-1}, depending on the speed of the wind blowing into room A from the lateral windows of the greenhouse. Under this condition, the whiteflies released were trapped mainly by the side wall screen, which indicates that the whiteflies flying inside room C were carried to the side wall screen by a blower at the door opening. Figure 5c shows the number of whiteflies trapped by the three screens under conditions of no wind. Whiteflies in a vessel flew up when the vessel was tapped and moved to the yellow-boarded screen of the central wall, but very few (less than 2 per cent) went to the side wall screen. No whiteflies moved to the screen attached to the door.

In the present system, the gray-netted DD-screen was an exhaust port of pest-free air to room A. This screen acted as an air filter to eliminate insects from the air passing through the screen. By combining this with the two other DD-screens (with the yellow and gray boards), we could direct the wind blowing inside room C toward the wind-permeable DD-screen to allow the air to flow to the next room (Figures 5a and 5b). It appears that the whiteflies entering room C were forcibly carried with the wind destined for the wind-permeable screen trap.

The primary aim of this study was to evaluate the feasibility of an airflow-oriented pre-entrance room guarded by DD-screens to prevent whiteflies from entering a greenhouse when workers go in and out of the entrance door. The current situation (*i.e.*, heavy infestation of hydroponic tomatoes by whiteflies released in one side of a two-room greenhouse) is highly risky, as numerous whiteflies can enter when the door is opened. Actually, the movement of whiteflies from room B to room A was demonstrated in a control experiment in which the DD-screens in room C were not charged; there were 85 whiteflies on 15 Y-plates in room A. Most whiteflies entered room A when the wind blew toward the second entrance door and the door opened and the whiteflies were carried inside by the wind. The results indicate that the three DD-screens in room C were able to capture whiteflies blown in by a fan blower (while the second entrance door was open) and, moreover, that the yellow-boarded screen opposite the entrance door was photo-attractive for trapping flying whiteflies after the door was closed. In addition, the opening of the third entrance door created a stream of air that carried the remaining whiteflies to the screen on the opposite wall. Eventually the results obtained were satisfactory; three separate experiments revealed no whiteflies in any Y-plates or on any tomato plants in room A. In contrast, many whiteflies were trapped by the screens of room C; the total number of whiteflies trapped in the three experiments was 246, 345, and 431, respectively, and most of these (93.1 ± 0.8 per cent) were trapped by the central wall screen. Thus, the present study demonstrated that our method of electrostatically guarding the entrance and windows of a greenhouse is a promising method to keep the greenhouse pest-free throughout the entire period of tomato cultivation.

Our district contains a large number of biotype-Q whiteflies carrying TYLCV (Matsuda et al., 2013). Using a commercially available PCR-based detection kit, we were able to easily distinguish these whiteflies from the virus-free biotype-B whiteflies released in the greenhouse. We detected a continuous increase in the number of biotype-Q whiteflies in room B, indicating invasion by outside viruliferous whiteflies. Moreover, the appearance of typical symptoms of TYLCV on the greenhouse tomatoes was another sign of invasion by whiteflies carrying the virus. These results suggested frequent entry of whiteflies into room A of the greenhouse. Despite this invasion of whiteflies, the guarded room remained pest free throughout the entire period of cultivation, indicating that electrostatic guarding with SD- and DD-screens was highly effective at excluding whiteflies from the greenhouse.

Our final concern was the risk of unintentional delivery of whiteflies into the greenhouse by workers. The whiteflies in this study seldom attached themselves to the workers, even when they wore yellow clothing and walked near infested tomato plants. Nevertheless, insect movement was frequent when the workers touched infested tomato plants. We frequently observed this insect transfer as part of the routine care of tomato plants, including the excision of auxiliary buds. Unfortunately, the conventional method of using air curtaining or air projecting (Carlson et al, 2006) to blow insects off workers may be ineffective at removing whiteflies that typically cling to clothing. Isaacs et al. (1999) reported that whiteflies can sense wind velocity and terminate take-off as the wind speed increases. Our alternative approach was to remove clothing in the pre-entrance room, close the door quickly (within 5 s) and, most importantly, refrain from touching infested plants before entering the greenhouse. The accidental delivery of whiteflies could be eliminated by strictly following these three guidelines.

4. Conclusions

Our method of electrostatically guarding the entrance area was reasonable from the standpoint of electrostatics and aeromechanics, and therefore, the complete exclusion of insects from the greenhouse could be expected in practical tomato cultivation. In one of our experiments one half of the two-room greenhouse was filled with a large number of whiteflies, and researchers were forced to pass through the pest-infested area to enter the other room of the greenhouse. Apparently this method carried a higher risk of pest invasion than the typical situation in which the researchers entered the greenhouse directly. This demonstration was useful to enable us to rigorously evaluate the effectiveness of the present guarding method.

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