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Research Article

ELIMINATION OF WHITEFLIES COLONISING GREENHOUSE TOMATO PLANTS USING AN ELECTROSTATIC FLYING INSECT CATCHER

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ABSTRACT

To control whiteflies attacking greenhouse tomatoes, we developed an efficient non-chemical method to control insecticide-resistant whiteflies using the attractive force of a newly devised racket-shaped electrostatic flying insect catcher (REIC). The REIC has two layers of insulated iron conductor wires (ICWs) in parallel arrays and two electrostatic direct current (DC) voltage generators that supply negative or positive voltages to the ICWs. Within each layer, the ICWs are arranged in parallel at 5-mm intervals, and connected to each other and to a negative or positive voltage generator. The negatively and positively charged ICWs are designated as ICW(–) and ICW(+), respectively. The two ICW layers are arranged in parallel with a 2-mm separation between the layers, and the ICWs of each layer are offset from the ICWs of the other layer. Adult whiteflies were blown into the space between the ICWs to determine the voltage range that captured all of the test insects. The result showed that at ≥4.0 kV, the force was strong enough that the ICWs captured all of the whiteflies, despite a wind speed of 3 m/s. The REIC is portable and easy to operate on site in a greenhouse. Whiteflies colonising tomato plants were forced to fly up in the air by gently tapping the plants, and the flying whiteflies were caught by the REIC waved once or twice in the air above the plants. The insect trapping operation was executed on greenhouse tomatoes at the time of daily plant care during the experimental period of 2 months. The results show that the tomato plants remained healthy until their fruit production, indicating that the REIC is a promising tool applicable to the practical control of insecticide-resistant whiteflies of greenhouse tomatoes.

INTRODUCTION

Infection by tomato powdery mildew conidia and attack by whiteflies which can pass through the conventional insect net of a greenhouse are a current threat to tomato cultivation. Fortunately, the problem of the powdery mildew infection can be solved by use of a corona discharge method to directly eradicate fungal colonies formed on tomato leaves before they mature to release progeny conidia (Nonomura *et al.* 2008). The remaining problem is how to protect tomato plants from infection by tomato yellow leaf curl virus (TYLCV) carried by whiteflies. A conventional pest control measures is the use of an electric field screen as an environmentally friendly tool to reduce the use of agrochemicals such as fungicides and insecticides. In fact, this system was highly effective in excluding pathogens and pests from spaces of plant cultivation when installed as screens in the windows of a greenhouse (Kakutani *et al*., 2012). However, such equipment is expensive for small scale farmers and thus we were requested to devise a low-cost method to control whiteflies.

To solve this problem, we developed an easy and efficient method to directly remove whiteflies colonising tomato plants in greenhouses to reduce the population size of these pests, thus reducing subsequent multiplication through their oviposition in the greenhouse. For this purpose, we devised a racket-shaped electrostatic flying insect catcher (REIC), which was portable and easy to operate on-site in the greenhouse at the time of daily care of plants. The present paper describes the capability of the REIC to trap whiteflies that fly off of colonised seedlings. In addition, we examined

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the REIC for its ability to control other major pests (western flower thrips, green peach aphids, and tomato leaf miner flies). Lastly, we applied this method to greenhouse tomatoes that were colonised by numerous whiteflies and demonstrated a drastic decrease in their population thereby minimising the secondary expansion of insecticide-resistant and viruliferous whiteflies which can lead to a serious loss of tomato fruit yield.

MATERIALS AND METHODS

Pest insects

Whitefly (*Bemisia tabaci* Gennadius, type Q, virus-free) adults were collected from greenhouse-grown tomatoes and reared on tomato plants in a temperature-controlled greenhouse (26 ± 2°C, relative humidity 35–55%) (Matsuda *et al.* 2013). Male and female adults that multiplied on the tomato plants were collected using an insect aspirator (Wildlife Supply, Binghamton, NY). Three further insect species were used to investigate pest control: Green peach aphids (*Myzus persicae* Sulzer), western flower thrips (*Frankliniella occidentalis* Pergande) and tomato leaf miner flies (*Liriomyza sativae* Blanchard). These insects were purchased from Sumika Technoservice (Hyogo, Japan). Adult western flower thrips and wingless adult female green peach aphids were reared on water-swollen seeds (Murai 1991) and 1-week-old broad bean seedlings (*Vicia faba* L. 'GB-Blend') (Murai and Loomans 2001), respectively. Hatched winged adult female green peach aphids and adult male and female western flower thrips were collected using an insect aspirator, and used in the experiments. Adult leaf miner flies were released on potted 1-month-old tomato seedlings in a greenhouse with the same conditions as described above. Pupae that fell onto the soil in the pots were collected in a Petri dish and hatched adult flies were collected using an insect aspirator. The average body size of the insects (i.e. length from head to wing tip, with 20 adults measured per species) was 0.86 ± 0.27 mm for the whiteflies, 1.95 ± 0.17 mm for the green peach aphids, 1.56 ± 0.18 mm for the western flower thrips, and 1.93 ± 0.47 mm for the leaf miner flies.

Construction of racket-shaped electrostatic flying insect catcher (REIC)

Iron conductor wire (2 mm diameter, 15–20 cm length) was insulated by passing it through a transparent insulator vinyl sleeve (1 mm thickness, 1×10^9 Ω·m) and was used to construct the REIC (Fig. 1A). Electrical components of the REIC included two layers of insulated iron conductor wires (ICWs) in parallel arrays and two electrostatic direct current voltage generators (DMS-P and DMS-N; Max Electronics, Tokyo, Japan) that supplied negative and positive voltages to the ICWs (Fig. 1B). The ICWs of each layer were arranged in parallel at 5-mm intervals and connected to each other and to a negative or positive voltage generator. The two ICW layers were arranged in parallel with a 2-mm separation between the layers, and the positions of the ICWs of each layer were offset between the layers (Fig. 1C). The ICWs of both layers were oppositely charged with equal voltages. The generators were operated with 12 V storage batteries to supply equal negative and positive voltages to the ICWs [the negatively and positively charged ICWs are hereafter represented as ICW(–) and ICW(+), respectively]. In this system, free electrons from ICW(+) were conducted to ICW(–). Cover sleeves were dielectrically polarised positively on the surface of the iron wire side and negatively on the outer surface of the insulator sleeve in ICW(–) and vice versa in ICW(+) (Matsuda *et al.* 2012). The opposing surface charges on the ICWs act as dipoles that form an electric field in-between. The two layers of ICWs were integrated in the frame of the racket surface (table tennis racket size) and two voltage generators together with a storage battery were put inside the hand grip (Fig. 1A).

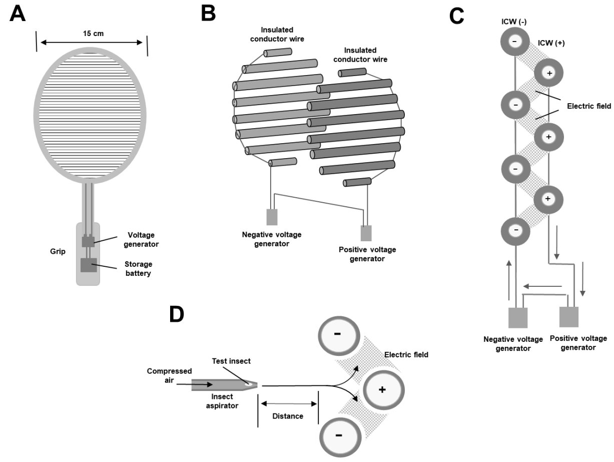

Fig 1 Diagram of the racket-shaped electrostatic flying insect catcher (REIC) (**A**), two layers of ICWs oppositely charged with two voltage generators (**B**), electric fields formed between ICW(–) and ICW(+) (**C**), and an insect blowing assay (**D**). The arrows show the direction of the flow of free electrons between ICW(–) and ICW(+) to form the electric field (**C**) and the direction of the path taken by the insects due to the electrostatic forces (**D**).

Determination of the optimal voltage for the REIC

The REIC was operated at voltages in the range 0.5–5.0 kV to investigate the required voltage to capture all of the test insects. Adult insects were blown into the space between the ICWs by compressed air (1.5 kg/cm^2) blown through the tip of an insect aspirator, as shown in Figure 1D. The distance between the tip of the aspirator and the surface of the ICWs was varied to provide wind speeds in the range 1–3 m/s. The wind speed was measured at the surface of the ICW using a high-sensitivity anemometer (Climomaster 6533; Kanomax, Tokyo, Japan). To confirm the successful capture of the insects with the ICW, we directed the blower (with a maximum wind speed of 7 m/sec at the ICW) at the captured insects for 10 min. Twenty adults were used for each voltage tested and for each insect species. The experiments were repeated three times and data are presented as the mean and standard deviation (SD). The significance of the data was analysed statistically, as described in the caption to Table 1.

Table 1. Percentage of test insects captured by the ICWs of a doubly charged racket-shaped electrostatic flying insect catcher (REIC)

Wind speed ($m\,s^{-1}$)	Test insects	Negative and positive voltages (kV) applied to ICWs						
		0	0.5	1	2	3	4	5
1	Whitefly	0	0	48.3 ± 5.8 a	91.7 ± 7.6 a	100 a	100	100
	Western flower thrips	0	0	0 c	13.3 ± 5.8 c	68.3 ± 5.8 b	100	100
	Green peach aphid	0	0	0 c	8.3 ± 2.9 c	63.3 ± 2.9 b	100	100
	Tomato leaf miner fly	0	0	0 c	6.7 ± 2.9 c	31.7 ± 5.8 c	100	100
2	Whitefly	0	0	11.7 ± 2.9 b	53.3 ± 5.8 b	100 a	100	100
	Western flower thrips	0	0	0 c	11.7 ± 2.9 c	38.3 ± 7.6 c	100	100
	Green peach aphid	0	0	0 c	8.3 ± 5.8 c	33.3 ± 2.9 c	100	100
	Tomato leaf miner fly	0	0	0 c	0 d	28.3 ± 5.8 c	100	100
3	Whitefly	0	0	0 c	46.7 ± 7.6 b	100 a	100	100
	Western flower thrips	0	0	0 c	0 d	30.0 ± 5.0 c	100	100
	Green peach aphid	0	0	0 c	0 d	26.7 ± 7.6 c	100	100
	Tomato leaf miner fly	0	0	0 c	0 d	8.3 ± 5.8 d	100	100

Seven to ten adult insects were used with each voltage and wind speed, and the means and standard deviations were calculated from five repetitions of the experiments. The letters on the means in each column indicate significant differences (i.e. $p < 0.05$) according to Tukey's method.

Capture of flying insects with the REIC

In the first experiment, the whiteflies (500 adults) were released on the abaxial leaf surfaces of three potted tomato plants (*Solanum lycopersicum* cv. Moneymaker, 2-month-old), using an insect aspirator. Tomato plants were placed in a closed-window greenhouse to avoid further insects entering the greenhouse. After 1–2 h for insect colonisation on leaves, the plants were gently tapped so that the whiteflies would fly up in the air. The insects flying over the plants were captured by waving the REIC once or twice above the plants. After captured insects were counted, the operations of plant-tapping and insect-capturing (trapping operation) were repeated until all released insects were trapped, because non-trapped insects flew back to the original colonised plants. The speed of the airflow that passed through the ICWs of the REIC was measured with an anemometer fixed to the surface of the ICWs. The airflow speed was between 0.2 and 2.8 m/sec, depending on the racket waving speed.

The second experiment was a practical assay in a greenhouse. An open-window greenhouse (whose windows were furnished with a conventional insect-proof net with mesh size of 1.5 mm) was divided into two rooms with a partition. Five hundred pest-free and healthy tomato seedlings (cv. Moneymaker) were grown for 40 d in an electrostatic nursery shelter (Fig. 2A) (Takikawa *et al.* 2016) and placed in another open-window greenhouse. They were transplanted into soil beds of each room and cultivated for 2 months until the fruits were cropped. In both rooms (rooms A and B), yellow sticky plates (Y-plates; Arysta Life Science, Tokyo, Japan) (total of 20 plates for each) were hung from a crossbeam at 2-m intervals to determine the number of whiteflies present in the room. The trapping operation described above was conducted in room B at the time of daily plant care (removal of lateral buds and watering) during the entire period of experiment, while control measures were not performed in room A. The experiment was initiated when vigorous and continuous invasion by whiteflies was detected in neighbouring greenhouses, where no treatments for pest control were conducted during the experimental periods in order to maintain a large population of whiteflies for their frequent movement to the test greenhouse.

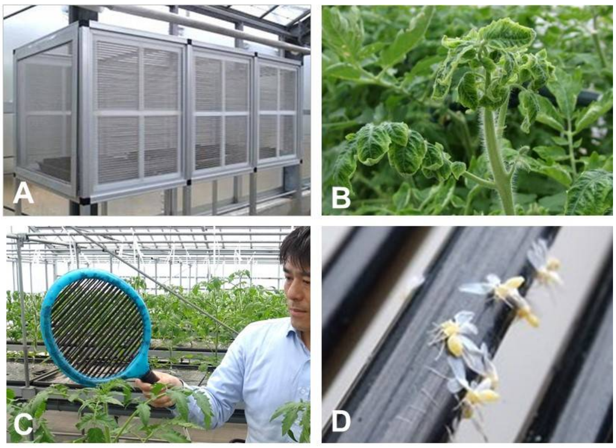

Fig 2 (**A**) An electrostatic nursery shelter used for raising pest-free healthy tomato seedlings. (**B**) Typical TYLCV symptoms (leaf curing and yellowing) detected in a tomato plant grown in room A of the test greenhouse. (**C**) An insect trapping operation with the REIC by a worker. (**D**) Whiteflies captured with the ICWs of the REIC during the insect trapping operation.

RESULTS AND DISCUSSION

The whitefly *B. tabaci* (biotype Q) is a major pest in tomato cultivation (Perring, 2001). The greatest economic threat is due to the transmission of damaging plant viruses, primarily the Geminiviruses (Cohen and Berlinger, 1986; Oliveira *et al.*, 2001). Whitefly has been difficult to control with insecticides because it feeds and oviposits mainly on the abaxial leaf surfaces (Sharaf, 1986) and because it has developed resistance to most classes of insecticides applied for its control (Prabhaker *et al.* 1985; Palumbo *et al.* 2001; Horowitz *et al.* 2004; Nauen and Denholm 2005). Physical methods could provide an alternative means of managing the pest, since they would be compatible with other components of integrated pest management, have little impact on the environment, and reduce pesticide use, thus slowing the development of insecticide resistance (Weintraub and Berlinger 2004). In the present study, we evaluated the effectiveness of the REIC as a physical method to control whiteflies colonising tomato plants.

Prior to the practical testing of the approach, we examined the ability of the REIC to capture released adult insects with various body sizes. Table 1 lists the percentage of insects captured by the REIC at various voltages in the range 0.5–5.0 kV and for various wind speeds in the range 1.0–3.0 m/sec. Higher voltages were required to be applied to the ICWs to capture insects with larger body sizes, and higher voltages were required at higher wind speeds. Raising the voltage applied to the ICWs increased the electrostatic attractive force. There were no significant differences in the captured numbers of the three insect species (i.e. whiteflies, western flower thrips, and green peach aphids) with similar body sizes for all wind speeds and voltages. For voltages in excess of 4.0 kV the ICWs captured all of the insects for all wind speeds investigated; in fact, the electrostatic forces were sufficient to capture the insects at wind speeds of up to 7 m/sec. These results demonstrate that the REIC at 4.0 kV could eliminate all major pest insects investigated under real-world conditions in the greenhouse. At lower voltages, however, the electrostatic forces were not sufficient to capture the insects, and either the whiteflies fluttered their legs, twisted their bodies, and then flew away from the ICW or were blown away from the ICW by the blower. Based on these observations, in subsequent experiments a voltage of 4.0 kV was applied to ensure successful capture of the insects at wind speeds of up to 3 m/sec, which corresponds to the maximum airflow speed when the REIC was waved.

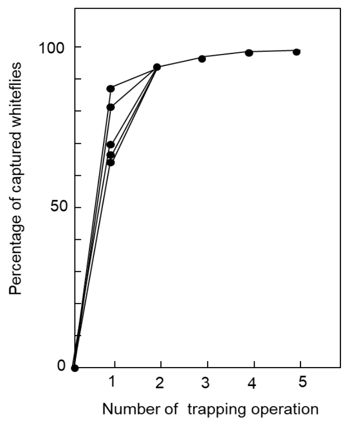

Fig 3 Percentage of captured whiteflies colonising tomato plants by number of trapping operations using the REIC.

Figure 3 shows the number of the whiteflies captured by the repeated trapping operations. The trapping operation was effective in directly removing whiteflies from the tomato plants, as whiteflies have the habit of flying up from the plant when shaken and immediately returning to the original plant. In fact, all of the insects released could be trapped by repeating the operation twice. Thus, the REIC was portable and easy to operate and appears to be effective for the removal of whiteflies on tomato plants in a greenhouse.

Biotype-Q whiteflies infected with TYLCV were prevalent in our district (Matsuda *et al*. 2013). A PCR-based whitefly detection assay showed that the ratio of biotype-Q whiteflies on the insect-adhesive yellow plates hung inside the neighbouring greenhouses increased gradually during the experimental period (data not shown). Moreover, the appearance of typical symptoms of TYLCV (yellowing and curling of tomato leaves) in the tomatoes was another sign of invasion by whiteflies carrying the virus. In fact, symptoms of TYLCV were detected in many tomato plants in the greenhouses (Fig. 2B). These results indicate the entry of outside virus-carrying whiteflies into these greenhouses, suggesting that the tomato plants in this study could suffer similarly from invasion by these whiteflies.

Our cultivation system for greenhouse tomatoes involves two stages; a nursery cultivation of young seedlings in an electrostatic nursery shelter for 30 days, and fruit production by the seedlings transplanted and cultivated in a non-guarded greenhouse for two months. In the present study, the healthy tomato seedlings were successfully obtained in the first stage and therefore our subsequent goal was to protect the tomato seedlings transferred to a non-guarded greenhouse from invading whiteflies by directly eliminating them with the REIC. The main point of this approach was to reduce the population of the whiteflies colonising the greenhouse to minimise the chance of their oviposition. Theoretically, the invading whiteflies are able to continue at least four generations during the experimental period (Helyer *et al*. 2004), and therefore it was very important to carefully and thoroughly eliminate the whiteflies at the initial stage after transplanting. In fact, the number of the whiteflies entering per day was very low (average number, 6.6, 8.3, and 2.8 whiteflies/day in three other greenhouses tested) so that we could directly eliminate them without missing any. Figure 2C shows a worker conducting the insect trapping operation with the REIC, and Figure 2D shows whiteflies captured by the ICWs of the REIC. The effectiveness of the REIC was very clear in comparison with the number of whiteflies captured on the yellow plates in both rooms of the same greenhouse. The results indicate that the number of whiteflies on the plates in the non-trapped room (room A) increased gradually during the 2-month experimental period: Average values of 15.4 ± 13.3, 72.3 ± 23.5, 256.2 ± 95.5, and 437 ± 172.1 whiteflies per plate were observed over 2-week intervals. In addition, symptoms of TYLCV were detected in 40 tomato plants in room A within 2 months after transplanting. These results indicated the entry of outside virus-carrying whiteflies into room A from the lateral windows, suggesting that room B of the same greenhouse suffered similarly from invasion by virus-carrying whiteflies. Nevertheless, in room B, the number of the whiteflies on the plates remained very low (1.7 ± 1.8, 5.5 ± 2.3, 13.9 ± 4.6, and 22.8 ± 4.9 whiteflies per plate), and no tomato plants showed the symptoms of TYLCV demonstrating that the trapping operation with the REIC solved the whitefly problem.

CONCLUSION

The primary contribution of this work was the use of basic electrostatics for controlling insect pests in crop production and protection. The REIC is a unique product developed for this purpose. The structure of the REIC is simple; no special technique is required for its construction. The REIC can operate normally at low electric power consumption to capture flying insect pests that cause severe damage in crops. This work demonstrated that the proposed REIC can easily eliminate whiteflies colonising greenhouse tomatoes leading to healthy growth of the tomato plants to the final stage of fruit production in an open-window greenhouse.

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