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Successful single-truss cropping cultivation of healthy tomato seedlings raised in an electrostatically guarded nursery cabinet with non-chemical control of whiteflies

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The present study presented an efficient non-chemical method of controlling insecticide-resistant whiteflies in a single-truss cropping system for hydroponic tomatoes. For this purpose, we used two kinds of electrostatic apparatus: an electrostatically guarded nursery cabinet (EGNC) to create a pest-free space by preventing the entry of whiteflies, and an electrostatic insect sweeper (EIS) to directly trap whiteflies colonising tomato plants. The EGNC was newly devised in this study. It had two layers of insulated conductor iron wires (ICWs) in parallel arrays and two electrostatic direct current voltage generators that supplied negative or positive voltages to the ICWs. Within each layer, the ICWs were placed 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 represented as ICW(–)s and ICW(+)s, respectively. Two IC Wlayers were placed parallel at a 2-mm interval and the ICWs in the layers were offset relative to each other. Adult whiteflies were blown into the space between the ICWs to identify the voltage range that would capture all of the test insects. The results 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 EGNC was practically applicable to a greenhouse that suffered from frequent invasion by numerous viruliferous whiteflies; we found that seedlings grew normally inside the EGNC during a40-day cultivation period. The EIS was also effective in protecting tomato plants subsequently transplanted to a non-guarded greenhouse. The EIS was handy and easy to operate on-site in a greenhouse, and it was used to trap whiteflies residing on tomato plants during daily plant care. Through this daily elimination of whiteflies, the whitefly population in a greenhouse was controlled to negligible levels during the experimental period (40 days), and nearly all tomato plants were able to produce normal fruit. Thus, the present study demonstrated that single-truss cultivation of greenhouse tomatoes could be performed with the aid of two electrostatic apparatuses to control insecticide-resistant whiteflies.

Keywords: physical control, electrostatic attractive force, insect pests

INTRODUCTION

We have used two systems for greenhouse tomato cultivation over the course of 3 years, rotated seasonally. From July to September (a very hot and humid season in Japan), we practice short-period cultivation of densely planted tomato seedlings, harvesting fruits from only the first trusses (single truss-cropping system) (Giacomelli et al. 1994).In the remaining seasons, we practice long-period cultivation of tomato plants, harvesting fruits from multiple-flower trusses. The latter, which is our usual system, has suffered from an unavoidable and formidable drop in yield during the summer season, which is characterised by high temperature and humidity. To solve this problem, we introduced the short-period cultivation system to our greenhouse cultivation trials.

The main threats to our tomato cultivation system have historically been powdery mildew (Matsuda et al. 2001, Kashimoto et al. 2003) and tomato yellow leaf curl virus (TYLCV) transmitted by whiteflies (Tanaka et al. 2008, Matsuda et al. 2013). In the summer season, however, powdery mildew infection seldom occurs; conversely, invasion by viruliferous whiteflies is very frequent and severe. Therefore, we sought to effectively control this pest during the summer season.

In the long-period cultivation system, tomato plants are grown in a greenhouse whose windows and entrances are guarded with an electric field screen to prevent the ingress of airborne pathogens and flying insect pests (Matsuda et al. 2006, Tanaka et al. 2008, Kakutani et al. 2012, Nonomura et al. 2012). This system is effective in excluding major pests from the greenhouse, including whiteflies, western flower thrips, green peach aphids, and tomato leaf-miner flies (Helyer et al. 2004), as well as conidia of the tomato powdery mildew (Matsuda et al. 2001, Kiss et al. 2001, Kashimoto et al. 2003).Thus, our electrostatic exclusion method helps to create a pest- and pathogen-free space inside the greenhouse. However, the equipment for this system is very expensive; for example, the installation of the electric field screen would likely be cost-prohibitive for small farmers. To provide a more cost-effective alternative method, we propose an efficient system for single-truss cropping cultivation of greenhouse tomatoes. This system consists of two pest-control operations with electrostatic apparatuses. In the first stage, healthy tomato seedlings are raised in an electrostatically guarded nursery cabinet(EGNC), and in the second stage of cultivation, whiteflies are directly eliminated using an electrostatic insect sweeper (EIS) (Takikawa et al. 2015).

In the above system, the cultivation of healthy seedlings in the first stage is critically important because these young seedlings are highly sensitive to TYLCV infection. Damage from the infection is so serious that the seedlings cannot grow and produce normal fruit during the second stage. In the present study, we describe an effective pest exclusion method using the EGNC, an easily assembled temporary cabin whose three faces are furnished with an electric field screen, and demonstrate its utility for raising pest-free tomato seedlings in an open-window greenhouse suffering from frequent invasion by whiteflies. Furthermore, we describe a method for the direct capture of whiteflies during the second stage of cultivation using the EIS, which is a portable electrostatic apparatus that traps whiteflies on leaves during daily plant care activities. The EIS can be operated onsite in a non-guarded greenhouse. Finally, we show that these electrostatic approaches are suitable for a short-period cultivation system based on the single-truss cropping method, and can be conducted without relying upon chemical control for insecticide-resistant viruliferous whiteflies. Thus, in the present study, we successfully present an experimental basis for a protected single-truss cropping system for hydroponic tomatoes.

MATERIALS AND METHODS

Pest insects

Adult whiteflies (B. Tabaci Gennadius, type Q, virus-free) were collected from greenhouse-grown tomatoes and reared on tomato plants in a temperature-controlled greenhouse (26 ± 2°C, relative humidity of 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 also used to investigate pest control: green peach aphids (M. persicae Sulzer), western flower thrips (*F. Occidentalis* Pergande), and tomato leaf-miner flies (*L. 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 (Viciafaba 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 subsequently used in the experiments. Adult leaf-miner flies were released on potted 1-month-old tomato seedlings in a greenhouse under the same conditions as described above. Pupae that fell onto the soil in the pots were collected in Petri dishes, and hatched adult flies were collected using an insect aspirator. The average body size of the insects (i.e., the mean length from the head to wing tip of 20 adults of each species) was 0.78 ± 0.09 mm for the whiteflies, 3.75 ± 0.27 mm for the green peach aphids, 1.46 ± 0.13 mm for the western flower thrips, and 3.93 ± 0.37 mm for the leaf-miner flies.

Construction of an electrostatically guarded nursery cabinet (EGNC)

The EGNC was a rectangular cabin (4 × 4 × 2 m) covered with a semi-transparent vinyl sheet whose three lateral walls were furnished with electric field screens (0.9 ×3.8m) (Figure 1A, Figure 4A). The electric field screens were constructed using an insulated conductor wire (ICW); an iron conductor wire (2-mm diameter, 3.6m length) was insulated by passing it through a transparent

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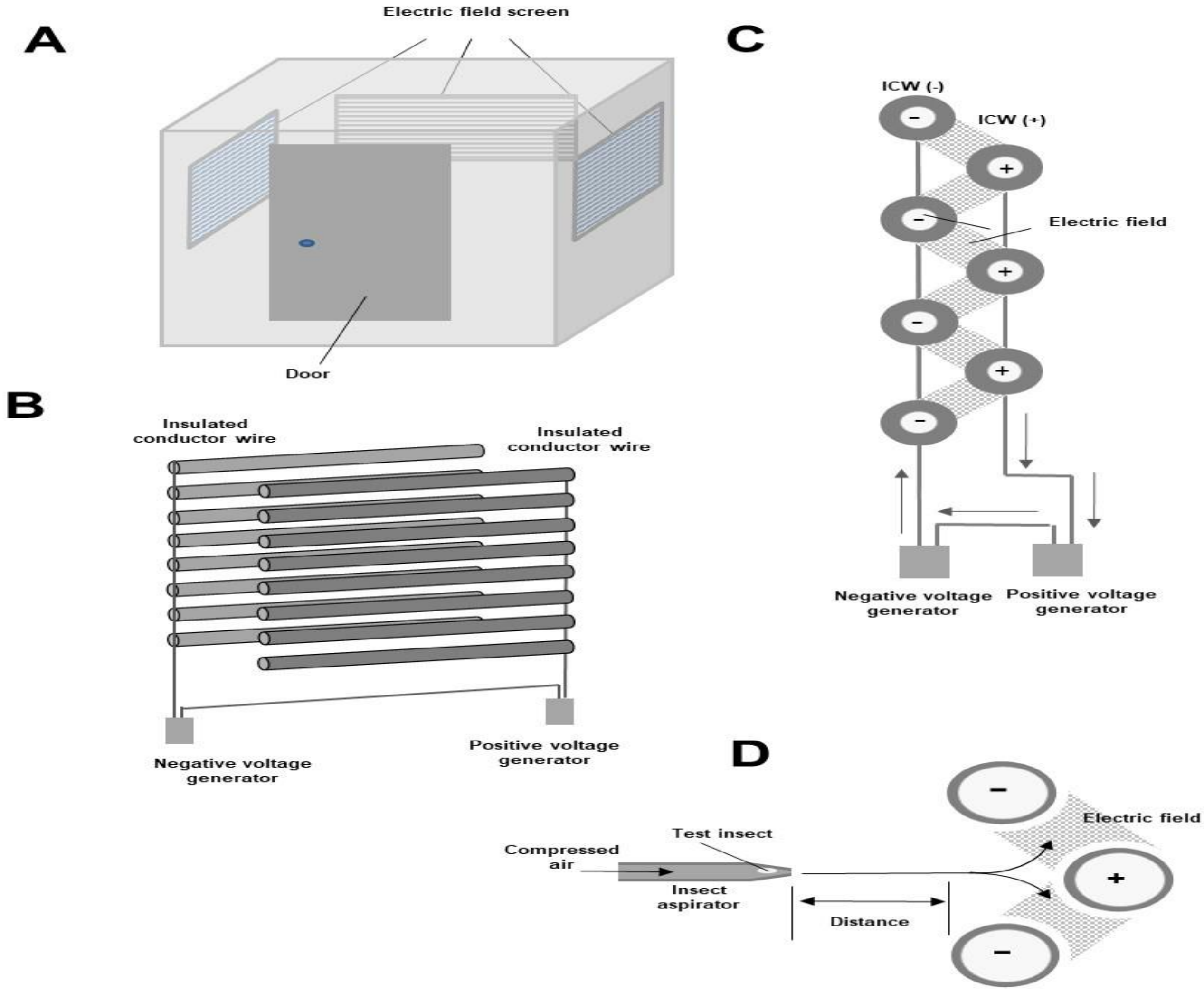
Figure 1. (A) Diagram of the electrostatically guarded nursery cabinet (EGNC) furnished with electric field screens on three walls,(B) two layers of ICWs oppositely charged with two voltage generators, (C) electric fields formed between ICW(–)s and ICW(+)s,and (D)an insect blowing assay. The arrows show (C) the direction of flow of free electrons between ICW(–)s and ICW(+)s to form the electric field and(D)the direction of the path taken by the insects due to the electrostatic forces.



insulator vinyl sleeve (1-mm thickness, 1×10^9 Ω·m). The electric field screen had two components: two layers of insulated conductor iron 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, respectively, to the ICWs (Figure. 1B). The ICWs of each layer were positioned parallel at a 5-mm interval and connected to each other and to a negative or positive voltage generator. Two ICW-layers were positioned parallel at a 2-mm interval, and the ICWs of the layers were offset relative to each other (Figure 1C). The ICWs of both layers were oppositely charged with equal voltages. The generators were operated to supply equal negative and positive voltages to the ICWs (the negatively and positively charged ICWs are hereafter represented as ICW(–)s and ICW(+)s, respectively). In this system, free electrons from ICW(+)s were pushed out to ICW(-)s. 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(–)s, and vice versa in ICW(+)s(Matsuda et al. 2012). The opposite surface charges on the ICWs act as dipoles that create an electric field between them. Two layers of ICWs were integrated into the aluminium frame, and two voltage generators were operated using 12V storage batteries, with power supplied by a 50W solar panel.

Optimal voltage for the electric field screen

The electric field screen was operated at voltages in the range of 1.0–5.0 kV to determine the voltage required to capture all of the test insects. Adult insects were blown into the space between the ICWs by sending compressed air (1.5 kg/cm^2) 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 of 1–3 m/s. 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/s 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 for Figure 2.

Greenhouse assay for capture of whiteflies with the EGNC and EIS

For the insect trapping assay at the first stage of single-truss cropping, 500 two-week-old cotyledonal tomato seedlings (Solanum lycopersicumcv. Money maker) were transferred to polystyrene plates floating on nutrient solution in a hydroponic culture trough that was set in the EGNC in an open-window greenhouse. Hydroponic culture was carried out for 40 days according to a previously described method (Nonomura et al. 2001). The number of whiteflies captured with the ICWs of the electric filed screens was counted every 10 days throughout the experiment.

For the second stage of the experiment, seedlings were transferred to hydroponic culture troughs in three non-guarded greenhouses. Plants were grown at a high density (12 to 15 plants per square meter) to achieve maximum yield, and topped above the ninth leaf position (the second leaf position above the first clusters) approximately 1 week after transfer. All lateral buds formed were removed throughout the 40-day propagation period. The whiteflies that invaded the greenhouse and colonised tomato plants were captured using the EIS according to a previously described method (Takikawa et al. 2015). Specifically, the EIS was negatively charged at 1.5 kV and gently slid along the leaves to capture the colonising insects (See Figure 4B). This insect-trapping operation was conducted during daily plant care activities for the entire period of the experiment. These experiments were initiated after we detected the severe and persistent invasion of neighbouring greenhouses by whiteflies.

Results and discussion

The whitefly B.tabaci (biotype B) is a major pest in tomato cultivation (Perring, 2001). It poses an economic threat through the transmission of damaging plant viruses, primarily the Geminiviruses (Cohen and Berlinger, 1986; Oliveira et al., 2001). Whitefly is difficult to control with insecticides because it feeds and oviposits mainly on the abaxial leaf surfaces (Sharaf, 1986) and because it has developed a 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, as they are 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 EGNS and EIS as physical methods for controlling insecticide-resistant viruliferous whiteflies that invade greenhouse tomato plants.

Prior to our experiment, we assessed the ability of the electric field screen of the EGNS to capture released adult insects of various body sizes. Figure 2 shows the percentage of insects captured by ICWs of the electric field screen at various voltages in the 1.0–5.0 kV range, and for various wind speeds in the 1.0–3.0 m/s range. Higher voltages were required to capture insects with larger body sizes, and higher voltages were required at higher wind speeds. The force of the ICWs became stronger with increasing applied voltage. There were significant differences in the capture rates for all insect species at all wind speeds at <3.0 kV. For voltages in excess of 4.0 kV, the ICWs captured all of the insects at the highest wind speed investigated (3 m/s) (Figure 3A-D). The electrostatic forces were sufficient to capture insects at wind speeds of up to 7 m/s. These results demonstrate that at 4.0 kV, the EGNC could eliminate all major pest insects under real-world conditions in the greenhouse. We note that the wind speed of 3 m/s was the highest observed in the greenhouse because the greenhouse windows were automatically closed when the outside wind speed reached 3 m/s. At lower voltages, however, the electrostatic forces were not sufficient to capture insects, and the insects were able to escape the ICW or were blown away from the ICW by the blower. Based on these observations, in subsequent experiments, we used a voltage of 4.0 kV to ensure successful capture of insects at wind speeds of up to 3 m/s.

TYLCV-carrying biotype-Q whiteflies are prevalent in our district (Matsuda et al. 2013). A PCR-based whitefly detection assay conducted in a neighbouring greenhouse during the experimental period showed that the ratio of biotype-Q whiteflies on the insect-adhesive yellow plates hung inside the neighbouring greenhouses increased gradually throughout the experiment (data not shown). Moreover, the appearance of typical symptoms of TYLCV (yellowing and curling of tomato leaves) signified invasion by whiteflies carrying the virus; indeed, symptoms of TYLCV were detected in many tomato plants in these greenhouses (Figure 4B). These results indicate that virus-carrying whiteflies invaded neighbouring greenhouses, suggesting that the tomato plants placed in the EGNC were at a similar risk for invasion by these whiteflies.

The present short-period cultivation system involves two stages: cultivation of young seedlings in an electrostatic nursery shelter for 40 days, and hydroponic

Figure 2. Capture of adult whiteflies (A), western flower thrips (B), green peach aphids (C), and tomato leaf miner flies (D) by the ICWs of the electric field screen doubly charged with 1.0-4.0 kV. Twenty adult insects were used at each voltage and wind speed, and the means and standard deviations were calculated from five experimental repetitions. The letters in each figure indicate significant differences (p< 0.05) according to Turkey's method.

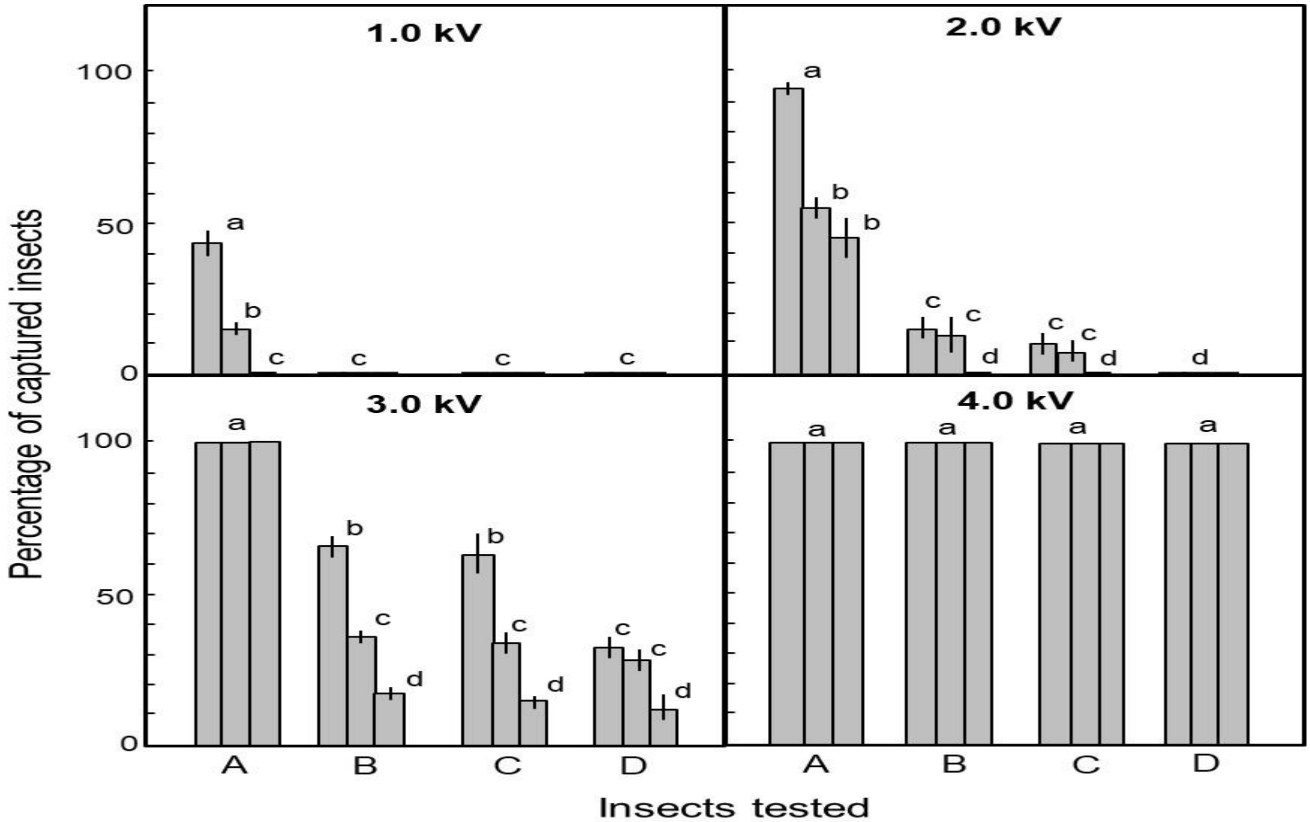


Figure 3. Insect pests captured with the ICWs of the electric field screen. The ICWs of the electric field screen were doubly charged with 4.0 kV, and adult whiteflies (A), western flower thrips (B), green peach aphids (C), and tomato leaf miner flies (D) were separately blown toward the ICWs at 3 m/sec.

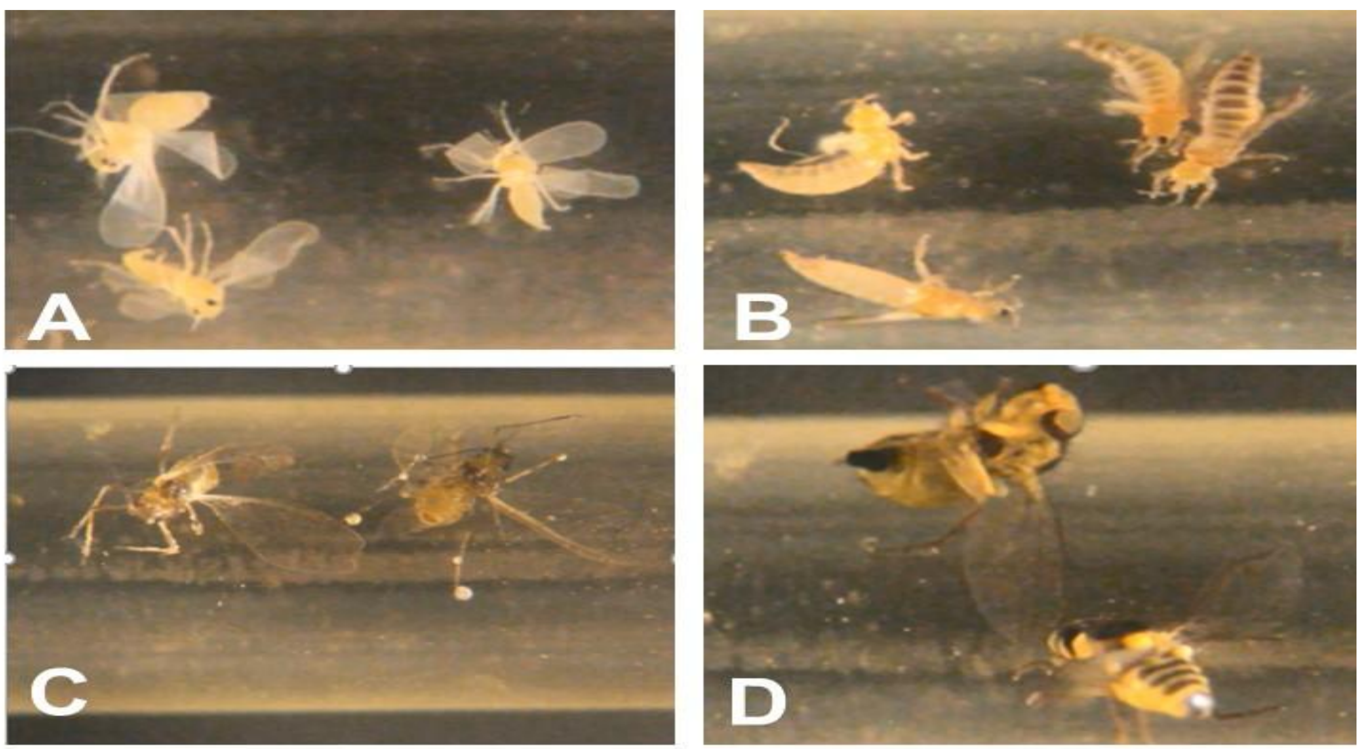
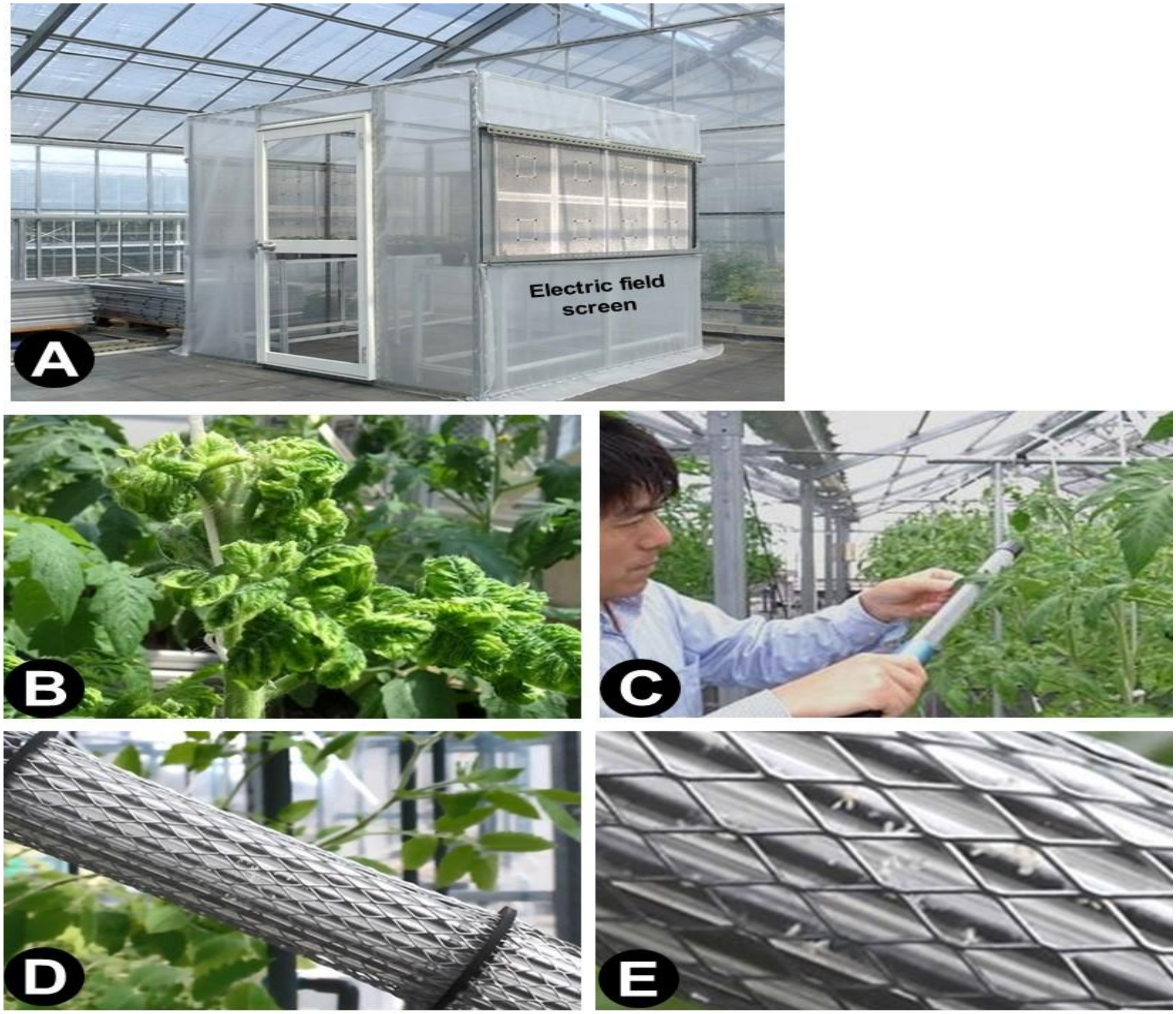


Figure 4.(A) The electrostatically guarded nursery cabinet (EGNC) furnished with electric filed screens on three walls, (B) typical symptoms (yellowing and curling of leaves) of TYLCV in tomato plants, (C) pest-trapping operation with the EIS at the time of routine plant care, (D)enlarged photograph of EIS, and(E) whiteflies captured with the EIS.



culture of topped seedlings in a non-guarded greenhouse for additional 40 days (until their first-truss fruits became ripe). During the first step of cultivation, we monitored the whiteflies that were captured with the electric field screens of the EGNC. We found that the ICWs successfully captured the whiteflies and prevented them from entering the cabinet. The number of captured whiteflies was12, 20, 15, and 34 at each of the successive 10-day monitoring intervals, and the inside of the EGNC remained pest-free throughout the experimental period.

We obtained healthy tomato seedlings from the first cultivation period. Accordingly, we were apprehensive about invasion by viruliferous whiteflies after transferring the healthy seedlings to a non-guarded greenhouse. To solve this problem, we attempted to directly eliminate whiteflies residing on the transplanted tomato plants using a second electrostatic apparatus (EIS). The goal of this approach was to reduce the whitefly population and limit their opportunities for oviposition. Theoretically, the experimental period spanned at least two whitefly generations (Helyer et al. 2004), and it was therefore very important to carefully and thoroughly eliminate whiteflies at an early stage after transplantation. We found that the pest-trapping activity using the EIS was easy to perform during daily plant care (Figure 4C). The number of whiteflies trapped per day (i.e. the number of the whiteflies entering the greenhouse per day) was low (average of 6.6, 8.3, and 12.8 insects/day in the three greenhouses tested), and the trapping procedure was very effective in capturing whiteflies on tomato plants (Figure 4D and E). Through this routine pest trapping procedure, we were able to prevent the whitefly population from growing through secondary multiplication. Ultimately, we were able to raise healthy tomato plants throughout the entire cultivation period, from raising healthy seedlings to cropping fruits.

CONCLUSION

The present work demonstrated an effective application of basic electrostatics for controlling insect pests during crop production. The EGNC is a unique product newly developed for this purpose, and the EIS in an apparatus that has been the subject of previous studies. The structure of the EGNC is simple, and no special technique is required for its construction. Both apparatuses can operate with low electric power consumption to capture insect pests capable of causing severe crop damage. The present study demonstrates that these methods can be applied to the single-truss cropping system for greenhouse tomatoes to effectively control insecticide-resistant viruliferous whiteflies.

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