

# 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

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An ozone-generative electrostatic spore precipitator was developed to protect nursery-stage seedlings of tomato from both airborne conidia of powdery mildew (*Oidium neolycopersici*) and root-infecting pathogen propagules of bacterial wilt (*Ralstonia solanacearum*) and fusarium crown and root rot (*Fusarium oxysporum* f.sp. *radicis-lycopersici*). The device was a cylindrical electrostatic spore precipitator (S2 cylinder) in which a positively charged straight conductor wire insulated with a transparent acrylic cylinder originated from a spore-precipitation cylinder (S1 cylinder) designed to physically control airborne conidia of tomato powdery mildew in greenhouses. The S2 cylinder consisted of two sites for conidial attraction and ozone production. The site for ozone production was located at the end of the cylinder, where an earthed copper conductor ring (as a cathode) was attached to the edge of the cylinder, responding to the anodal tip of a positively charged central conductor wire. Distinct types of discharge (corona, corona-streamer, streamer and arc discharge) occurred between the two electrodes and were dependant on the voltages applied to the wire and the distances between the electrodes. The highest ozone production was observed through streamer discharge. The remaining portion of the S2 cylinder, which was dielectrically polarized by a positively charged wire, created a non-uniform electric field outside the cylinder to attract conidia that came into the generated field. Hydroponic culture troughs to raise tomato seedlings in a nursery greenhouse were paralleled with S2 cylinders. The aim was to control rhizosphere pathogens *R. solanacearum* and *F. oxysporum* f.sp. *radicis-lycopersici* and to prevent them entering the hydroponic system during cultivation, while at the same time trapping *O. neolycopersici* conidia in the spaces between the cylinders. The results indicated that susceptible tomato plants in culture troughs attached to the S2 cylinders remained uninfected by both rhizosphere and aerial pathogens throughout the experimental period (2 and 3 weeks, respectively). This suggests that the present system will enable the dual control of both these pathogens in hydroponic systems in greenhouses.

**Keywords:** fusarium crown and root rot of tomato, ozone production, physical control of pathogens, polarized dielectric insulator, tomato bacterial wilt, tomato powdery mildew

## Introduction

While a greenhouse environment is excellent for growing tomatoes, it is even better for propagating disease-causing organisms. Because of the higher temperature, higher relative humidity and lush green foliage, diseases are

constant threats once they enter a greenhouse. Management of disease at the seedling nursery stage is important to prevent the establishment of diseases that may affect cropping. In greenhouses, powdery mildew of tomato (*Oidium neolycopersici*) has frequently been detected on hypocotyls, cotyledons and first leaves of nursery-stage seedlings, as well as on well-developed plants in the propagation stage (Matsuda *et al.*, 2001; Kashimoto *et al.*, 2003). Powdery mildew infection of hypocotyls and cotyledons of young seedlings causes severe damage and

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loss of seedlings. Weekly spraying with fungicides is thus standard practice during the entire period of tomato cultivation. In addition to powdery mildew, root-infecting pathogens, such as the bacterial wilt pathogen (*Ralstonia solanacearum*) and the fusarium crown and root rot pathogen (*Fusarium oxysporum* f.sp. *radicis-lycopersici*), occur frequently in hydroponically cultured tomato seedlings, rapidly spreading to seedlings in the same hydroponic culture troughs because of the circulation of the nutrient solution and high-density planting (Matsuda *et al.*, 1998; Nonomura *et al.*, 2001b; Xu *et al.*, 2006). However, the application of chemicals to hydroponic nutrient solution is legally regulated in Japan. Hence, there is a need to develop non-chemical measures to control rhizosphere pathogens of hydroponically cultured tomatoes while controlling airborne conidia of powdery mildew.

Ozone is an effective antimicrobial agent which kills viruses, bacteria and fungi in water (Katzenelson *et al.*, 1974; Burleson *et al.*, 1975; Farooq *et al.*, 1977; Haas & Gould, 1979; Khadre *et al.*, 2001) and therefore has been used on its own to control rhizosphere pathogens in hydroponics (Vanachter *et al.*, 1988; Yamamoto *et al.*, 1990; Runia, 1994; Vestergård, 1994) or in combination with other chemical and physical techniques (Vestergård, 1988; Wakoh *et al.*, 1992; Runia, 1995; van Os, 2001; Schnitzler, 2004; Minuto & Garibaldi, 2005). In Japan, an ozone sterilization technique has attracted great interest from growers, but commercial ozone generators are too costly for agricultural use.

A cylindrical electrostatic spore precipitator to control conidia of tomato powdery mildew was developed (Matsuda *et al.*, 2006). The precipitator consisted of a straight conductor wire, an insulator cylinder and an electrostatic voltage generator. The conductor wire was coaxially held in the centre of a cylinder and negatively charged to dielectrically polarize a cover cylinder. The negative charge on the outer surface of the polarized dielectric insulator produced a non-uniform electric field outside, to make a dipole in conidia entering into the field. In a practical application of this method, cylinders were attached in parallel to a frame covering a hydroponic culture trough. The cylinders then prevented airborne conidia from passing through spaces between them (Matsuda *et al.*, 2006). In addition to this conidial attraction, it was found that with slight modification the cylinder could be improved to produce ozone, without compromising its ability to attract airborne conidia of powdery mildew.

In a non-uniform electric field, discharge occurs between oppositely charged conductors (Griffith, 2004; Halliday *et al.*, 2005). Discharge causes not only the surrounding air to be ionized, but also generates ozone (Chen & Davidson, 2002). The major aim of the present work was to remodel an original spore-precipitation cylinder (Matsuda *et al.*, 2006) into a new system that could produce ozone. For this, the central copper conductor wire of the cylinder was used as an anode and a cathodic copper ring was attached to the edge of the cylinder. The resultant cylinder was a new type of ozone generator

that functioned as an electrostatic spore precipitator, and which could trap conidia of tomato powdery mildew and inactivate or kill pathogens in the nutrient solution. In the present study, this new system was applied to a small-scale hydroponic culture apparatus used for raising tomato seedlings in a nursery greenhouse. The successful dual control of one aerial and two rhizosphere pathogens of tomato in hydroponics with this new system is described.

## Materials and methods

### Hydroponic culture for raising tomato seedlings

Seeds of pear tomato (*Lycopersicon esculentum* var. *pyriforme*) cv. Yellow Pear were germinated and placed in polyurethane sponge supports ( $3 \times 3 \times 3$  cm<sup>3</sup>) in a multi-pack tray ( $30 \times 60$  cm<sup>2</sup>) (128 wells per tray). Six trays with seeds were soaked in nutrient solution in a hydroponic culture trough set in a temperature-controlled growth chamber ( $26 \pm 2^\circ\text{C}$ ,  $80 \mu\text{mol m}^{-2} \text{s}^{-1}$  with a 12-h photoperiod) for 10 days, and then transferred to a hydroponic culture trough ( $0.6 \times 1.8$  m<sup>2</sup>) in a nursery greenhouse to raise seedlings to the third-true-leaf stage for 1 month. Nutrient solution (100 L) of pH 6.0–6.5 with an electrical conductivity of  $1.2 \text{ mS cm}^{-1}$  was circulated through a trough at a flow rate of  $20 \text{ L min}^{-1}$  according to a method described previously (Nonomura *et al.*, 2001a). A portion (10 mL) of the solution was sampled every 3 days to normalize any deficient nutritional components in the solution.

### Pathogens

The following tomato pathogens to which tomato cv. Yellow Pear is highly sensitive (Nonomura *et al.*, 2001b; Matsuda *et al.*, 2005a; Xu *et al.*, 2006) were used in experiments: isolate K-101 of *Ralstonia solanacearum* (Toyoda *et al.*, 1991); isolate Kin2003 of *Fusarium oxysporum* f.sp. *radicis-lycopersici* (Xu *et al.*, 2006); and isolate KTP-01 of tomato powdery mildew pathogen *Oidium neolycopersici* (Kashimoto *et al.*, 2003).

The bacteria were grown at  $26^\circ\text{C}$  for 48 h on PCG agar (10 g bacto-peptone, 1 g casamino acid and 10 g glucose in 1 L water, solidified with 1.5% agar) and stored in sterilized water at room temperature as described by Toyoda *et al.* (1991). Fluidal colonies formed on the medium were selected and suspended in sterilized water. This procedure was repeated three times, and finally a fluidal colony was suspended in water as inoculum.

Microconidia of *F. oxysporum* f.sp. *radicis-lycopersici* were inoculated onto Czapek agar and incubated at  $20^\circ\text{C}$  for 4 days. Colonies obtained from the isolate were subcultured several times at weekly intervals, and monoconidial isolates were cultured in Czapek liquid medium to collect newly formed microconidia (Xu *et al.*, 2006). The conidia were washed with sterile water by low-speed centrifugation and suspended in water to prepare the inoculum.

Conidia of *O. neolycopersici* were maintained on leaves of 1-month-old seedlings of Yellow Pear as

described by Kashimoto *et al.* (2003). Newly produced conidia of 8-day-old powdery mildew colonies on leaves were dusted onto leaves of tomato plants by tapping or brushing the colony-forming leaves.

### Ozone-generative electrostatic spore precipitator

An original spore-precipitation cylinder (S1 cylinder) (Matsuda *et al.*, 2006) was modified to construct the new spore-precipitation cylinder (S2 cylinder) that could produce ozone. An S1 cylinder contains a straight copper conductor wire (2 mm in diameter and 1.0 m long) covered with a transparent acrylic insulator cylinder (10 mm in diameter and 1.0 m long) and held with insulating silicon stoppers in the middle and at both ends of the cylinder (Fig. 1a). In an S2 cylinder (10 mm in diameter and 2.0 m long), a silicon stopper at one end was set 10 cm inside from the cylinder edge and a copper wire (2 mm in diameter and 1.95 m long) passed through it (Fig. 1b). A copper ring, linked to an earthed line, was attached to a cylinder edge to cause a discharge between the wire end and the ring (Fig. 1c). Ozone gas was produced at the site of discharge (site A in Fig. 1b). Single or multiple S2 cylinders (10–50 cylinders) were inserted into a polypropylene airflow pipe to collect the ozone produced. The air containing ozone was transferred to an ozonometer or an air-mixing tank in which air containing ozone was mixed with further air to dilute the ozone to the desired levels with an aspirator (aspirator A) (Fig. 1d). A back-flow preventer was attached to the entrance of the airflow pipe to prevent the inside air from running back to the entrance when the aspirator stopped.

The central conductor wires were linked in parallel to terminals of a connector line of an electrostatic voltage generator, LS40 (voltage-range 1–50 kV; maximum current 3 mA) (Kansai Electronics) (Fig. 1d) and were positively charged with different voltages. The charged wire created a non-uniform electric field (field I) in a space between the wire and the inner surface of the cylinder to dielectrically polarize the cylinder (negative on the inner surface, positive on the outer surface) (site B in Fig. 1b), which produced a non-uniform electric field outside (field II) (Fig. 1e). Field II was the region to create an attractive force between the cylinder and conidia that came into this field (Matsuda *et al.*, 2006).

### Assay for ozone production

An S2 cylinder was placed in a temperature-controlled chamber (25°C) and the conductor wire was positively charged with different potentials (10–30 kV). The distance (distance A in Fig. 1c) between the wire tip (anode) and a cylinder-edge ring (cathode) was changed from 1 to 50 mm to produce different types of discharge with each voltage applied. Discharge was classified into four types according to a criterion described by Goelian *et al.* (1997): corona, corona-streamer, streamer and arc (spark) discharges. A pale-blue light radiated by ionized air through the discharge was detected with a charge-

coupled device camera using a previously described method (van Veldhuizen & Rutgers, 2002). The air in the airflow pipe (connected to a single S2 cylinder) was collected at the lowest useful airflow rate (1.0 L min<sup>-1</sup>) of aspirator A to determine ozone productivity ( $\mu\text{g min}^{-1}$ ); the air was then transferred to a flow cell of an ozone monitor EG-700E3 (Ebara Jitsugyo) and the UV absorption at 254 nm was measured by a standard method (Francis, 2002). The following experiment was conducted with an optimal tip distance of 33 mm, the highest useful voltage (30 kV) and the lowest airflow rate (1.0 L min<sup>-1</sup>).

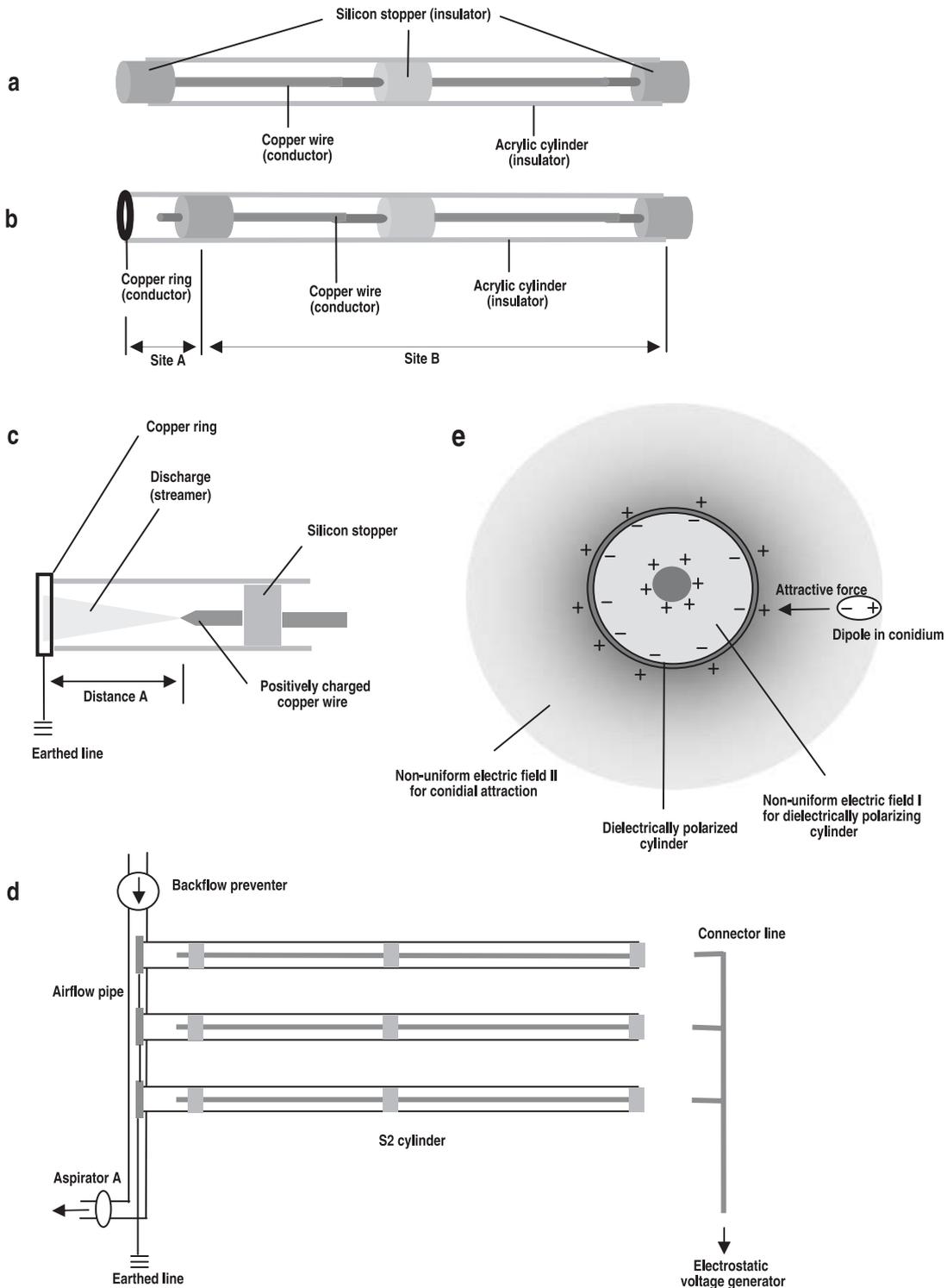
To determine the effect of ozone production by the S2 cylinder, it was tested in chambers at constant temperatures ranging from 5 to 45°C. Moreover, between 10 and 50 cylinders were used to clarify the levels of ozone produced.

During the aspiration of the inside air, the outer-surface positive charge (kV) of the cylinder was measured with an electrostatic field meter FMX-002 (Simco) according to a method described previously (Matsuda *et al.*, 2006).

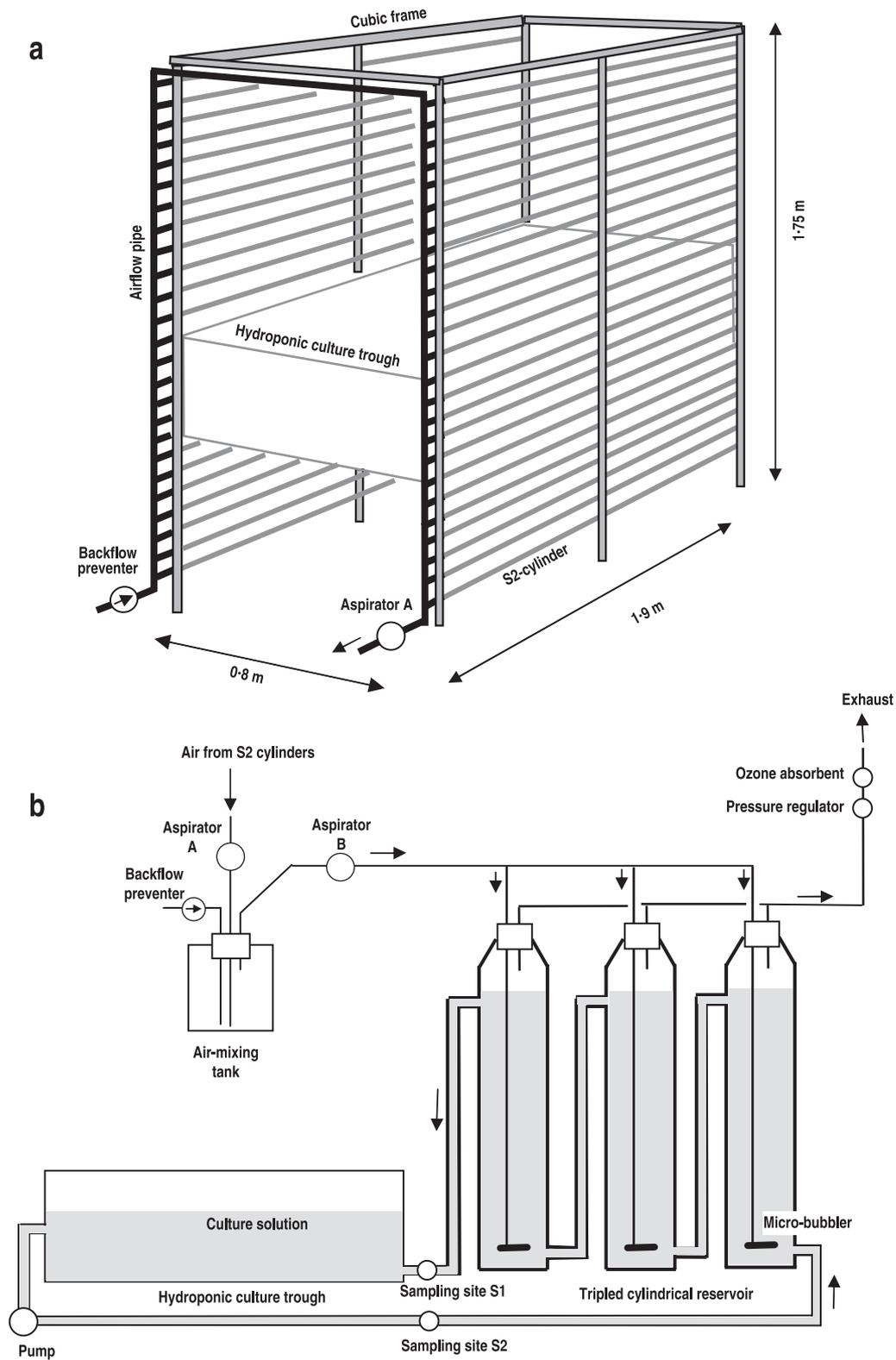
### Application of S2 cylinders to greenhouse hydroponic culture troughs

To evaluate the present system in hydroponic tomatoes, a culture trough in a nursery greenhouse was covered with a cubic frame (1.75 × 0.8 × 1.9 m<sup>3</sup>) in which 25 S2 cylinders were attached 60 mm apart to one side and 25 cylinders at the same spacing to the opposite side, giving a total of 50 cylinders (Fig. 2a). Other lateral faces and a roof of the frame were shielded with a transparent acrylic plate. Intervals between the cylinders were fixed at 60 mm as described by Matsuda *et al.* (2006). Conductor wires of all cylinders were linked in parallel with a connector wire of an electrostatic generator and positively charged with 30 kV. S2 cylinders were attached to an airflow pipe that was linked to aspirator A. An outlet of aspirator A was connected to a 5-L tank in which outside air entering via an inlet tube was mixed with air containing ozone (Fig. 2b). A backflow preventer was attached to the entrance of the inlet tube. An additional aspirator (aspirator B) was set at the end of the outlet tube of the air-mixing tank to transfer the air in the box to a reservoir (95-L tripled cylindrical tank). By changing the airflow rate of aspirator B, the volume of the outside air integrated into the air-mixing tank was regulated.

The reservoir cylinders were linked in parallel to an outlet tube of the air-mixing tank. The air in the air-mixing tank was simultaneously released into a nutrient solution in three reservoir cylinders through a micro-bubbler MB-150 (Nomura Electronics), which produced fine bubbles (20–25  $\mu\text{m}$  in diameter), set at the end of the airflow line (Fig. 2b). An exhaust from the reservoir was passed through an airflow regulation valve [opened when the inside pressure reached 0.5 kg cm<sup>-2</sup> (approx. 0.05 MPa)] and then through an activated charcoal adsorbent to trap ozone leaked from the solution. A portion (100 mL) of the ozonized culture solution was collected at two sites (sampling sites S1 and S2) in the circulation line in the hydroponic system to monitor the change of ozone



**Figure 1** Schematic representation of a new spore-precipitation cylinder (S2 cylinder) for ozone production. (a) The S1 cylinder originally devised for conidial attraction (Matsuda *et al.*, 2006). (b) The S2 cylinder carrying a discharge site (site A) at the end of the cylinder and a spore-attraction site (site B) in the remaining portion. (c) Discharge between the anode (positively charged copper conductor wire) and the cathode (earthed copper ring). (d) S2 cylinders linked in parallel to a connector wire of an electrostatic voltage generator at one end and to an airflow pipe at the opposite end. (e) Possible mode of conidial attraction. A positively charged central copper wire produces a non-uniform electric field (I) inside the cylinder to dielectrically polarize the cylinder. The polarized cylinder produces a non-uniform electrostatic field (II) outside. Field II causes the dipole in conidia that come into this field. Opposite charges between the cylinder surface and the conidia create an electrostatic force to attract conidia to the cylinder.



**Figure 2** Ozone-generative spore-precipitation cylinders (S2 cylinders) attached to a hydroponic culture trough. (a) Culture trough covered with a cubic frame installed with a total of 50 cylinders (60-mm intervals) attached to two lateral faces. (b) Conveyance of the air in the airflow pipe to an air-mixing tank with aspirator A and then to a tripled cylindrical reservoir with aspirator B. Arrows show the direction of airflow or water circulation.

content. The sample was bubbled with fresh air for 30 min to distil ozone from the solution. Ozone released was measured with the ozone monitor detailed earlier.

### Control of rhizosphere pathogens by ozone produced by S2 cylinders

Multi-pack trays with germinated seeds (six trays with a total of 768 seeds) were positioned in the ozonized nutrient solution, and 1 week later, when the seedlings were at the cotyledonal stage, propagule suspensions of *R. solanacearum* and *F. oxysporum* f.sp. *radicis-lycopersici* were introduced into the solution at a final density of  $10^6$  CFU mL<sup>-1</sup>. This inoculation treatment was repeated daily for 1 week. Samples (100 µL) of the inoculated nutrient solution were taken at 1, 3, 5 and 7 days after the last inoculation and spread onto PCG agar containing 2,3,5-triphenyltetrazolium chloride (TTC) for *R. solanacearum* (Toyoda *et al.*, 1988) or Komada's agar (a selective medium for *F. oxysporum*) (Komada, 1975) for *F. oxysporum* f.sp. *radicis-lycopersici*, all in Petri dishes. After 3 days of incubation at 26°C for *R. solanacearum* and 20°C for *F. oxysporum* f.sp. *radicis-lycopersici*, colonies were counted and the density of pathogen propagules in the nutrient solution was estimated. Two hundred Petri dishes were used in each sampling. Data were given as means and standard errors of three replications. Incidence of wilting in test plants was recorded 7 and 14 days after the last inoculation. At the end of the experiment (14 days after the first inoculation), wilted plants were confirmed to be infected with the pathogens; the stems of wilted seedlings in bacteria-inoculated troughs were excised and dipped in sterilized water to confirm a stream of bacterial exudates; surface-sterilized stem segments of fusarium-inoculated plants were placed onto Komada's agar to confirm the presence of *F. oxysporum* f.sp. *radicis-lycopersici*. Mean incidence of plants with disease symptoms was recorded, and data given as means and standard deviations of five separate experiments.

### Trapping of airborne conidia with ozone-generative S2 cylinders

The tomato seedlings in troughs were inoculated with conidia of *O. neolycopersici* to confirm the feasibility of the spore precipitator attracting conidia in the air during ozone production. In this experiment, four troughs (A–D) were used as an experimental unit; trough A was covered with a total of 50 positively charged S2 cylinders (25 on one side and the other 25 on the opposite side of the trough), trough B with 50 positively charged S1 cylinders, trough C with 50 negative-charged S1 cylinders and trough D with non-charged S2-cylinders. All cylinders in troughs A–C were charged with the same potential (30 kV). At the stage when test seedlings had developed cotyledons (1 week after culturing), five *O. neolycopersici*-inoculated tomato plants (2-month-old seedlings), producing abundant conidia on their leaves, were placed 2 m in front of the test troughs, and until the end of experiment the conidia were disseminated from the leaves by an electric fan ( $1.0$  m s<sup>-1</sup>

wind velocity) towards the troughs, according to a previously described method (Matsuda *et al.*, 2006). Incidence of leaves with mildew symptoms was recorded in test seedlings every 7 days. Disease incidence was expressed as the percentage of mildewed leaves in the test plants. At the end of the experiment (3 weeks), the number of conidia attracted to the cylinders was counted using a high-fidelity digital microscope (Matsuda *et al.*, 2005b). Data were given as means and standard errors of five separate experiments.

### Results

Four types of discharge (a, arc; b, streamer; c, corona-streamer; d, corona discharge) were produced by S2 cylinders when the wire tip was variously positioned in relation to the ring (linked to an earthed line) (Fig. 3).

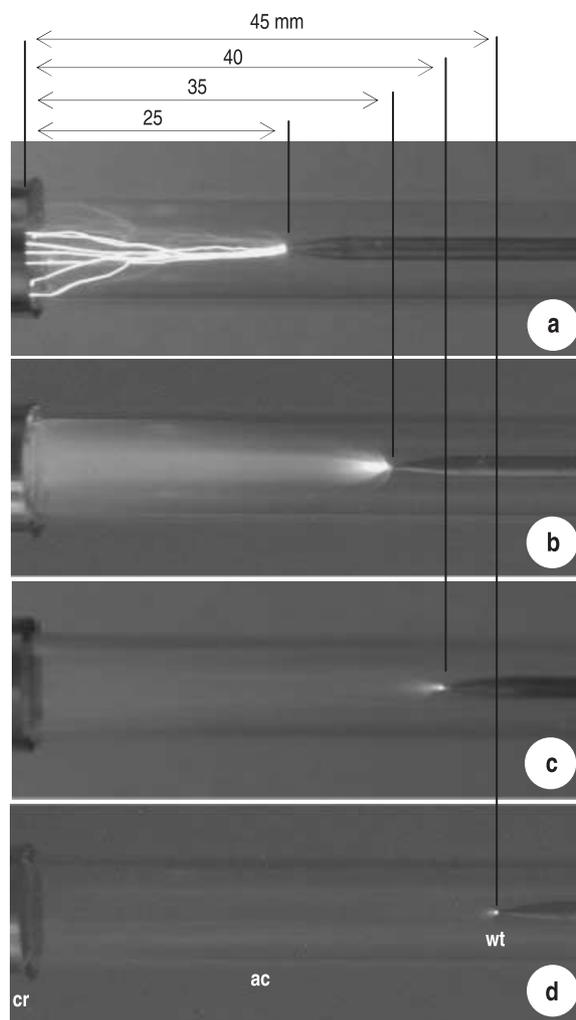


Figure 3 Different types of discharge produced at the discharge site of an S2 cylinder. The S2 cylinder (ac) was positively charged with 30 kV, and a conductor wire tip (wt) was placed at different positions away from an earthed conductor ring (cr). a–d represent arc, streamer, corona-streamer and corona discharge, respectively.

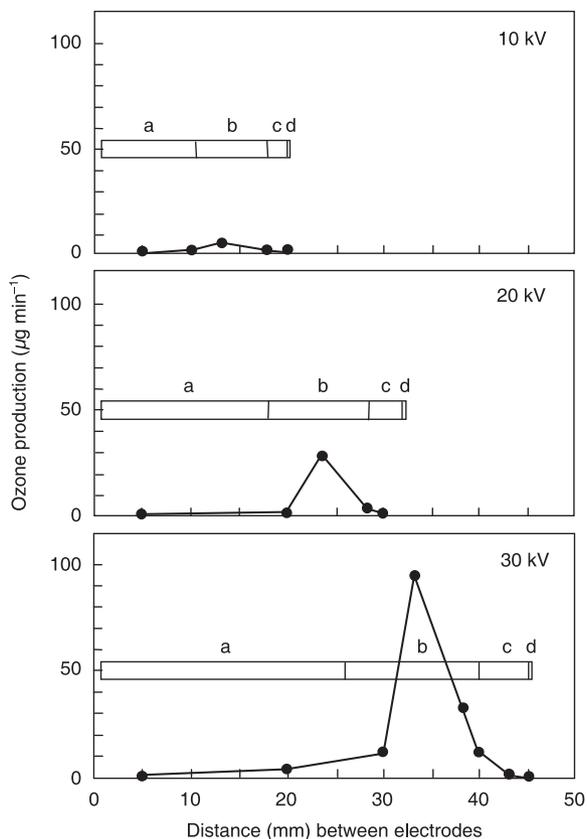


Figure 4 Relationship between ozone production and the distance between a wire tip (anode) and a ring (cathode) of an S2 cylinder under different voltage conditions. An S2 cylinder was positively charged with 10, 20 or 30 kV, and the distance between electrodes changed for each voltage applied. a–d represent the regions causing arc, streamer, corona-streamer, and corona discharge, respectively. Data are given as means of five replications.

Ranges of the four types of discharge under different voltage conditions are given in Fig. 4. The ranges became wider and more distant from the ring in direct proportion to an increase in the voltage applied to the conductor wire. Ozone production was highest in the streamer-discharge region at all voltages tested. The highest ozone production ( $94.6 \pm 3.1 \mu\text{g min}^{-1}$ ) was attained when the conductor wire was positively charged with 30 kV and its tip end was positioned 33 mm from the ring.

Ozone production by S2 cylinders was similar at temperatures in the range 5–45°C (Fig. 5a). Ozone concentration increased proportionally to the increasing number of S2 cylinders used (Fig. 5b). Fifty S2 cylinders provided ozone at approximately  $4.8 \text{ mg min}^{-1}$ .

The positive charge at site B of S2 cylinders remained unchanged ( $28.5 \pm 0.1 \text{ kV}$ ) during the electrification, and ozone was produced at all tested temperatures and airflow rates. The surface potential of the S2 cylinders was similar to that of the S1 cylinders ( $28.7 \pm 0.1 \text{ kV}$ ).

It was possible to change the concentration of ozone sent to the nutrient solution by altering the airflow rate of

aspirator B. Different levels of ozone at sampling site S1 were actually obtained with a constant airflow rate ( $1.0 \text{ L min}^{-1}$ ) of aspirator A and with changed airflow rates ( $1.0$ – $2.0 \text{ L min}^{-1}$ ) of aspirator B (Fig. 6). Ozone levels at sampling sites S1 and S2 were very similar in all airflow combinations of aspirators A and B. During the experimental period, the levels of ozone at both sites were constant in each combination.

Using the ozonized nutrient solution, tomato seedlings were protected from rhizosphere pathogens inoculated into the nutrient solution (Table 1). In the case of ozone supply by aspirator B at airflow rates of  $1.0$  and  $1.5 \text{ L min}^{-1}$ , all hydroponic tomato seedlings remained free of symptoms, showing normal growth throughout the experiment. At a higher airflow rate of aspirator B ( $2.0 \text{ L min}^{-1}$ ), however, the suppressive effect was insufficient, with wilting in approximately 55% (*R. solanacearum*) and 30% (*F. oxysporum* f.sp. *radicis-lycopersici*) of the test plants. The wilt diseases also developed in plants grown in non-ozonized nutrient solution. Both pathogens were recovered from non-ozonized inoculated nutrient solution, at levels of  $10^5$ – $10^6 \text{ CFU mL}^{-1}$  (*R. solanacearum*) and  $10^4$ – $10^5 \text{ CFU mL}^{-1}$  (*F. oxysporum* f.sp. *radicis-lycopersici*). Finally, 45–80% and 35–55% of test plants wilted after infection with the bacterial and fungal pathogens, respectively.

The spore precipitator with 50 S2 cylinders was examined for its ability to protect hydroponic tomato seedlings from airborne conidia of tomato powdery mildew (Table 2). In trough A, installed with positively charged S2 cylinders, tomato seedlings remained uninfected by the conidia throughout the entire period of the experiment (3 weeks). Also, on tomato seedlings in troughs B (with positively charged S1 cylinders) and C (with negative-charged S1-cylinders), no powdery mildew colonies appeared on leaves of the test plants during the experiment. In trough D (with non-charged S2 cylinders), powdery mildew colonies appeared on 80% of test plant leaves within 7 days and had extended to almost all leaves by the end of the experiment.

## Discussion

The single-truss system is a method of tomato cropping (Giacomelli *et al.*, 1994) in which plants have their growing point removed after the production of 2–3 leaves above the first fruit cluster, and all lateral shoots are removed as the plants are grown at a high density of 12–15 plants  $\text{m}^{-2}$  to achieve maximum yields. The most interesting advantage of single-truss crop production is the short time from planting to harvest. The production of at least four or five crops per year per greenhouse can be achieved. In Japan, the single-truss system in tomato cultivation has been a popular trend during the last decade (Okano *et al.*, 1999; Sato *et al.*, 2004) because of its potential to produce high quality fruit without yield losses, and because of the lower use of chemicals in shorter time periods (approximately 45–60 days). At the same time, this trend calls for a stable supply of disease-free

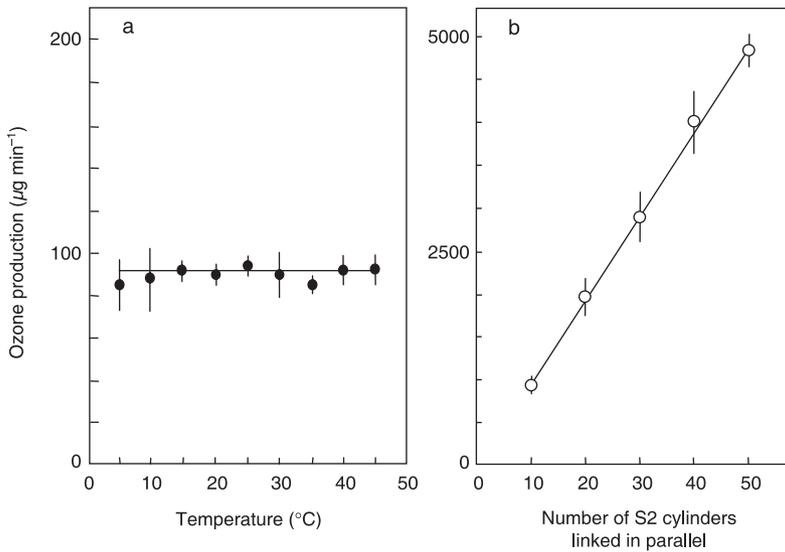


Figure 5 Ozone production by an S2 cylinder. Single (a) or multiple S2 cylinders (b) were attached to an airflow pipe and positively charged with 30 kV. (a) A single cylinder placed at different temperatures and the air collected at 1.0 L min<sup>-1</sup>. (b) Between 10 and 50 S2 cylinders attached to an airflow pipe and the air collected at 25°C at a flow rate of 1.0 L min<sup>-1</sup>. Data are given as means and standard errors of five replications.

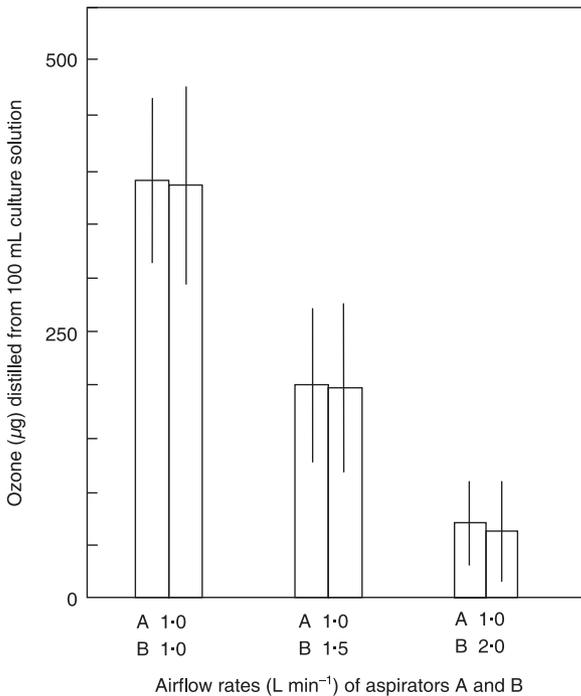


Figure 6 Ozonation of a hydroponic nutrient solution. The air from 50 S2 cylinders was conveyed to an air-mixing tank at a constant rate (1.0 L min<sup>-1</sup>) of aspirator A, and then the air in the tank was transferred to a reservoir at different flow rates (1.0 to 2.0 L min<sup>-1</sup>) of aspirator B. The ozonized nutrient solution was collected at sampling sites S1 and S2 (see Fig. 2) to examine the change of ozone concentrations. Left and right columns in each combination represent sampling sites S1 and S2, respectively. All experiments were conducted in a nursery greenhouse. Data are given as means and standard errors of five replications.

tomato seedlings grown in pathogen-free nursery areas. The standard Japanese greenhouse (20 × 7.2 m<sup>2</sup>) has three rows of growing tables (1 × 16 m<sup>2</sup>) carrying hydroponic troughs in which a total of approximately 700–750

seedlings can be grown. The scale of nursery seedling production used in the present study (approximately 800 seedlings) is sufficient for such a supply. One of the most serious problems is infection of nursery-stage seedlings with pathogens, which causes not only the immediate loss of seedlings, but also spreads diseases among fruiting plants. Effective measures to control pathogens at the nursery stage are thus essential to sustain the single-truss system for tomato cropping.

The major aim of the present work was to protect nursery-stage tomato seedlings from aerial and rhizosphere pathogens. For this purpose, an existing electrostatic spore-precipitation cylinder (S1 cylinder) (Matsuda *et al.*, 2006) was remodelled into a cylindrical ozone generator (S2 cylinder), while retaining the capability to attract airborne conidia. The usual commercial apparatus for generating ozone are variations of a high-voltage electric discharge method (Katzenelson *et al.*, 1974; Burleson *et al.*, 1975; Vestergård, 1988, 1994; Yamamoto *et al.*, 1990; Khadre *et al.*, 2001; van Os, 2001; Kim *et al.*, 2002; Minuto & Garibaldi, 2005; Takayama *et al.*, 2006). The device used in this study was based on the same physical principle: a high-voltage discharge site was prepared at the end of the spore-precipitation cylinder. The new cylinder consisted of two sites: one for ozone production (site A) and the other for spore attraction (site B). The length of the S2 cylinder site B was equal to the longer side of a cubic frame attached to a hydroponic culture trough. This site was responsible for preventing airborne conidia from passing through the spaces between the cylinders. This was essential to retain a surface charge large enough to create an attractive electrostatic force. Data indicated that the surface charge of site B remained unchanged during ozone production, suggesting that the non-uniform electric field was efficient. In fact, the system effectively prevented powdery mildew conidia from passing through the spaces between the cylinders, even after the S2 cylinders were integrated into the system. Thus, this study presents a new

**Table 1** Protection of hydroponic tomato seedlings from rhizosphere pathogens *Ralstonia solanacearum* and *Fusarium oxysporum* f.sp. *radicis-lycopersici* with ozone generative electrostatic spore precipitation cylinders

Pathogens <sup>a</sup> inoculated into hydroponic culture solution	Airflow rates (L min <sup>-1</sup> ) of aspirators A and B <sup>b</sup>		Recovered pathogens (CFU mL <sup>-1</sup> ) <sup>c</sup>				Wilted seedlings (%)	
	A	B	1	3	5	7 <sup>d</sup>	7	14 <sup>d</sup>
<i>Ralstonia solanacearum</i> K-101	0	1	8.2 ± 1.1 × 10 <sup>6</sup>	7.1 ± 1.5 × 10 <sup>5</sup>	6.8 ± 1.1 × 10 <sup>5</sup>	5.7 ± 1.2 × 10 <sup>5</sup>	20.8 ± 8.1	62.7 ± 18.0
	1	2	4.5 ± 1.6 × 10 <sup>4</sup>	7.7 ± 1.7 × 10 <sup>3</sup>	4.3 ± 0.6 × 10 <sup>3</sup>	1.2 ± 0.9 × 10 <sup>3</sup>	11.5 ± 5.7	54.3 ± 12.2
	1	1.5	0	0	0	0	0	0
	1	1	0	0	0	0	0	0
<i>Fusarium oxysporum</i> f.sp. <i>radicis-lycopersici</i> Kin2003	0	1	7.3 ± 1.3 × 10 <sup>5</sup>	5.2 ± 1.6 × 10 <sup>5</sup>	4.3 ± 1.8 × 10 <sup>5</sup>	9.3 ± 1.0 × 10 <sup>4</sup>	9.3 ± 0.7	45.3 ± 10.5
	1	2	1.5 ± 0.8 × 10 <sup>4</sup>	8.6 ± 1.3 × 10 <sup>3</sup>	1.3 ± 0.4 × 10 <sup>3</sup>	9.5 ± 0.8 × 10 <sup>2</sup>	2.6 ± 0.4	27.2 ± 8.3
	1	1.5	0	0	0	0	0	0
	1	1	0	0	0	0	0	0

<sup>a</sup>Bacterial wilt pathogen (*R. solanacearum* K-101) and fusarium crown and root rot pathogen (*F. oxysporum* f.sp. *radicis-lycopersici* Kin2003) were inoculated into hydroponic culture solution ozonated at various concentrations; 100 mL inoculum suspension (10<sup>9</sup> CFU mL<sup>-1</sup>) was poured into the circulated hydroponic culture solution (100 L) at the seedling cotyledonal stage. Inoculation was conducted daily for 1 week.

<sup>b</sup>The hydroponic culture solution was aerated with the ozone-containing air from an airflow pipe with 50 S2 cylinders at different airflow rates of aspirators A and B.

<sup>c</sup>A portion of the inoculated hydroponic culture solution was sampled at various periods after the last inoculation and spread onto TTC-containing PCG agar for *R. solanacearum* or Komada's agar for *F. oxysporum* f.sp. *radicis-lycopersici*.

<sup>d</sup>Days after the last inoculation.

Data are given as means and standard errors of five separate experiments.

**Table 2** Protection of hydroponic tomato seedlings from *Oidium neolycopersici* conidia with positively charged electrostatic spore-precipitation cylinders

Hydroponic culture troughs <sup>a</sup>	Charge on cylinder surface <sup>b</sup>	Ozone production	Powdery mildew incidence (%) on seedlings in cylinder-attached hydroponic troughs <sup>c</sup>			Number of conidia on cylinders <sup>e</sup>
			7	14	21 <sup>d</sup>	
A (S2)	positive	yes	0	0	0	18450.9 ± 5233.6
B (S1)	positive	no	0	0	0	27230.5 ± 8333.9
C (S1)	negative	no	0	0	0	19230.3 ± 4521.7
D (S2)	non-charged	no	80.3 ± 4.1	89.1 ± 3.5	92.3 ± 2.8	443.3 ± 285.1

<sup>a</sup>The S1 cylinder was originally developed to trap airborne conidia of tomato powdery mildew (Matsuda *et al.*, 2006) and was modified here to construct a new electrostatic spore-precipitation cylinder (S2 cylinder) producing ozone. Both cylinders carry a copper conductor wire insulated with a transparent acrylic cylinder. The wire was charged to dielectrically polarize the cylinder. A total of 50 cylinders were attached to a cubic frame covering a hydroponic culture trough. Conductor wires of all cylinders in troughs A and B were positively charged with 30 kV, and those in trough C were negatively charged with the same potential. S2 cylinders in trough D were not charged. Negatively charged S1 cylinders were used to compare conidial attraction, and non-charged cylinders were used as a negative control.

<sup>b</sup>Surface charges of S1 or S2 cylinders dielectrically polarized by the charged conductor wire.

<sup>c</sup>Inoculum tomato plants, producing abundant conidia, were placed 2 m in front of the test troughs. The conidia were disseminated towards test plants in troughs by an electric fan. This spore dissemination was initiated at the cotyledonal stage of the seedlings and continued until the end of the experiment. Disease incidence was expressed as the incidence of leaves with mildew symptoms in test seedlings.

<sup>d</sup>Days after the inoculation of conidia was initiated.

<sup>e</sup>At the end of the experiment (21 days after the first inoculation), conidia on cylinders were directly observed with a high-fidelity digital microscope (Matsuda *et al.*, 2006).

Data are given as means and standard errors of five separate experiments.

and simple ozonizer that possesses the capability to capture airborne conidia facilitating the simultaneous control of rhizosphere pathogens in hydroponic systems for tomato cultivation.

Rhizosphere pathogens in hydroponic tomatoes are basically similar to those in soil culture (Jenkins & Averre, 1983; Schnitzler, 2004). In order to kill the pathogens in the nutrient solution, a certain concentration of ozone is

introduced into the solution (Yamamoto *et al.*, 1990; Runia, 1994, 1995; van Os, 2001). However, the solubilization of ozone in the solution is greatly affected by environmental factors such as temperature, the volumes and shapes of hydroponic culture troughs, and by salt concentrations in the solution (Vanachter *et al.*, 1988). Moreover, it is difficult to maintain a stable aqueous concentration of ozone, because of its easy transformation to oxygen (Khadre

*et al.*, 2001). Previous research (Farooq *et al.*, 1977; Haas & Gould, 1979; Vanachter *et al.*, 1988; Khadre *et al.*, 2001; Kim *et al.*, 2002) showed that a small bubble size is essential to obtain improved microorganism–bubble contact and to increase the gas–liquid interfacial area. In the present study a micro-bubbler was used to create fine bubbles (approximately 20  $\mu\text{m}$  in diameter) of ozonic air from the S2 cylinders. In the circulation system, small bubbles containing ozone subsisted in the nutrient solution during circulation. Indeed, the amounts of ozone recovered at the site of the sponge supports in the multi-pack tray were negligibly low (0.01–0.03  $\mu\text{g h}^{-1}$ ) (data not shown). This ozonized nutrient solution was useful to prevent the nutrient solution from being contaminated with pathogens. The ozone productivity (approximately 290  $\text{mg h}^{-1}$ ) of 50 S2 cylinders was considerably lower than that of existing commercial ozone generators (10–40  $\text{g h}^{-1}$ ) (Vanachter *et al.*, 1988; Yamamoto *et al.*, 1990; Wakoh *et al.*, 1992; Runia, 1994; Kim *et al.*, 2002), but was sufficient to kill the bacterial and fungal pathogens entering the nutrient solution.

Discharge occurs in a non-uniform electric field between oppositely charged conductor electrodes (Griffith, 2004; Halliday *et al.*, 2005), and during this stage the air in this field is ozonized (Chen & Davidson, 2002). The structure of S2 cylinder site A was simple, involving an earthed copper ring newly attached to an edge of the cylinder and a tip of a conductor wire passing through a stopper (see Fig. 1b,c). In this device, the earthed ring and conductor wire tip worked as cathodic and anodic electrodes, respectively, and generated an electric field in the space between the two electrodes. The device generated distinct types of discharge (corona, corona-streamer, streamer and arc discharge) (Goelian *et al.*, 1997), depending on the distances and potentials between the electrodes. The data show that the highest levels of ozone were generated by streamer discharge.

The size (0.6  $\times$  1.8  $\text{m}^2$ ) of the hydroponic culture trough used in the present study was a standard unit for raising approximately 700 nursery-stage seedlings in hydroponics. Fifty S2 cylinders were effective to protect seedlings in this unit from both aerial and rhizosphere pathogens. The use of transparent cylinders and the attachment of the cylinders to the two lateral faces of the guard box were useful to minimize the loss of light for photosynthesis by the seedlings. In fact, the seedlings within an enclosed frame covered by S2 cylinders grew normally, without an increase in production time compared with the seedlings grown in non-covered hydroponic apparatus (data not shown).

The diurnal change of temperature in the greenhouses in this study was between 5 and 18°C in winter and between 16 and 39°C in summer. For successful commercial application to such greenhouses, S2 cylinders must stably express their capability under changeable temperature conditions. The present study revealed that S2 cylinders can produce ozone constantly between 5 and 45°C, suggesting that the cylinders are effective under the usual temperature changes found in these greenhouses.

Seedling roots can tolerate high concentrations, whereas shoots can only tolerate very low concentrations of ozone in the air (Vestergård, 1994; Moeder *et al.*, 2002; Grantz *et al.*, 2003). Additionally, high aerial concentrations of ozone are toxic to humans (Brady & Holum, 1988). In the present study, the ozonic air from the cylinders escaped largely at the bubbling stage, and therefore trapping this escaped ozone would be an essential step for the safe use of the system. An activated charcoal adsorbent (Vestergård, 1994) would be useful for this purpose.

Another issue to be considered is the occurrence of nutrient deficiency diseases in tomato plants that are cultured in ozonized nutrient solutions, since ozone preferentially oxidizes some nutritional minerals to make them insoluble (Vanachter *et al.*, 1988; Wakoh *et al.*, 1992). In a preliminary study, minerals such as ammonium, manganese and ferrous ions were shown to frequently be at suboptimal levels when the nutrient solution was ozonized (data not shown). The periodic addition of these nutrients was necessary to accomplish normal tomato growth under the present hydroponic culture conditions.

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