

## Physical control of powdery mildew (*Oidium neolycopersici*) on tomato leaves by exposure to corona discharge

T. Nonomura, Y. Matsuda, K. Kakutani, Y. Takikawa, and H. Toyoda

**Abstract:** We devised a cylindrical electrostatic discharge generator to physically eradicate tomato powdery mildew colonizing tomato leaves. The generator consists of a copper needle with a pointed tip, an insulating acrylic cylinder, and an electrostatic voltage generator. The needle is insulated with a vinyl sleeve, except for the pointed tip, and is coaxially fixed in the cylinder and connected to the voltage generator. The needle is negatively charged, and the treated plant is earthed. In initial tests, a corona, characterized by a blue glow, formed at the needle tip as the probe was brought closer to the leaf surface. The distance at which this occurred increased from 16 to 50 mm as the voltage was increased from 5 to 30 kV. If the probe was brought too close to the leaf surface, an arc discharge occurred that caused injury to the leaf. Powdery mildew colonies were destroyed by 2-second exposures at probe distances intermediate to where corona discharge was initiated and where arcing occurred. A probe distance of 25 mm and 30 kV for a 2-second burst was selected to further test the efficacy of the probe for controlling powdery mildew in a greenhouse environment. Tomato plants were grown hydroponically in two open-window greenhouses under a first-truss cropping system. Colonies appeared on tomato leaves 10 to 14 days after transplanting. During the following 2 weeks, these colonies produced abundant progeny conidia that secondarily infected neighboring plants. Corona discharge treatment in one greenhouse, at the stage when colonies first became visible, completely suppressed the spread of the disease compared with a non-treated greenhouse in which disease spread rapidly. The present discharge generator is portable and easy to operate on-site as a part of routine care of hydroponically cultured tomatoes in greenhouses and provides a non-chemical method to control powdery mildew disease.

*Key words:* disease control, tomato powdery mildew, electrostatic field, single-truss cropping.

**Résumé :** Nous avons conçu un générateur cylindrique de décharges électrostatiques pour éradiquer physiquement l'oïdium colonisant les feuilles de la tomate. Le générateur est composé d'une aiguille de cuivre pointue à une extrémité, d'un cylindre isolant d'acrylique et d'un générateur de voltage électrostatique. L'aiguille, sauf sa pointe, est isolée à l'aide d'un manchon de vinyle et est fixée coaxialement dans le cylindre et branchée au générateur. L'aiguille est chargée négativement et la plante est reliée à la terre. Lors des tests initiaux, un effluve, caractérisé par une lueur bleue, s'est formé à la pointe de l'aiguille lorsqu'on approcha la sonde de la surface d'une feuille. La distance à laquelle ceci s'est produit a augmenté de 16 à 50 mm quand le voltage a été augmenté de 5 à 30 kV. Si l'on approchait la sonde trop près de la surface de la feuille, il se produisait une décharge en arc qui blessait la feuille. Deux secondes d'exposition suffisaient pour détruire les colonies d'oïdium lorsque la distance à la feuille se situait environ à mi-chemin entre celle permettant la formation de l'effluve et celle à laquelle se produisait la décharge en arc. Une distance de la sonde à la surface de la feuille de 25 mm et une tension de 30 kV appliquée pendant 2 secondes ont été choisies pour vérifier, en serre, encore davantage l'efficacité de la sonde et du traitement. Les tomates étaient produites en culture hydroponique dans deux serres ouvertes, selon la méthode de la grappe unique. Les colonies sont apparues sur les feuilles des tomates de 10 à 14 jours après la transplantation. Dans le cours des deux semaines suivantes, les colonies produisirent d'abondantes conidies qui infectèrent les plantes avoisinantes (infection secondaire). Dans une des serres, le traitement à l'effluve, au stade où les colonies ont commencé à paraître, inhiba complètement

Accepted 27 October 2008.

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la propagation de la maladie, comparé à l'autre serre où la maladie se propagea rapidement. Le générateur de décharges électrostatiques est portable et facile à utiliser. Son utilisation trouve facilement sa place parmi les soins de routine donnés aux tomates cultivées en serres hydroponiques et pourvoit un moyen de lutte non chimique contre l'oïdium.

*Mots-clés* : lutte contre les maladies, oïdium, champ électrostatique, méthode de la grappe unique.

## Introduction

The single-truss system is a method of fruit cropping used in hydroponic cultivation of tomatoes (Giacomelli et al. 1994). In this system, the growing point is removed from the plants after the production of 2 to 3 leaves above the first fruit cluster, and all lateral shoots are removed as the plant grows. Plants are grown at a high density (12 to 15 plants/m<sup>2</sup>) to achieve maximum yields. One of the main advantages of single-truss crop production is the short time from planting to harvest. This enables the production of at least four to five crops per year per greenhouse. In Japan, the single-truss system of tomato cultivation is a relatively new trend and has increased in popularity during the last decade (Okano et al. 1999; Sato et al. 2004). In this system, growers can produce high quality fruit with low yield losses, plant growth is easily regulated, and fungicide use is lower during the short periods (45–50 days) of plant propagation. Two cultivation steps are required in the single-truss system. Seedlings are first raised at a nursery and then propagated for cropping. In our system, an ozone generative electrostatic spore precipitator is used to protect the seedlings at the nursery stage from air-borne conidia of tomato powdery mildew [*Oidium neolycopersici* L. Kiss], rhizosphere propagules of fusarium crown and root rot [*Fusarium oxysporum* Schlechtendahl: Fries f. sp. *radicis-lycopersici* Jarvis & Shoemaker], and bacterial wilt [*Ralstonia solanacearum* Smith] (Shimizu et al. 2007).

Once seedlings are transferred to hydroponic troughs in the non-guarded propagation greenhouse, they are frequently infected with tomato powdery mildew (Kashimoto et al. 2003a; Matsuda et al. 2001). We conducted a 3-year survey on the occurrence of powdery mildew on seedlings during plant propagation throughout different seasons of the year. Our survey revealed that powdery mildew first appeared on a few leaves, and then progeny conidia rapidly developed and spread the disease to neighboring plants. The disease spreads quickly throughout the crop in well-ventilated greenhouses, especially when chemical control is insufficient. At present, chemical methods are effective and are essential to control the powdery mildew pathogen. Nevertheless, new fungicide-tolerant lines of the pathogen can easily arise as a result of frequent fungicide application. In fact, a preliminary survey revealed that azoxystrobin-insensitive isolates of tomato powdery mildew are already present on naturally infected tomato leaves, although some fungicides (benomyl, fenarimol, pyrazophos, thiabendazol, and triforine) were effective against the pathogen (Mori et al. 2004). With fewer fungicides coming on the market because of environmental concerns, we are now focusing on developing physical methods for environmentally safe disease control. In the present study, we describe the efficacy

of the point-by-point eradication of powdery mildew colonies on leaves of propagation-stage tomato seedlings. This was achieved via localized exposure to corona discharge from a portable electrostatic discharge generator.

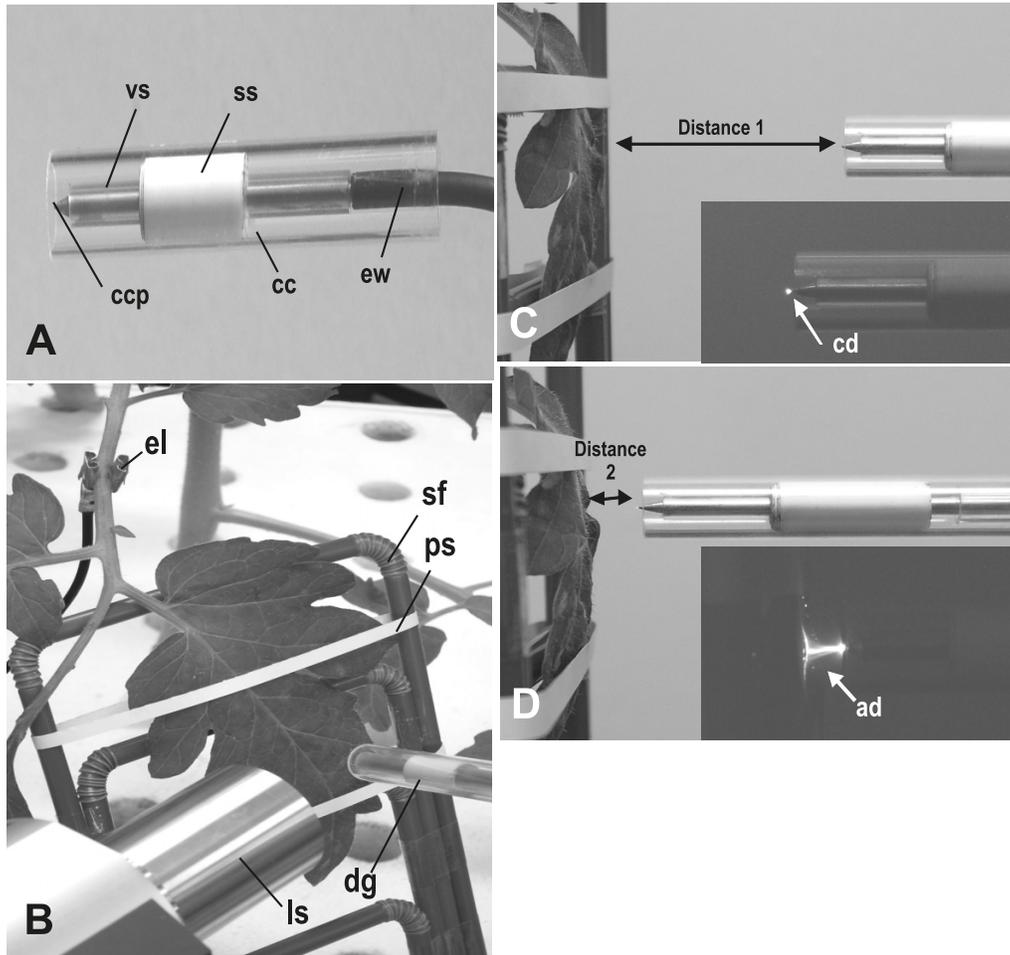
In an electric field between cathodic and anodic conductors, an electrostatic discharge occurs in direct proportion to the voltages applied to the conductors, and in a reverse proportion to distances between the conductors (Griffith 2004; Halliday et al. 2005). The discharge of electrified conductors ionizes the surrounding air (Griffith 2004; Halliday et al. 2005); this ionized atmospheric field (plasma field) involves free electrons and positively ionized gases (Chen and Davidson 2002). Herrmann et al. (1999) reported that the plasma stream exposure destroys surface-colonizing microbes. We attempted to create a corona discharge between a powdery mildew colony and a charged conductor probe. Plants are able to conduct electrical currents, and can therefore be oppositely electrified as an electrified conductor probe nears the plant tissue (Mizuno and Washizu 1995). We constructed a portable electrostatic discharge generator that consists of a conductor needle covered with an insulator cylinder. This device creates a constant corona discharge that can be targeted to individual powdery mildew colonies on infected leaves. Using this system, we successfully controlled powdery mildew in greenhouse tomatoes topped for single-truss cropping.

## Materials and methods

### Plant and hydroponic culture

Germinated seeds of tomato (*Lycopersicon esculentum* Mill., 'Moneymaker') were placed on polyurethane sponge supports (3 cm × 3 cm × 3 cm) soaked in a hydroponic nutrient solution. After 10 days, the seedlings were transferred to a polystyrene plate floated on the same nutrient solution in a hydroponic culture trough in a pathogen-free nursery greenhouse (26 ± 3 °C) (Kashimoto et al. 2003a). Hydroponic culture was carried out as described previously (Nonomura et al. 2001). After 1 month of cultivation, 100 tomato seedlings were transferred to hydroponic channels (1.5 m × 2 m) in a temperature-controlled greenhouse (8 m × 12 m; 26 ± 4 °C). In addition, 1500 seedlings were equally distributed between two open-window propagation greenhouses (one for electrical discharge experiments and the other for the non-exposed control). Each greenhouse had three rows of hydroponic culture channels (8 m × 12 m) on growing tables (80-cm high). The seedlings in the greenhouses were physically supported by hanging them from an overhead wire. Plants were topped above the ninth leaf position (the second leaf position above the first clusters) approximately 1 week after the transfer, and all lateral buds

**Fig. 1.** Cylindrical discharge generator for eradication of *Oidium neolycopersici* colonizing tomato leaves. (A) Discharge generator consisting of a vinyl sleeve (vs)–insulated copper wire conductor probe (ccp) with a pointed tip, coaxially held in the cover cylinder (cc) with an insulating silicon stopper (ss) and linked to an electric wire (ew) of an electrostatic voltage generator. (B) Leaf and branch of hydroponically cultured tomato seedling, held on a supporting frame (sf) with paper strips (ps) and touched with an earthed line (el), and a lens (ls) of a high-fidelity digital microscope and a discharge generator (dg). (C and D) Production of corona and arc discharges between earthed tomato leaf and probe tip of the discharge generator. Corona (C) and arc discharge (D) produced by electrostatic discharger generator in an electric field between earthed leaf and tip end of the probe (30 kV). Insert photographs in C and D represent corona (cd) and arc discharge (ad), respectively, taken against a dark field. Distance 1, 30 mm; distance 2, 3 mm.



that formed were removed throughout the 45-day propagation period.

### Pathogen and inoculation

For the powdery mildew pathogen, we used conidia of *O. neolycopersici* Kiss (isolate KTP-01) (Kashimoto et al. 2003a). To maintain the pathogen, conidia were dusted onto leaves of fresh 2-month-old tomato seedlings ‘Money-maker’ with a paintbrush every 2 weeks. ‘Money-maker’ is highly susceptible to KTP-01 (Matsuda et al. 2005). The powdery mildew colonies that formed on the leaves of these inoculated plants were used to inoculate test plants. Voucher material of the fungus used is preserved in the Herbarium Preservation Section of Kinki University (Nara, Japan).

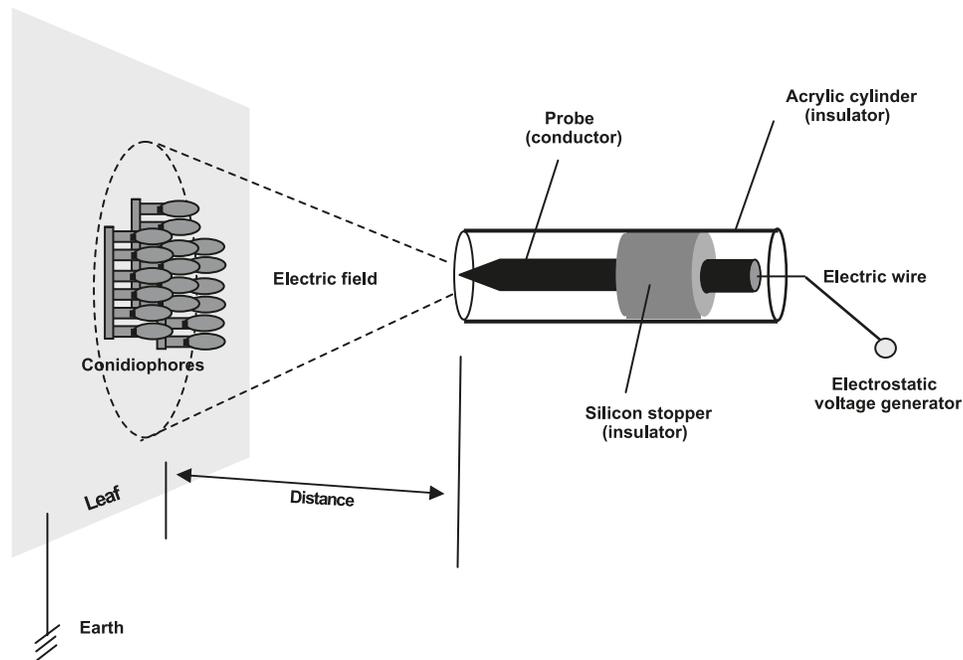
In the inoculation test, newly produced conidia from 10-day-old colonies were dusted onto leaves of 40-day-old seedlings that were hydroponically cultured in the inoculation greenhouse. Under these inoculation conditions, between one and eight powdery mildew colonies formed on

inoculated leaves. Colonies appeared 4 to 5 days after inoculation and began to produce conidia on conidiophores 2 days later. Leaves bearing one or two mildew colonies were used for the corona discharge experiments.

### Electrostatic discharge generator

An electrostatic discharge generator (Fig. 1A) was constructed to expose powdery mildew colonies on leaves to corona discharge. A copper wire with a pointed tip (length, 5 cm; diameter, 2 mm; diameter of the pointed tip, 30  $\mu$ m) was used as a cathodic conductor probe. Except for the pointed tip, the wire was insulated with a vinyl sleeve (1-mm thickness). The wire was linked to an electrostatic voltage generator HVA10K-102 (Logy Electric Co. Ltd., Tokyo, Japan). The conductor probe was coaxially held in an acrylic cylinder (insulator) with an insulating silicon stopper. The probe was negatively charged with a potential of 5 to 30 kV.

**Fig. 2.** Schematic diagram of spatial relationship between charged probe tip and earthed tomato leaf. Distance between tip and leaf was adjusted to determine tip position at which conidiophores were instantly destroyed by corona discharge.



### Discharge production

We used non-inoculated tomato seedlings to determine the distances from the leaf at which the probe tip generated arc or corona discharges. We tested various voltage conditions using this system. An electric field between the leaf and the charged probe tip was formed by touching an earthed electric wire to the branch of the test leaf (Fig. 2). Because the charged probe generates an electrostatic force that attracts the leaf toward the probe tip (Moriura et al. 2006), the leaf was fixed on a supporting frame with paper strips (Fig. 1B). The tip end of the charged probe was slowly moved toward the leaf, and the distances at which arc or corona discharge occurred under different voltage conditions were recorded to determine the relationship between the distance and the voltage applied. Thirty leaves of five plants were used to test each voltage, and data represent means and standard deviations of three replicates. The pale-blue light radiated by ionized air through corona and arc discharges was detected in the dark with a charge-coupled device (CCD) camera as described elsewhere (van Veldhuizen and Rutgers 2002).

### Instant destruction of conidiophores by corona discharge

Inoculated tomato seedlings in the temperature-controlled greenhouse were used in this experiment. A branch of a leaf bearing a single colony was touched with the earthed electric wire, the colony on the leaf was treated with the discharge generator, and a 2-second corona discharge was generated. The conidiophores in the colony were observed using a high-fidelity digital microscope KH-2700 (Hirox, Tokyo, Japan) while gradually moving the charged probe toward the leaf, to determine the distance at which instantaneous breakdown of conidiophores occurred (Fig. 2). The distance was measured under different voltage conditions,

using 50 leaves of 10 plants with a single colony for each voltage. Data are means and standard deviations of five replications. Digital microscopic observation was conducted as described previously (Moriura et al. 2006).

### Assay for mildew colony development

The probe was negatively charged with 30 kV. Single colonies on 20 different leaves of five plants in each experiment were subjected to corona discharge. Both exposed and non-exposed leaves of inoculated plants were detached 7 and 14 days after corona discharge. Leaves were decolorized with lactophenol alcohol and then stained to detect superficial hyphae and conidiophores with aniline blue as described previously (Kashimoto et al. 2003b). The number of conidiophores per colony was counted, and images of the stained hyphal colonies under a light microscope were analyzed to determine the area of the colony (Adobe Photoshop software, v. 6.0, Adobe; and Scion Image software, Scion). Data are means and standard deviations of three replications.

### Histochemical analysis

Leaves of inoculated tomatoes bearing mildew colonies were detached at various times after corona treatment and dipped in 70% ethanol for fixation and decoloration. The samples were frozen and 20  $\mu\text{m}$  sections were prepared at the infection sites. The sections were dipped in 70% ethanol saturated with Sudan IV for 60 min to stain suberin (Krishnamurthy 1999). Excess stain was removed with ethanol, and the specimens were then mounted in dilute glycerin for light microscopy. Non-exposed leaves with powdery mildew colonies were used as a control.

### Corona discharge to control powdery mildew in greenhouse tomatoes

The ability of the corona discharge method to eradicate powdery mildew naturally infecting the leaves of tomato plants was further evaluated in the propagation greenhouses. In greenhouse A, powdery mildew colonies were treated as soon as they became visible on leaves. The petiole of a leaf was connected to an earthed line, and the powdery mildew colony was exposed to corona discharge for 2 second (30 kV negative charge). The distance between a leaf and the probe tip was maintained at 25 mm by a spacing cylinder fixed to the tip end of the discharge generator (Fig. 3A). Treatment was carried out on all colonies on test plants during the entire experimental period (45 days). Powdery mildew colonies on seedlings in greenhouse B were left untreated. The number of powdery mildew colonies on leaves were counted every 5 days from the first day after seedlings were transferred into the greenhouses. Experiments were repeated three times in different seasons over 2 years: mid-June to late July in 2007 and mid-September to late October in 2006 and 2007.

### Ozone estimation

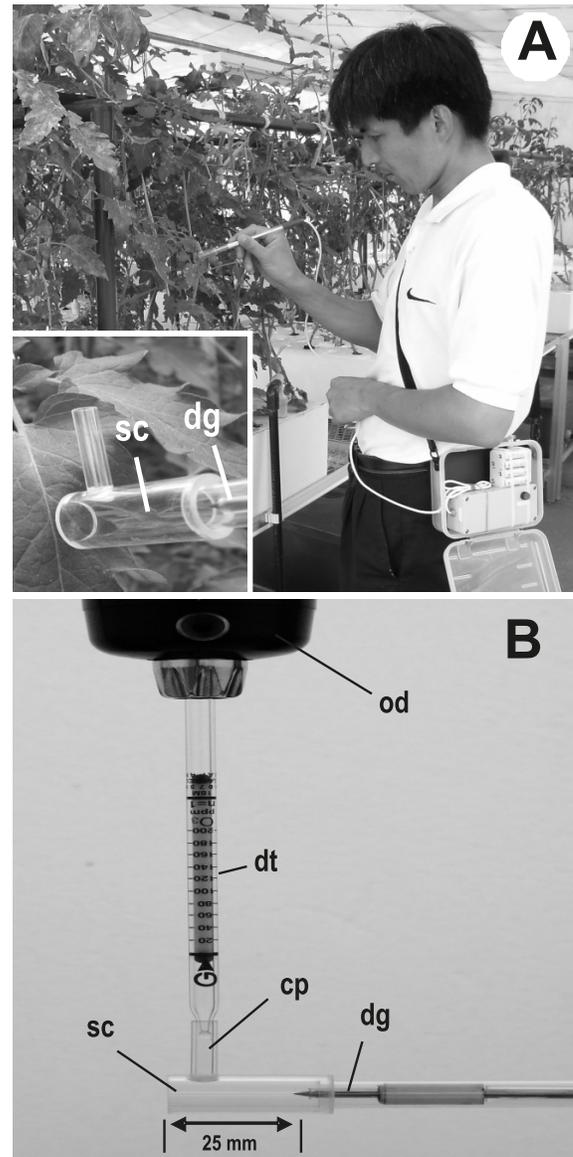
To determine levels of ozone produced from corona discharge, the air of the cylinder was aspirated into an ozone detector tube (lowest detection limit, 0.01 ppm) (Gastec, Kanagawa, Japan) attached to an outlet tube of the cylinder (Fig. 3B). Ozone estimation was conducted using 50 leaves with and without powdery mildew colonies. Data are means and standard deviations of three replicates.

### Results

Using the discharge generator, we produced two types of discharge between the probe tip and a leaf: corona discharge around the probe tip (Fig. 1C) and arc discharge between the tip and leaf (Fig. 1D). Figure 4 shows the relationship between voltage applied to the probe and the probe tip position that caused corona and arc discharges. At a given voltage, corona discharge was initially detected around the probe tip when it was moved closer to a leaf. The corona intensified as the probe tip continued to near the leaf, followed by an arc discharge. At any given voltage, the distance for these discharges was constant, irrespective of leaves used. Also, at any given voltage, instantaneous breakdown of conidiophores in target colonies was observed at distances where corona and arc discharges occurred. Figure 5 shows the same conidiophores on a leaf before and after exposure to corona discharge. Each conidiophore was destroyed immediately (within 2 seconds). Leaf tissues were not affected by exposure to the corona discharge, but when arc discharge occurred, incrustation (cork formation) developed at the exposure sites 3 to 5 days later. Histochemical analysis of sectioned leaf specimens revealed that this cork formation was the result of suberization within cell walls of epidermal and/or mesophyll cells.

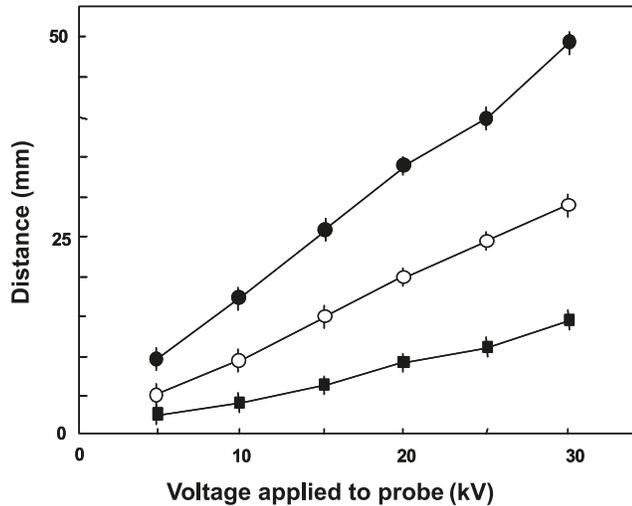
Table 1 summarizes powdery mildew colony development and conidiophore production after corona discharge from the probe at various distances from the leaf. Colony development and conidiophore production were completely suppressed when colonies were 16 to 30 mm from the

**Fig. 3.** Spacing cylinder on discharge generator, and estimation of ozone produced in the spacing cylinder. (A) A portable corona discharge generator with a battery-operated voltage generator. Insert photograph shows leaf of hydroponically cultured tomato seedling, exposed to corona discharge with the discharge generator (dg) with spacing cylinder (sc) at the tip. (B) Spacing cylinder (sc) ensuring 25 mm distance from discharge generator, linked to a detector tube (dt) of ozone gas detector (od) via a connecting pipe (cp).

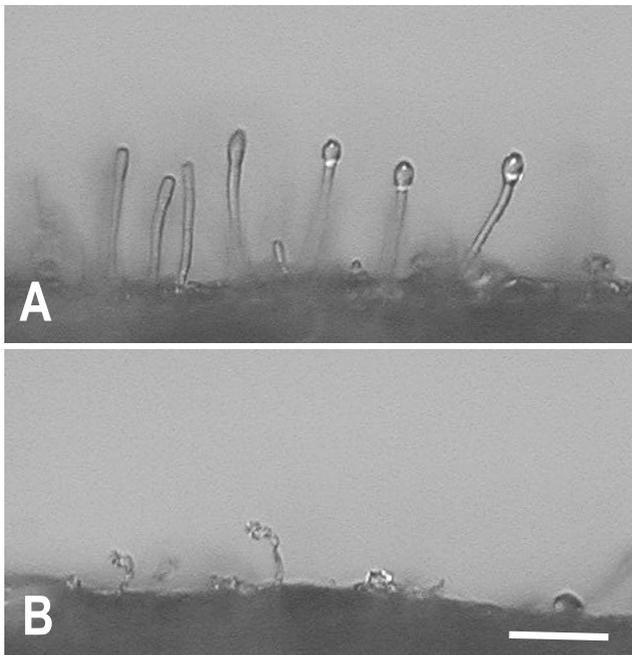


probe tip at discharge, although only partial breakdown of conidiophores was observed at 30 mm. On the other hand, conidiophores that were 31 to 50 mm from the probe tip at discharge were not destroyed instantaneously, and therefore this distance was ineffective at suppressing colony growth and conidiophore development. Colony growth remained active, similar to non-exposed mildew colonies at a probe distance of more than 51 mm from the leaf. In subsequent experiments, therefore, the charged probe tip (30 kV) was

**Fig. 4.** Relationship between voltage applied to discharge probe and probe tip position from leaflet that results in corona (solid circle) or arc discharge (solid square). Open circle indicates tip positions of probe causing instantaneous breakdown of conidiophores of *Oidium neolycopersici*.



**Fig. 5.** Digital micrographs of *Oidium neolycopersici* conidiophores on tomato leaves (A) before and (B) after exposure to a 2-second corona discharge (probe at 30 kV and 25 mm from leaflet surface). Scale bar represents 50  $\mu$ m.



positioned 25 mm from the leaf by attaching a spacing cylinder to the discharge generator (Fig. 3A).

Ozone gas produced through a 2-second discharge of the 30 kV probe at 25 mm was  $0.025 \pm 0.05$  ppm ( $0.05 \text{ mg/m}^3$ ); the amounts were similar among the 150 leaves tested, irrespective of whether leaves bore mildew colonies.

The discharge probe was used to destroy powdery mildew that was naturally infecting leaves of tomato plants in the propagation greenhouse (Fig. 6). In the experiment, a total of 41 250 leaves of 750 plants were surveyed for colony production. Powdery mildew colonies first appeared on 5 leaves of different seedlings 10 days after seedlings were transferred to the propagation greenhouse (greenhouse A). Throughout the experimental period, the number of colonies was counted every 5 days and was between 3 and 5 colonies (Fig. 6A). Approximately 30 colonies (average of three separate experiments, 32.6 colonies) appeared on the leaves of about 30 plants (32.0 plants). All colonies occurred singly on the leaves and were exposed to corona discharge as soon as they became visible. As a result, development of all colonies was effectively suppressed. In contrast, powdery mildew colonies on non-treated leaves in the control greenhouse developed rapidly. Colonies appeared on leaves of plants during the first 20 days at a similar rate to that observed in the treated greenhouse, but then colony numbers increased sharply (Fig. 6B), especially on leaves of plants neighboring those that originally produced colonies. Finally, more than 10 000 colonies (average, 10 068.0 colonies) were formed on 20 000 leaves (24 456.7 leaves) of 400 plants (444.7 plants).

## Discussion

We demonstrated instant destruction of tomato powdery mildew conidiophores on leaves of tomatoes by short-term exposure to electrostatic corona discharge. Corona discharge occurs preferentially around the pointed tip of electrified conductors (Griffith 2004; Halliday et al. 2005) and depends on the distance between the cathodic and anodic conductors (Marode 1975). In our study, we found that the spacing between the negatively charged probe tip (cathode) and the earthed leaf (anode) was a matter of primary importance to effectively create the corona discharge. The spacing cylinder was an essential device to retain this interval, because the charged probe produces an electrostatic attractive force that draws the leaf toward the probe (Moriura et al. 2006). In fact, the leaves came within the arc discharge range if the probe tip was not equipped with the spacing cylinder. Arc discharges damaged leaves, causing suberin deposition in both epidermal and mesophyll cell walls at the site of exposure. Suberin is often produced in plant tissues as a response to injury (Faulkner and Kimmins 1975; Krishnamurthy 1999; McDougall 1993). Thus, the spacing cylinder effectively prevented injury to the treated leaves and ensured that an optimal probe distance was maintained.

In an electrostatic field, the discharge of electrified conductors produces not only a plasma stream but also an ionized atmospheric field (Chen and Davidson 2002). Ozone is a major component of the ionized gases (Chen and Davidson 2002) and is a well-known decontaminant of surface-colonizing microbes (Brady and Holum 1988; Francis 2002). Ozone gas has been shown to kill bottle gourd and cucumber powdery mildews colonizing host leaves (Khan and Khan 1998, 1999). In these experiments, several intermittent exposures with ozone at a high concentration (0.2 ppm) were needed to completely eradicate the pathogens. This level of ozone is considerably higher than

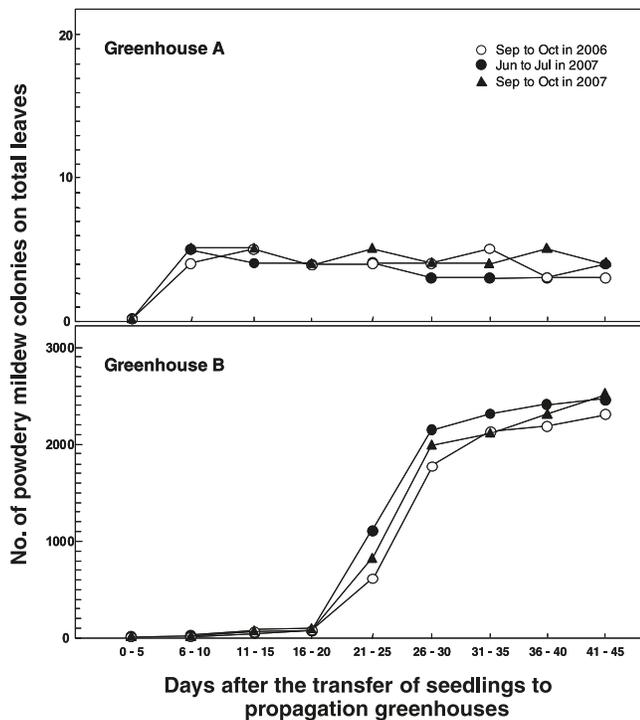
**Table 1.** Effect of corona discharge on colony development and conidiophore production by *Oidium neolycopersici* infecting tomato leaves.

Corona discharge exposure	Position of probe tip from leaf (mm)	Instant breakdown of conidiophores	Colony area (cm <sup>2</sup> )		No. of conidiophores per colony	
			7 d	14 d	7 d	14 d*
Treated	20	Yes	0.03±0.02 a	0.03±0.02 a	0 a	0 a
	25	Yes	0.04±0.02 a	0.04±0.01 a	0 a	0 a
	30	Yes (partially induced)	0.10±0.02 b	0.12±0.03 b	238.8±4.7 b	422.6±7.1 b
	35	No	0.17±0.02 c	0.23±0.02 c	423.6±1.6 c	671.6±1.6 c
	40	No	0.25±0.02 d	0.34±0.03 d	559.0±5.4 d	981.8±6.6 d
	45	No	0.26±0.02 d	0.34±0.02 d	569.5±3.5 d	1004.8±3.5 d
	50	No	0.25±0.02 d	0.35±0.03 d	575.8±53.4 d	1059.1±95.0 d
Non-treated	–	No	0.26±0.01 d	0.36±0.02 d	571.3±49.5 d	1070.8±86.1 d

**Note:** Values followed by different letters are significantly different ( $P < 0.05$ ; Tukey's method).

\*Days after exposure.

**Fig. 6.** *Oidium neolycopersici* colonies on leaves of tomato seedlings hydroponically cultured in propagation greenhouses. A total of 1500 seedlings were distributed between two propagation greenhouses, and colonies formed by natural infection of the pathogen were recorded every 5 days. All colonies on leaves of seedlings in greenhouse A were exposed to the corona treatment as soon as they became visible, while seedlings in greenhouse B remained untreated during the 45-day experimental period.



that produced inside the cylinder (0.025 ppm) within the short exposure time (2 seconds) of our corona discharge generator. This strongly suggests that ozone was not the only factor that killed the powdery mildew conidiophores colonizing tomato leaves. Herrmann et al. (1999) reported that the plasma stream produced during corona discharge effectively destroyed surface-colonizing microbes, which suggests that the plasma stream within our probe also plays a role in destroying powdery mildew on the leaf surface.

In the present single-truss cropping system, the plants are topped to leave first fruit-clusters. Because the height of the average single-truss plant does not exceed ~50 cm, plants can be grown in hydroponic culture channels on tables at a convenient working height for routine care. In particular, the removal of lateral buds must be carried out regularly to promote growth of the first-fruit cluster, because the topped seedlings develop lateral buds that grow very quickly. Under such a system, the total amount of foliage is much reduced, compared with systems where the plant is allowed to grow continuously. The corona discharge generator described in this study is portable and easy to operate, and the exposure treatment could be integrated as a part of routine crop care; the corona discharge exposure can be applied as soon as powdery mildew colonies are visible. In these colonies, conidia on conidiophores are immature, so killing them at this stage effectively suppresses the subsequent production and release of mature conidia (Oichi et al. 2004).

Tomatoes grown in open-window greenhouses become infected with air-borne conidia of powdery mildew that enter through the windows. In the non-treated greenhouse, the number of colonies on plants increased dramatically 30 days after transfer. Clearly, this infection was caused by the conidia that were abundantly released from colonies of non-treated infected plants. In a previous assay, we found that approximately  $10^4$  conidia were continuously released from conidiophores of single colonies during their 3-week lifetime (T. Nonomura et al., unpublished data). These conidia are readily wind-dispersed to neighboring plants, especially in a well-ventilated greenhouse (Oichi et al. 2006). In the present study, we treated all colonies one by one before the colonies released progeny conidia. This was carried out during the routine operation of lateral bud excision. As a result, this one-by-one treatment of colonies inhibited the spread of the disease by the conidia released from infected tomato plants in the greenhouse. This strategy of disease control was very successful, and only a small number of colonies appeared as a result of natural infection by conidia entering the greenhouses during the entire experimental period. The number of powdery mildew colonies was low and within limitations of routine treatment. The single-truss cropping system is repeated 5 to 6 times per year, and fungicide spraying is usually conducted every 2 weeks, i.e., three times during the 45-day cultivation period, to completely control powdery mildew. Our corona

discharge generator is a promising alternative disease control tool for complete eradication of powdery mildew without fungicidal treatment.

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