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ORIGINAL PAPER

Quantitative analysis of the lifelong production of conidia released from single colonies of *Podosphaera xanthii* on melon leaves using electrostatic techniques

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Abstract

Using an electrostatic rotational spore collector, we consecutively collected all of the conidia produced from single colonies of melon powdery mildew (*Podosphaera xanthii* Pollacci KMP-6 N) on leaves of living melon plants throughout the lifetime of the colony in a natural environment, and counted all conidia that were attracted to insulators. The collector consisted of an insulated round plastic container, a conductor (copper) film, an insulator (collector) film, an electrostatic voltage generator and a timer mechanism. Negative charge was supplied from the voltage generator to the conductor film, and the negatively charged conductor film caused dielectric polarization of the insulator film. The insulator film, which creates an attractive force for trapping conidia that enter the field, was placed ca. 2 cm from the apex of the single colony. Released conidia were successfully attracted to the electrostatically activated insulator films. Each collector film was exchanged for a new insulator film at 24 h intervals until KMP-6 N ceased to release conidia from single colonies. During a colony's lifespan, KMP-6 N released an average of 12.6×10^4 conidia from each of the single colonies at ca. 744 h. Additionally, we found that 1) the number of conidia released from single colonies in daytime was larger than that in night-time, 2) conidia were released from single colonies for ca. 2–4 h longer in spring or summer than in autumn or winter, and 3) release of conidia from KMP-6 N decreased as light intensity declined. Thus, conidial release from conidiophores is affected by day-length and light intensity.

Keywords Catenated conidia · Conidiophores · Dielectric polarization · Electrostatic spore collector · Electrostatic field

Introduction

Powdery mildew disease caused by the obligate fungus *Podosphaera xanthii* (Schlechtend.:Fr.) Pollacci is a serious threat to Cucurbitaceae plants in many countries (Sowell Jr 1982; Reifschneider et al. 1985; Mohamed et al. 1995; Hosoya et al. 1999; Del Pino et al. 2002; Křístková et al. 2004, 2009; Tomason and Gibson 2006; Pérez-García et al. 2009; Hong et al. 2018). Powdery mildew fungi are primary causes of agricultural problems that significantly reduce cucurbit productivity (McGrath and Thomas 1996; Hosoya et al. 1999; Pérez-García et al. 2009). Recently, a severe outbreak of powdery mildew occurred on the leaves of melon plants (*Cucumis melo* L., cv. Earl's Favourite) cultivated hydroponically in a greenhouse in Japan (Takikawa et al. 2015). We isolated a fungus from the powdery mildew-infected melon leaves and identified the isolate based on morphological (Reifschneider et al. 1985; Braun 1987; Braun and Cook 2012; Cosme et al. 2012) and genetic (ribosomal DNA internal transcribed spacer sequences, rDNA-ITS) characteristics as cucurbit powdery mildew fungi (Hirata et al. 2000; Chen et al. 2008). We designated the isolate *Podosphaera xanthii* (also known as *Sphaerotheca fusca*; anamorph: *Oidium* subgenus *Fibroidium*; Braun et al. 2001) Pollacci KMP-6 N, and found that it was not *Golovinomyces orontii*

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(also known as *Erysiphe cucurbitacearum*; anamorph: *Oidium* subgenus *Reticuloidium*) (Takikawa et al. 2015). *P. xanthii* KMP-6 N was highly pathogenic and infective to the commercial cultivars of Cucurbitaceae plants on which it was tested (Takikawa et al. 2015).

Powdery mildew fungi produce progeny conidia on conidiophores, which are thought to be a possible source of infection into host plants and can lead to further dissemination of powdery mildew diseases over a large area by dispersal of mature conidia from conidiophores (by wind) in their natural environment (Aylor 1990; Brown and Hovmøller 2002). Therefore, to clarify the mechanism of conidial dispersal from the conidiophores, we thoroughly studied the conidiogenesis of powdery mildew fungi on host plant leaves with a high-fidelity digital microscope (Oichi et al. 2004, 2006; Moriura et al. 2006b; Takikawa et al. 2015). We demonstrated that the melon powdery mildew KMP-6 N isolate produces catenated conidia consisting of a maximum of six immature conidia, one undivided conidial cell (the upper cell), one generative cell (gc) and one basal cell (bc), and that the first mature conidium and subsequent conidia are released from the conidiophores by division of the upper cell and septation in the gc, respectively. Consequently, we clarified that a conidiophore of KMP-6 N produced an average of 36 conidia during its lifetime (an approximately 90-h period) in a natural environment (greenhouse conditions) (Takikawa et al. 2015). Conversely, we found that KMP-6 N piled up approximately 38–40 conidia on conidiophores in a chain, without releasing mature conidia from conidiophores in an indoor environment (in growth chambers) or in darkness (Suzuki et al. 2018). Based on the reports by Takikawa et al. (2015) and Suzuki et al. (2018), we have been greatly interested in determining when and how many progeny conidia are produced on and released from conidiophores in single colonies of the melon powdery mildew pathogens. Therefore, in the present study, we focused on quantifying the production of progeny conidia by individual colonies throughout their life, irrespective of whether it was day or night, in a natural environment. This information will be crucial to understanding the ecological mechanism of the expansion of infection by powdery mildews in a natural environment, and will help to develop ecofriendly methods for controlling powdery mildews in host plants without the use of artificial chemicals.

To successfully achieve our goal of developing ecofriendly management methods, we utilized electrostatic force. Griffith (2004) and Halliday et al. (2005) described the electrification of insulators through dielectric polarization caused by a charged conductor, such that the polarized dipole insulators produce a non-uniform electric field around them, creating an electrostatic force. Additionally, Matsuda et al. (2006) reported that conidia that come into the electric field are attracted to, and trapped on, the polarized dipole insulators. Based on the principles of electrostatics, we previously devised electrostatic spore collectors using a dielectrically polarized insulator plate or probe, and efficiently and successfully collected all mature conidia produced from single colonies of powdery mildew on barley (*Blumeria graminis* f. sp. *hordei* Marchal race 1) (Moriura et al. 2006a) and tomato (*Pseudoidium neolycopersici* L. Kiss, syn. *Oidium neolycopersici* L. Kiss) (Nonomura et al. 2009) during their lifetime. In this study, we devised a new electrostatic rotational spore collector (a dielectrically polarized insulator drum, i.e., a rotary drum) for collecting all conidia produced during the development of single KMP-6 N colonies growing on live melon seedlings in a natural environment, and counted the total number of conidia attracted on the insulators during the lifetimes of the colonies. This is the first report describing the lifelong production of conidia from single colonies of melon powdery mildew fungi on living leaves.

Materials and methods

Plant materials and cultivation

Melon (*Cucumis melo* L., cv. Earl's Favourite) seeds were supplied by Yuasa Experimental Farm, Kindai University (Wakayama, Japan). The seeds were placed on wet filter paper inside Petri dishes, and germinated for 3–4 days in a growth chamber (LH-240 N; Nippon Medical and Chemical Instruments, Osaka, Japan) under continuous illumination (22.2 μmoL m^{-2} s^{-1}; 380–750 nm) with white (full-spectrum) fluorescent lamps (FL40SS W/37; Mitsubishi, Tokyo, Japan) at 25 ± 2 °C. The germinated seedlings were then placed on polyurethane cubic sponge supports (3 × 3 × 3 cm^3); these were inserted into 30-mL cylindrical plastic containers (3 cm in diameter, 5 cm in length) containing 20 mL hydroponic nutrient solution (4.0 mM KNO_3, 1.5 mM $Ca(NO_3)_2$, 1.0 mM $MgSO_4$, 0.66 mM $NH_4H_2PO_4$, 0.057 mM FeEDTA, 0.048 mM H_3BO_3 and 0.009 mM $MnSO_4$) (Takikawa et al. 2015) and incubated for 14 days under controlled conditions (25 ± 1 °C; 40–50% relative humidity [RH]; and continuous illumination of 59.5 μmoL m^{-2} s^{-1}).

The 14-day-old seedlings were transferred to a polystyrene plate (68.0 × 44.5 × 2.5 cm^3) floating in the hydroponic nutrient solution, in a hydroponic culture trough (70.0 × 46.0 × 20.0 cm^3) on a growing Table (100 cm high) in a pathogen-free nursery greenhouse (10.0 × 6.0 m^2; 26 ± 3 °C) (Matsuda et al. 2006). The plants were used for maintaining a melon powdery mildew isolate and conducting experiments to collect all conidia from a single colony of the isolate with an electrostatic rotational spore collector (a rotary drum).

Fungal materials, inoculation and incubation

A melon powdery mildew isolate (*Podosphaera xanthii* Pollacci KMP-6 N; Takikawa et al. 2015; Suzuki et al.

2018) was used in this study. Mature conidia were collected from conidiophores on KMP-6 N-infected melon leaves using a pencil-type electrostatic insulator probe. The insulator probe, an ebonite rod with a pointed tip (7 cm in length, 4 mm in diameter, 5 μm in diameter at the tip), was mounted on the micromanipulator of a KH-2700 digital microscope (Hirox, Tokyo, Japan). The conidia were inoculated onto the true leaves of 14-day-old healthy melon seedlings (cv. Earl's Favourite), as described previously (Takikawa et al. 2015; Suzuki et al. 2018). The isolate was maintained for 14 days by incubation in an electrostatic screen chamber (ES-chamber; an apparatus for preventing airborne pathogens from entering the chamber), installed in a greenhouse (10.0 × 6.0 m^2) at 26 ± 2 °C, 50–70% RH under illumination of 190.6–400.4 μmoL m^{-2} s^{-1} (Matsuda et al. 2006), and in a growth chamber (LH-240 N; Nippon Medical and Chemical Instruments) at 25 ± 1 °C, 70–80% RH under continuous illumination of 22.2 μmoL m^{-2} s^{-1} (Takikawa et al. 2015). Voucher material (KMP-6 N) of the fungus used in this study is preserved in the Herbarium Preservation Section of Kindai University (Nara, Japan). Alternately, single conidia were inoculated onto 14-day-old melon leaves using the insulator probe described above and, at 5 days after inoculation, melon leaves with a single fungal fleck were selected for the experiments (Fig. 1a).

Conidial collector

The rotational spore collector consisted of a conductor (copper) film (250 × 10 × 0.5 mm^3) wound around an insulated round plastic container (5 cm in height, 8 cm in diameter); a direct current (DC) HVA 10K202PA electrostatic voltage generator (Logy Electric, Tokyo, Japan); a transparent collector (insulator) film (260 × 60 × 0.5 mm^3) made with polypropylene (Hapila, Tokyo, Japan); and a WH3311 timer mechanism (Matsushita Electric Works, Osaka, Japan) (Figs. 1b and 2a). The conductor film was connected to the negative terminal of the electrostatic voltage generator. The current was supplied from the voltage generator to the conductor film. The outer insulator film, which was negatively polarized and charged with static electricity (5.2 × 10^{-1} nC), was placed approximately 2 cm (distance A in Fig. 2b) from the apex of the single colony formed on a melon leaf to collect all released conidia, as described previously (Moriura et al. 2006a). The negative charge on the outer surface of the electrified insulator film generates an electrostatic field and creates an attractive force to trap conidia that come into the field, as shown in Fig. 2b (Matsuda et al. 2006; Moriura et al. 2006a; Suzuki et al. 2018). The insulator film, which took 24 h to complete a rotation at the collection site, was removed from the apparatus each 24 h and a new insulator film wound around the conductor film of the rotary drum. The number of conidia attracted to the insulator film from five individual 5-day-old fungal colonies on each melon leaf was counted under a KH-2700 digital microscope.

Electrostatic activation of the insulator film

The transparent collector (insulator) film was dielectrically polarized by providing the impressed potential supplied from the voltage generator to the conductor film (positively on the conductor film side; negatively on the opposite, conidium-collection side; Fig. 2b). The potential of the conductor film was controlled by the voltage generator, and the potential difference (kV) between the insulator surface and ground level

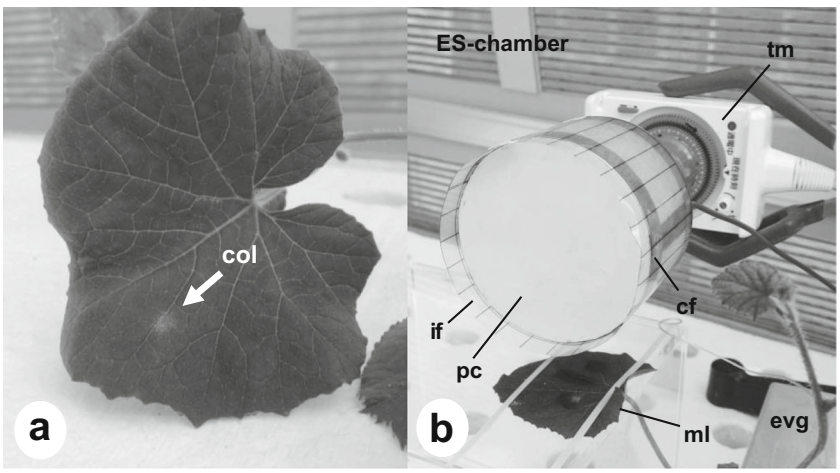

Fig. 1 Electrostatic device for the consecutive collection of conidia released from single colonies of *Podosphaera xanthii* KMP-6 N on melon leaves. **a** Melon seedling hydroponically grown in a greenhouse, with a single colony (col) of the pathogen on the inoculated leaf (5 days after inoculation). **b** Timer-controlled rotational spore collector used for the consecutive collection of conidia. The electrostatic conidial collection apparatus (rotary drum) consists of an insulated round plastic container (pc), a conductor (copper) film (cf), a transparent insulator (collector) film (if), an electrostatic voltage generator (evg), and a timer mechanism (tm). The collector and melon seedling with a single colony on a leaf (ml) were placed in an electrostatic screen chamber (ES-chamber) installed in a greenhouse

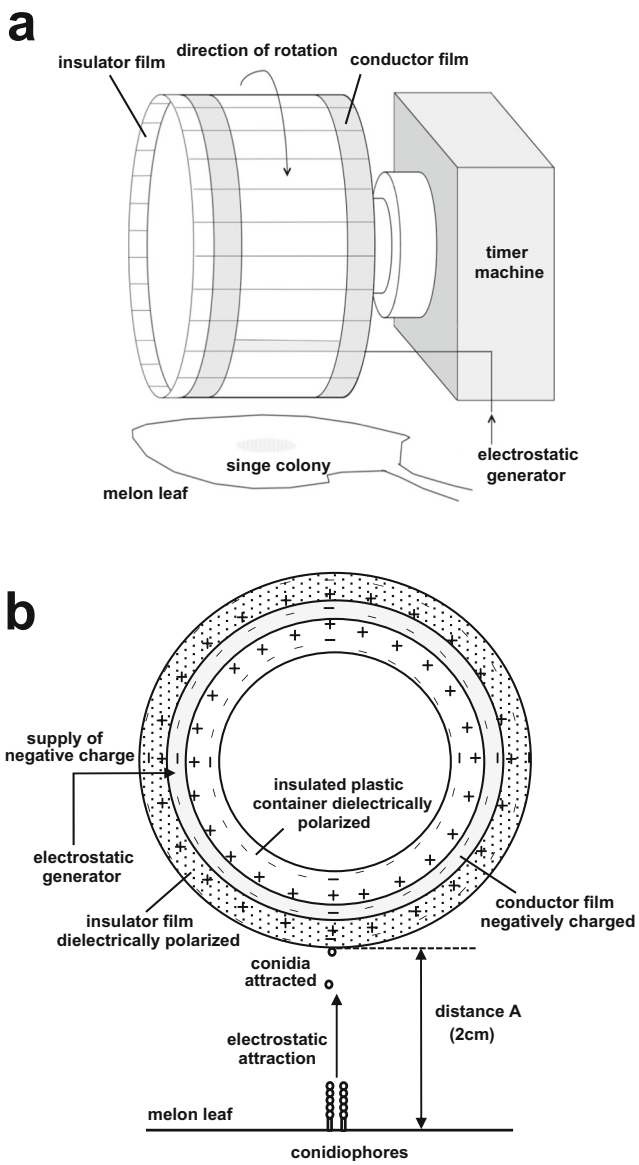

Fig. 2 A schematic diagram of the timer-controlled rotational spore collector with electrostatically activated insulator films. **a** Position of melon seedling with a single KMP-6 N colony, the conductor film connected to the electrostatic voltage generator, the insulator film on a single colony formed on a melon leaf, and a timer machine. **b** Possible mode of electrostatic collection of conidia released from conidiophores in a single colony. The electrostatic voltage generator produced a negative charge, which was transferred to the conductor film. The negative charge on the conductor film induced a positive "image charge" on the surface of the insulator film. Dielectric polarization produced a negative surface charge on the opposite side of the insulator film. An electrostatic field was formed around the dielectrically polarized insulator film. Conidia were directed to the insulator (collector) film by electrostatic attraction

(the voltage) was measured using an electrostatic field meter (FMX-002; Simco, Kobe, Japan). The surface electrostatic charge (nanocoulombs, nC) of the insulator film was measured by touching the film surface with the probe (tip diameter 50 μm) of the coulometer (NK-1001; Kasuga Denki, Kanagawa, Japan).

Consecutive collection of total conidia released from single colonies

Experiments were carried out to estimate the total number of conidia released from a single colony over a 30-day period after inoculation. Mature conidia from the greenhouse were collected from conidiophores with the electrostatic insulator probe, as described above (Takikawa et al. 2015), and transferred onto well-developed, young leaves of 14-day-old melon seedlings (cv. Earl's Favourite). A melon seedling with a single colony on a leaf, grown in an ES-chamber installed in a greenhouse (25 ± 1 °C; 45–55% RH) (Matsuda et al. 2006), was placed under the electrostatic conidial collection apparatus (rotary drum) (Fig. 1b). The collection apparatus was operated continuously for 24–29 days. The insulator film was continuously charged (5.2×10^{-1} nC) on a single colony formed on the leaf until being replaced by the next insulator film (film change duration, 30 s). A total of 24–29 films were used during each experiment. The number of conidia deposited on each film was counted every 5 h after collection using the KH-2700 digital microscope. The number of conidia collected per h was estimated by pooling the counts for each 60-min interval. The experiment was repeated five times (in May, June, October, November and December) in 1 year.

Germination rates of conidia collected using apparatus

Germination rates of the collected conidia were tested in a similar experiment. A melon seedling with a single colony on a leaf was placed under the collecting apparatus each month, as described above, and the apparatus was operated as above. The 1st (5-day-old colonies after inoculation), 6th (10-day-old), 11th (15-day-old), 16th (20-day-old) and 21st (25-day-old) insulator films were removed immediately after collection, placed in a humid box (RH 95–99%) and incubated at 25 ± 1 °C for 12 h. The number of germinated conidia was counted using a KH-2700 digital microscope. Data are presented as means and standard deviation of five replicates (for each collection time in May, June, October, November and December).

Observation of single KMP-6 N colonies by microscopy

The conidiophores of a colony on a melon leaf, and the conidia attracted to the insulator film, were viewed using the objective zoom lens MX-5030RZII (×250) of the KH-2700 digital microscope. The zoom lens was focused on the side of the leaf and the film. Digitized images of the conidia and conidiophores were obtained with a 1/2" Interline transfer charge-coupled device (CCD) camera and adjusted using Adobe Photoshop image-processing software (ver. 5.0; Adobe Systems, San Jose, CA, USA).

The fifth colonies on different leaves were used for each collection of conidia, as described above. After the final collection, leaf segments (approximately 5 × 5 cm^2 in size) were cut from the KMP-6 N-inoculated plants. The segments were fixed and decolored in a boiling alcoholic lactophenol solution (containing 10 mL glycerol, 10 mL phenol, 10 mL lactic acid, 10 mL distilled water and 40 mL 99.8% ethanol) for 1–2 min, and then stained with 0.1% Aniline Blue (Nacalai Tesque, Tokyo, Japan) dissolved in distilled water, as described previously (Sameshima et al. 2004). Subsequently, the stained colonies were observed using a BX-60 light microscope (Olympus, Tokyo, Japan) and photographed using an EOS KISSX6i digital camera (Canon, Tokyo, Japan) mounted on the microscope. The total number of conidiophores in five individual colonies was calculated. In addition, the mycelial areas of their colonies were calculated using ImageJ software (NIH, Bethesda, MD, USA). Data are presented as the means of five replicates.

Electrostatic collection of conidia released from single colonies under different light intensities

Mature conidia were inoculated onto young leaves of 14-day-old melon seedlings (cv. Earl's Favourite), according to the methods described above. A melon seedling with a single colony on a leaf, grown in a greenhouse installed with ES-chambers, was placed under the electrostatic collection apparatus in the greenhouse (25 ± 1 °C; 45–55% RH). Conidia were consecutively collected from the same powdery mildew colonies over a period of 5 days; the first collection was conducted when target colonies were 12 days old on each leaf, and the last was conducted when colonies were 17 days old. Insulator film was continuously charged (5.2×10^{-1} nC) on a single colony formed on the leaf, until being replaced by the next insulator film (film change duration was 30 s). A total of five films were used during each experiment. The number of conidia deposited on each film was counted every 5 h after collection using the KH-2700 digital microscope. The number of conidia collected per h was estimated by pooling the counts obtained at each 60-min interval.

In this experiment, the maximum photon flux density (PFD) in the daytime was set to four, by placing sheets of transparent black film around the apparatus. The stages were 190.96–400.40 μmoL m^{-2} s^{-1} (no cover), 19.20–313.80 μmoL m^{-2} s^{-1}, 0.20–2.12 μmoL m^{-2} s^{-1}, 0.02–0.10 μmoL m^{-2} s^{-1} and 0 μmoL m^{-2} s^{-1} (in darkness). The light intensity was measured using an LI-250A light meter (LI-COR, Tokyo, Japan) fitted with a quantum sensor that measures photosynthetically active radiation (400–700 nm).

Results

Observation of colonies and conidiophores using digital microscopy

Conidia released from a single colony were electrostatically attracted to the negatively polarized insulator film (Fig. 3a). Observation by light microscopy revealed that the attracted conidia were 23–33 × 15–22 μm, hyaline, ellipsoid-ovoid to doliiform, and contained fibrosin bodies (Fig. 3b). We also observed conidiophores in the colonies under a KH-2700 digital microscope. KMP-6 N produced catenated conidia consisting of a maximum of six immature conidia (C1–6), followed by a divided conidial cell (dc), one gc and a bc (Fig. 3c).

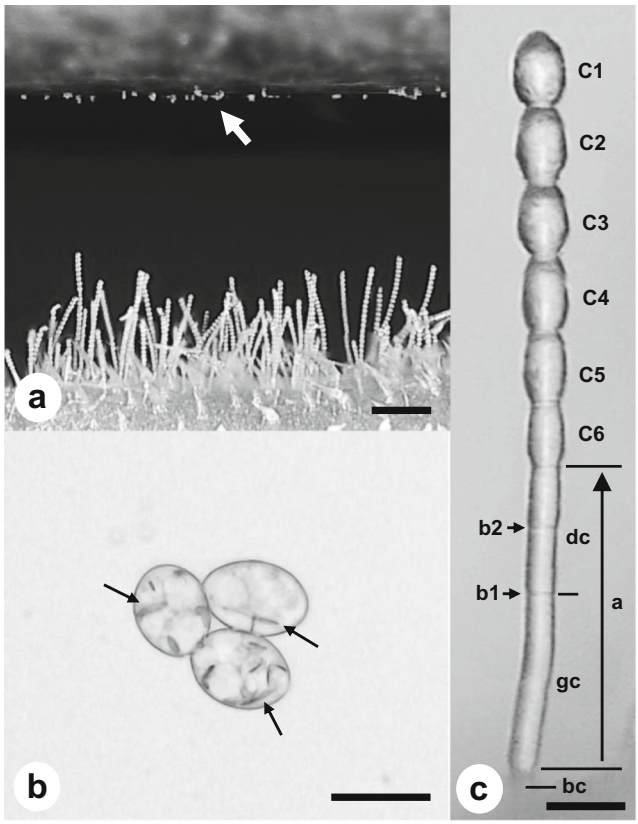

Fig. 3 Micrographs of conidia electrostatically attracted to the insulator film from conidiophores of a 10-day-old powdery mildew colony on a melon leaf. **a** Digital micrograph showing attraction of conidia released from conidiophores to an electrostatic insulator film. The insulator film (arrowed) carrying a charge of 1.0 nC was placed 600 μm from the apex of the conidiophore. Bar represents 200 μm. **b** Light micrograph showing conidia collected on an insulator film. Arrows show fibrosin bodies in single conidia. Bar represents 20 μm. **c** Digital micrograph showing a conidiophore possessing a developing chain of six immature conidial cells (C1–C6), a divided conidial cell (dc), one generative cell (gc) and one basal cell (bc) on hyphae on the leaf surface. Conidial cells were successively increased as the gc repeatedly elongated (arrow a) and divided twice via septation (arrows b1 and b2). Mature conidia were released when the septum between the apical conidium and next conidial cell had constricted fully. Bar represents 20 μm

Germination rates of conidia attracted to insulator films

Germination rates of the conidia electrostatically collected from five individual colonies each month were measured and the average and standard deviation of five replicates (collected on the 1st, 6th, 11th, 16th and 21st insulator films) were calculated. The germination rates were 96.5 ± 0.8, 98.5 ± 0.1, 94.5 ± 0.2, 95.0 ± 0.5 and 96.2 ± 0.4% in May, June, October, November and December, respectively.

Estimation of total conidia released from single colonies under greenhouse conditions

The lifelong production of conidia released from five single colonies was estimated from the total conidia collected in 1 h (Fig. 4). Conidia were first collected from 5-day-old colonies, and continued to be collected for approximately 4 weeks for all fungal colonies. The expansion of colonies ceased 14–15 days after inoculation, but thereafter mature conidia were continuously released from the colonies for approximately 2 weeks (13–14 days) (Fig. 4). Table 1 shows the colony areas, the number of conidiophores in single colonies, the duration of conidial secession and the total conidia released by individual colonies throughout their lifetime. Between 11×10^4 and 14×10^4 conidia were released per colony, with an average of 12.6×10^4 conidia released in a lifespan of approximately 744 h. Next, we compared the numbers and time periods of release of conidia from individual colonies (20-day-old colonies) in a single day (Fig. 5). The results show a distinct difference between the number of conidia released during the daytime versus the night-time in each month. The number of conidia released during the daytime was higher than that during the night-time. The periods of the daytime and the night-time in each month (shown in Fig. 5) were 14 h 6 min (from 4:50 am to 6:56 pm) and 9 h 54 min (from 6:56 pm to 4:50 am) on 20 May; 14 h 29 min (from 4:44 am to 7:13 pm) and 9 h 31 min (from 7:13 pm to 4:44 am) on 20 June; 11 h 10 min (from 6:06 am to 5:16 pm) and 12 h 50 min (from 5:16 pm to 6:06 am) on 20 October; 10 h 14 min (from 6:35 am to 4:49 pm) and 13 h 46 min (from 4:49 pm to 6:35 am) on 20 November; and 9 h 50 min (from 6:59 am to 4:49 pm) and 14 h 10 min (from 4:49 pm to 6:59 am) on 20 December, respectively. Overall, the time period of conidia release was longer by approximately 4.5 h in June than it was in December.

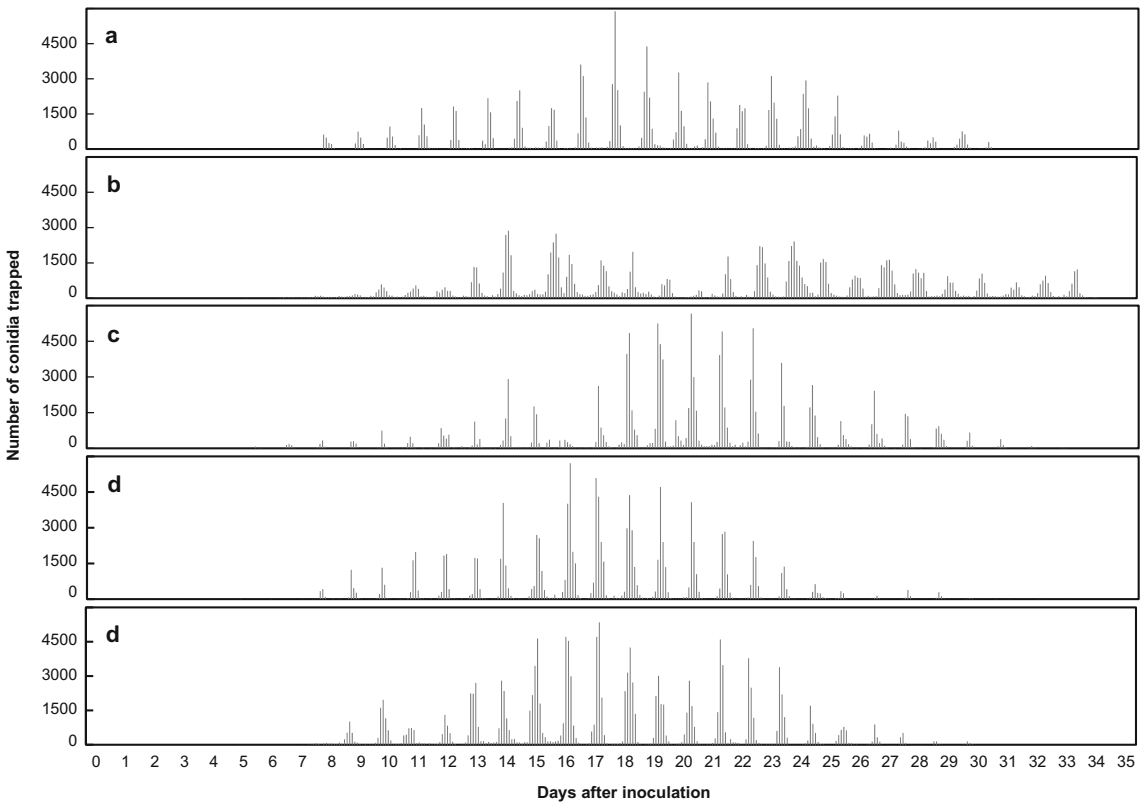

Fig. 4 Number of mature conidia trapped from a single colony of *Podosphaera xanthii* KMP-6 N on melon leaves during the entire period of conidial secession, using a timer-controlled rotational spore collector in May **(a)**, June **(b)**, October **(c)**, November **(d)** and December **(e)**. The data are plotted in 1-h periods

Table 1 Development of individual colonies of *Podosphaera xanthii* KMP-6 N on melon leaves for the duration of conidiation by conidiophores, assessed by direct counting of conidia continuously trapped on electrostatically activated insulator films

Colony [a]	Colony area (cm^2)	Number of conidiophores in a single colony	Duration of conidial secession (day)	Total conidia collected
a	1.9	1261	26	129,285
b	1.6	1273	29	132,861
c	1.8	993	27	117,473
d	1.7	1135	24	118,023
d	2.2	1393	24	136,364
Means	1.8	1211	26	126,801

[a] Refer to Fig. 4 for individual colonies (a-e)

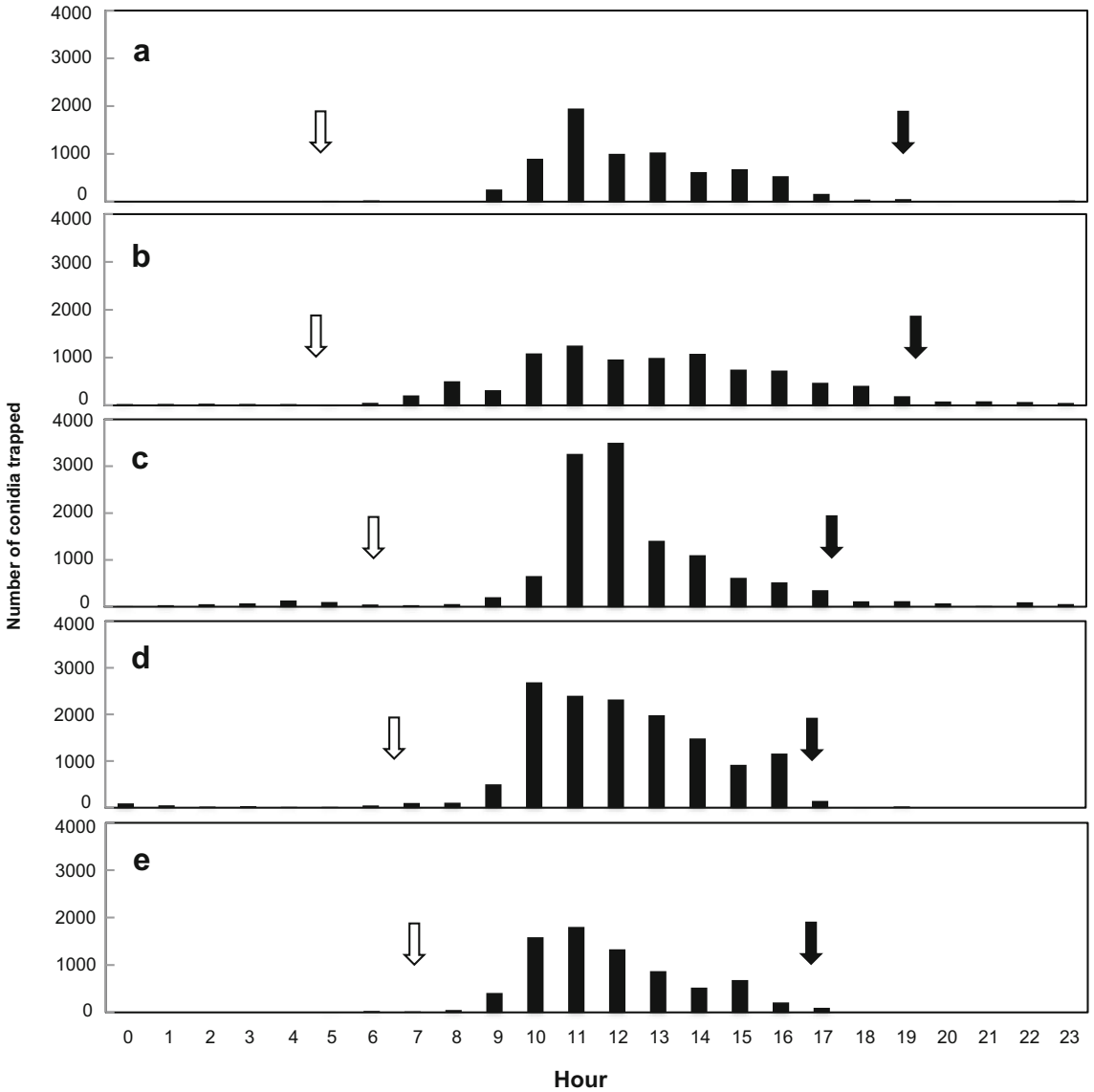

Fig. 5 Number of mature conidia trapped from a single colony of *Podosphaera xanthii* KMP-6 N on melon leaves over a period of 24 h, 20 days after inoculation, using a timer-controlled rotational spore collector in May **(a)**, June **(b)**, October **(c)**, November **(d)** and December **(e)**. The data are plotted in 1-h periods. Open and closed arrows indicate the times of sunrise and sunset, respectively

Estimation of the number of conidia released from single colonies under different light intensities

Using the electrostatic spore collector (rotary drum), we counted the number of conidia consecutively collected from colonies grown in greenhouses under different light intensities between 12 and 17 days post-inoculation. The number of conidia released from five single colonies was plotted as the total conidia collected in 1 h (Fig. 6). As shown in Fig. 6, the total number of conidia collected under the maximum light intensity of 400.40 μmoL m^{-2} s^{-1} in the daytime was highest, while the number of conidia collected in continuous darkness (0 μmoL m^{-2} s^{-1}) was lowest. Consequently, the number of conidia attracted to the insulator films decreased as the light intensity declined. The total numbers of conidia continuously collected in the greenhouses for 5 days (when the colonies were aged from 12 to 17 days) were 43,727, 3352, 1019, 506 and 148 conidia under the maximum light intensities of 400.40, 313.80, 2.12, 0.10 and 0 μmoL m^{-2} s^{-1} in the daytime, respectively. Thus, the release of conidia from single colonies was affected by the light intensity.

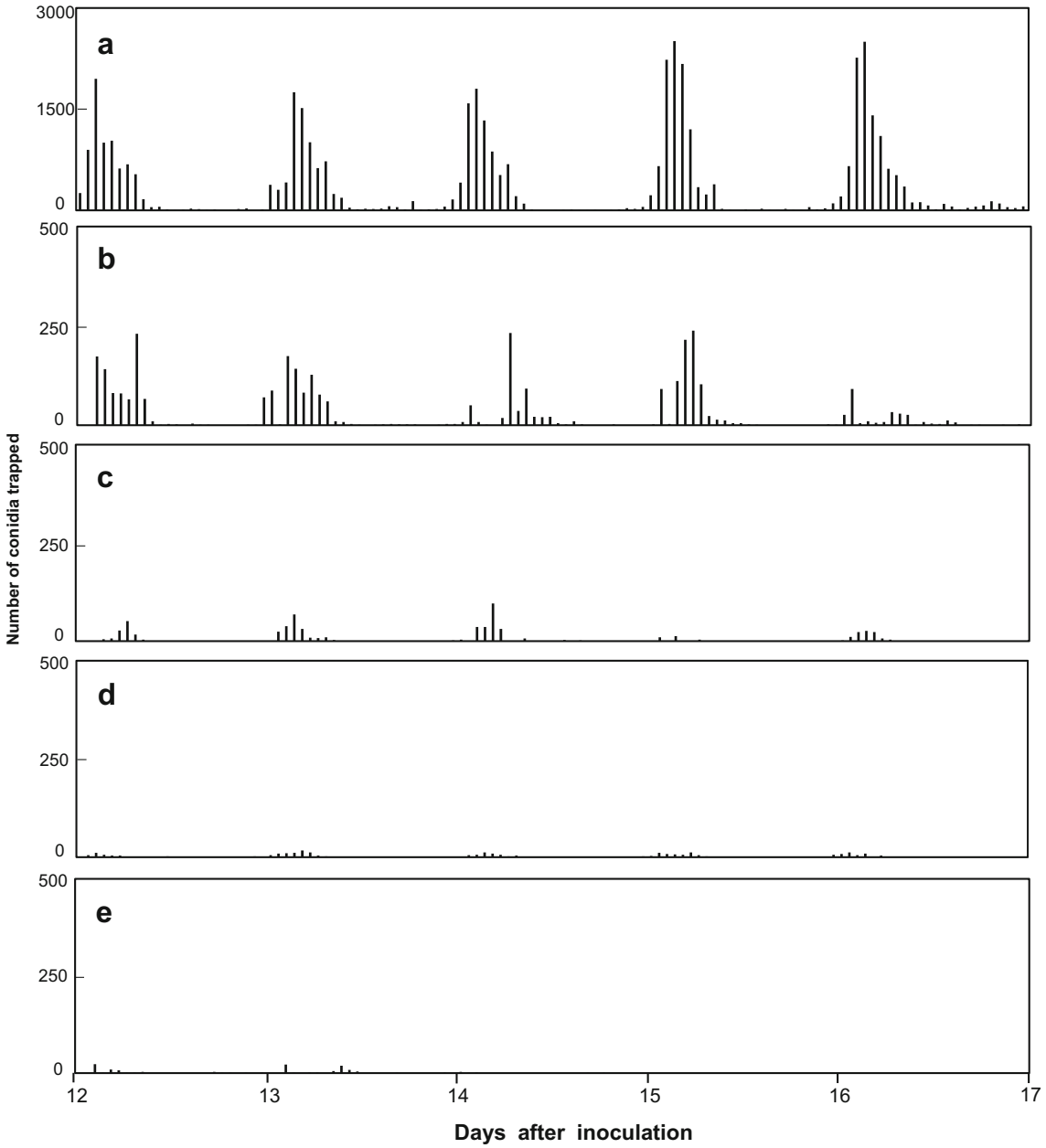

Fig. 6 Number of mature conidia trapped from a single colony of *Podosphaera xanthii* KMP-6 N on melon leaves grown under different light intensities in the daytime for a period of 5 days (from 12 to 17 days post-inoculation), using a timer-controlled rotational spore collector. The data are plotted in 1-h periods. Photon flux density (PFD) in the daytime was set to 190.96–400.40 μmoL m^{-2} s^{-1} **(a)**, 19.20–313.80 μmoL m^{-2} s^{-1} **(b)**, 0.20–2.12 μmoL m^{-2} s^{-1} **(c)**, 0.02–0.10 μmoL m^{-2} s^{-1} **(d)** and 0 μmoL m^{-2} s^{-1} **(e)**, while that in the night-time was 0 μmoL m^{-2} s^{-1}

Discussion

Some researchers (Mizuno and Washizu 1995; Griffith 2004; Halliday et al. 2005) have reported effective polarization of conidia, with an opposite "image charge" induced on the side facing the charged probe, and that these opposing charges created an electrostatic force between the conidia and insulators. Moreover, Leach (1976) reported that violently projected, wind-dispersed fungal spores become electrically charged at the moment of release. Based on the fundamental principles of static electricity, our previous studies revealed that the conidia of powdery mildew can be successfully attracted to both negatively and positively polarized insulators (insulator cylinders, probes and plates) by the exploitation of electrostatic force (Matsuda et al. 2006; Moriura et al. 2006a, 2006b; Shimizu et al. 2007; Nonomura et al. 2009). For example, using barley powdery mildew fungi (*B. graminis* f. sp. *hordei* Marchal race 1 KBP-01), we investigated the optimal electrostatic conditions for attracting conidia released from colonies on host leaves to an insulator plate (Moriura et al. 2006a), i.e., the relationship between voltage and charge induced on the surface of the insulator plate, and the relationship between the surface charge of the insulator plate and the largest separation distance that attracted conidia (the ability to attract conidia). Similarly, in the present study, the charge on the insulator film was directly proportional to the voltage on the conductor film, and the electrostatic force required to attract the conidia varied in proportion to the distance from the conidiophores (data not shown). Consequently, we achieved the optimized electrostatic conditions for attracting conidia by placing the KMP-6 N-inoculated melon leaf less than 2 cm from the insulator film at 5.2×10^{-1} nC.

By combining the electrostatic and digital microscopic techniques, we observed and analyzed the conidiogenesis and colony development of a powdery mildew fungus on host leaves. We counted all conidia produced on single conidiophores and released from single colonies of powdery mildew fungi throughout the fungal lifetime, using negatively polarized insulator plates (Moriura et al. 2006a) and probes (Moriura et al. 2006b; Nonomura et al. 2009; Takikawa et al. 2015). Moriura et al. (2006a, 2006b) reported that barley powdery mildew (*Blumeria graminis* f. sp. *hordei* Marchal race 1 KBP-01) produces approximately 29–38 mature conidia on a single conidiophore and releases approximately 1.2×10^5 conidia from a single colony throughout its lifetime. Also, Oichi et al. (2004) and Nonomura et al. (2009) reported that tomato powdery mildew (*Pseudoidium neolycopersici* L. Kiss KTP-01) produces approximately 8–10 mature conidia on a single conidiophore and releases approximately 5×10^3 conidia from a single colony throughout its lifetime. Moreover, Takikawa et al. (2015) reported that melon powdery mildew (*Podosphaera xanthii* Pollacci KMP-6 N) produces approximately 34–38 mature conidia on a single conidiophore throughout its lifetime. In this study, we successfully achieved consecutive monitoring of conidial production by individual living conidiophores and colonies on host leaves. A crucial element of the present study is that we quantitatively demonstrated the lifelong production of conidia released from single colonies of melon powdery mildew (*Podosphaera xanthii* Pollacci KMP-6 N) with a negatively polarized insulator drum. The dielectrically polarized insulator drum did not detach immature conidia from conidiophores. In addition, KMP-6 N elongated hyphae, formed conidiophores, and produced living progeny conidia from the generative cells of the conidiophores without any detrimental effect on their survival, even when exposed to an electrostatic force throughout their lifetime. The conidia collected on the insulator films under the voltage conditions used in this study maintained their highly germinative capacity. Our results reveal that the lifelong production of mature conidia released from single KMP-6 N colonies was similar to the production from single KBP-01 colonies described by Moriura et al. (2006a). Both powdery mildew fungi formed catenate conidia on conidiophores; barley powdery mildew KBP-01 formed chains of eight conidia per conidiophore (Moriura et al. 2006b), whereas melon powdery mildew KMP-6 N formed chains of six conidia per conidiophore (Takikawa et al. 2015). On the other hand, Hirata (1967) studied chemically fixed samples of powdery mildewed barley leaves that were collected at various stages after inoculation, and reported that a single colony produced up to 2.0×10^5 conidia in its lifetime. Our working hypothesis is that powdery mildew fungi forming catenate conidia are likely to continually release over 1.0×10^5 conidia per colony throughout a lifetime. To further test this hypothesis, we propose the use of electrostatic spore collection apparatus to count and compare the number of mature conidia released from single colonies throughout the lifetime of powdery mildew fungi from another genus.

Another important finding of this study is that we observed the vigorous release of conidia from KMP-6 N colonies in the daytime, whereas the colonies released very few conidia at night. The elucidation of the ecological characteristics of conidial release from the conidiophores of KMP-6 N was not previously possible without the use of this electrostatic spore collector system. In our previous study, Suzuki et al. (2018) reported that 1) KMP-6 N formed chains of a maximum of six conidia on each conidiophore under red light (620–750 nm), whereas KMP-6 N formed continuous chains (often greater than seven conidia in length) without fully formed constrictions between the conidia under blue light (450–495 nm) or in darkness, and 2) KMP-6 N vigorously released conidia from colonies under red light, whereas few conidia of KMP-6 N were released from conidiophores under blue light or in darkness. The conidial release in the night-time that we report in this study was similar to the conidial release under blue light or in darkness described by Suzuki et al. (2018). The light responses of cucurbit

powdery mildew fungi and other powdery mildew fungi, including the same *Podosphaera* genus, are likely to be different. In fact, the numbers of conidia formed on conidiophores were similar under both greenhouse conditions (similar to those in the natural environment) and growth chamber conditions (the indoor environment), and in darkness for many other powdery mildew isolates: a single conidium for tomato powdery mildew KTP-03 and KTP-04 (*Pseudoidium* genus) (Nonomura et al. 2013), Japanese Mallotus powdery mildew KMP-01 (*Oidium* genus) (Nonomura et al. 2013), and red clover powdery mildew KRCP-4 N (*Erysiphe* genus) (Takikawa et al. 2011), and eight conidia for barley powdery mildew KBP-01 (*Blumeria* genus) (Moriura et al. 2006b). We have generally assumed that powdery mildew fungi disperse mature conidia produced on conidiophores by wind throughout the day, for wide expansion of powdery mildew diseases in the natural environment. However, in this study KMP-6 N decreased the release of mature conidia from conidiophores in the night-time, unlike in barley (Moriura et al. 2006a) and tomato powdery mildew fungi (Nonomura et al. 2009). Interestingly, conidial release from colonies largely reflected day length during the season in this study. The time of sunrise in May and June was 1.5–2 h earlier than in November and December, whereas the time of sunset in May and June was 2–2.5 h later than in November and December (see Fig. 5). In addition, the conidial release was largely affected by light intensity; conidial production gradually reduced as light intensity (PFD) decreased (see Fig. 6). Our findings in this study provide important insights into the physiological and ecological mechanisms of cucurbit powdery mildew fungi. It is possible that KMP-6 N has receptors for light sensitivity on conidiophores, and responds to light intensity by constricting conidial cells at the top of the conidiophores. Thus, it is evident that light factors are crucial in determining the release of conidia from conidiophores by KMP-6 N.

In melon powdery mildew (*Podosphaera xanthii* Pollacci), this is the first report to our knowledge that describes 1) direct determination of the duration of conidial secession, 2) a precise count of the total conidia that seceded from live individual colonies during their lifetime, 3) the periods of conidial release from colonies throughout the day, and 4) the responsivity to light intensity of conidia release from colonies. Thus, the method used in this report is a useful tool for quantitative analysis of the conidiogenesis of powdery mildews. In the future, we will attempt to study the lifelong production of all conidia from single colonies of other powdery mildews with the electrostatic system under natural conditions, and to compare conidial productivity and light sensitivity among powdery mildew fungi.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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