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An apparatus for collecting total conidia of *Blumeria graminis* f.sp. *hordei* from leaf colonies using electrostatic attraction

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Conidia from living conidiophores of barley powdery mildew (*Blumeria graminis* f.sp. *hordei*) on host leaves were collected consecutively using an electrostatic spore collector. The collector consisted of an electrical conductor plate linked to an electrostatic voltage generator and insulator plates placed abreast on a timed conveyer. The conductor plate was negatively charged by the potential supplied from the voltage generator. The negatively charged conductor plate caused dielectric polarization of the insulator plate, and the surface charge on the insulator plate attracted mature conidia abstricted from conidiophores on colonies growing on leaves placed 2 cm from the insulator plate. The surface charge on the insulator plate was proportional to the voltage applied to the conductor plate. Under optimized conditions, abstricted conidia were attracted to the electrostatically activated insulator plates without any detriment to their survival. During a colony's life span of *c.* 460 h, conidia were released throughout the day and *c.* 12×10^4 conidia were collected during the lifetime of the colony. This is the first report on the direct quantification of progeny conidia produced by powdery mildew infecting host leaves.

Keywords: barley powdery mildew, conductor, dielectric polarization, electrostatic induction, insulator, spore collector

Introduction

The powdery mildew fungi are among the most ubiquitous plant pathogens. Their parasitism is characterized in part by the formation of superficial hyphae that develop dense layers of conidiophores in which generative cells successively produce abundant asexual spores (conidia) (Jarvis *et al*., 2002). Mature conidia are abstricted from the apex of conidiophores and easily dispersed by wind to infect neighbouring host plants (Aylor, 1990; Brown & Hovmøller, 2002; Oichi *et al*., 2004). Because of the high fertility of the powdery mildew fungi, interest has been directed towards quantifying the production of progeny conidia by individual conidiophores throughout their life. Conidiophores on the leaf surface are the most suitable targets to investigate conidium production, but consecutively monitoring the development of living conidiophores and production of conidia is often difficult, not only because of the abundance of conidiophores in the colonies, but also because the conidia are formed in chains. Furthermore, the conidiophores vary in maturity and number of conidial cells, especially at the mid- and latter stages of conidiogenesis (Agrios, 1988).

In the present study, powdery mildew of barley, *Blumeria graminis* f.sp. *hordei*, was used as a model for fungi that form conidia in chains, to clarify the life-long conidiogenesis by living conidiophores on barley leaves. The aim was to devise a reliable technique effectively and successively to collect the conidia released from conidiophores during their lifetime. An apparatus is described that collects conidia released from colonies growing on leaves sequentially on collector plates. Conidia are directed to the collector plates by electrostatic attraction. The apparatus can be used to study the release of conidia during the lifetime of an individual colony.

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Materials and methods

Plant, pathogen and inoculation

Seeds of barley (*Hordeum vulgare* cv. Gose-shikoku) were germinated on a water-soaked filter paper and placed in a sponge cube (1 cm^3). The sponge cube and seed were

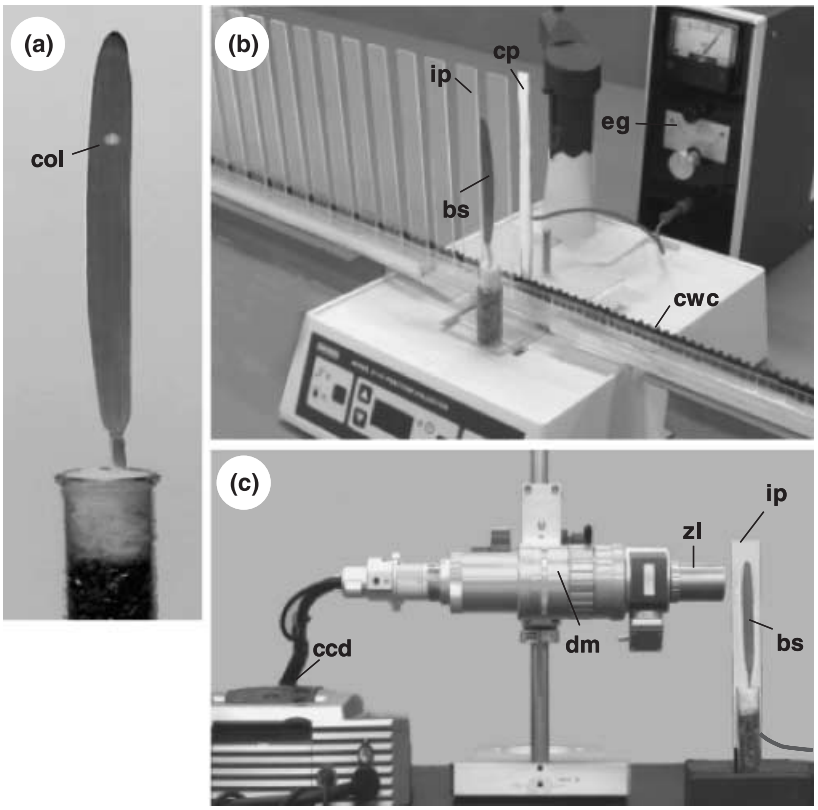

Figure 1 Electrostatic device for consecutive collection of conidia released from conidiophores of *Blumeria graminis* f.sp. *hordei* on intact barley leaves. (a) Barley seedling grown in a test tube containing fertilizer-supplied vermiculite, with a single colony (col) of the pathogen on the inoculated leaf (10 days after inoculation). (b) A timer-controlled conveyer (cwc) of insulator plates (ip) used for collection of conidia; conductor (aluminium) plate (cp); barley seedling (bs); and an electrostatic voltage generator (eg). The parallel insulator plates on the conveyer moved horizontally in a stop–start way. The conveyer was constructed from a column-chromatographic fraction collector. (c) Zoom lens (zl) of a high fidelity digital microscope (dm) connected to a CCD camera (ccd). Attraction of conidia from the conidiophore to the insulator plate was observed on the flank of the leaf and the plate.

inserted into the top portion of a 10-mL test tube with autoclaved, fertilizer-soaked vermiculite, and incubated for 14 days in a growth chamber at 20°C under continuous illumination at 56·1 mol m^{-2} s^{-1} with fluorescent lamps. Primary leaves of 12-day-old seedlings were inoculated with newly produced conidia of barley powdery mildew (*B. graminis* f.sp. *hordei*, race I). For inoculation, single conidia were placed on leaves using a micromanipulator, and 3 days after inoculation leaves with a single fungal fleck were selected for experiments (Fig. 1a). Alternatively, barley leaves were inoculated by dusting conidia onto barley leaves to obtain several colonies on the same leaves, according to the method described by Toyoda *et al.* (1987).

Automatic spore collector

The spore collector consisted of a conductor (aluminium) plate (120 × 20 × 0·5 mm); an electrostatic voltage generator; an insulator (polyethylene terephthalate) plate (150 × 30 × 0·5 mm); and a timed plate conveyer (Fig. 1b). The conductor plate was connected to an electrostatic voltage generator KTK-01 (Marushin Electronics), and the transparent collector plates (insulator plates) were placed abreast on the conveyer and moved in a stop–start way. Each insulator plate was stopped for 10 min at the collection site, where the insulator plate was positioned in front of the conductor plate with a barley leaf on the opposite side (Figs 1b and 2a).

Electrostatic activation of the insulator plate

The conductor plate was negatively charged by the impressed potential supplied from the voltage generator, and the electrostatic force generated by the conductor plate was used to polarize the insulator plate dielectrically (positively on the conductor plate side; negatively on the opposite, spore-collection side; Fig. 2b) while the insulator plates were stopped at the collection site. The potential of the conductor plate was controlled by the voltage generator, and the potential difference (kV) between the insulator surface and ground level was measured using an electrostatic field meter FMX-002 (Simco). The surface electrostatic charge (nanocoulombs, nC) of the insulator plate was measured by touching the plate surface with the probe (tip diameter 50 μm) of the coulometer (Nakamura Scientific).

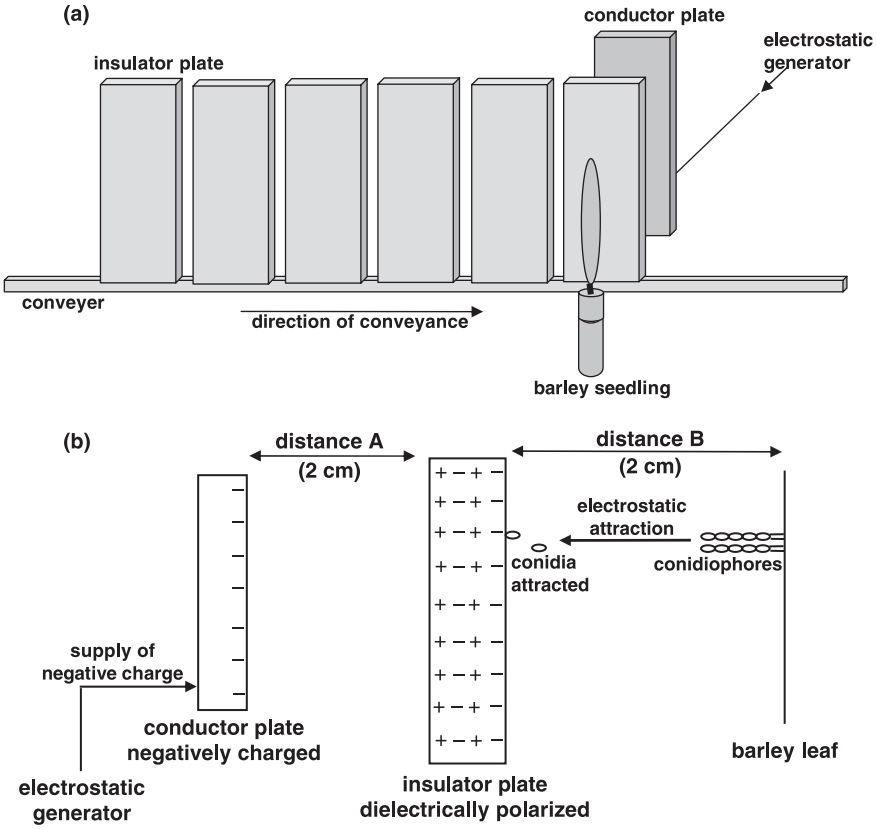

Figure 2 A schematic diagram for the automatic spore collector with electrostatically activated insulator plates. (a) Position of barley seedling; the conductor plate connected to the voltage generator; and the insulator plates on the conveyer. (b) Possible mode of electrostatic collection of abstricted conidia. The voltage generator produced a negative charge, which was transferred to the conductor plate. The negative charge on the conductor plate induced a positive 'image charge' on the surface of insulator plate. Dielectric polarization produced a negative surface charge on the opposite side of the insulator plate. Conidia were directed to the collector by electrostatic attraction.

Relationship between surface charge and attractive force of the insulator plate

For this experiment, the distance between the conductor and insulator plates was fixed at 2 cm (distance A in Fig. 2b). First, the conductor plate was connected to the negative terminal of the voltage generator, and the surface charge on the insulator plate was measured for different voltages (between 0·5 and 7·5 kV) to determine the relationship between voltage and surface charge. The relationship between surface charge and ability to attract conidia was examined in two ways. The distance at which conidia were first attracted to the charged insulator plate was measured for different surface charges (between 0·5 and 9·0 nC) by gradually moving a charged plate towards the apex of a conidiophore on a 10-day-old colony growing on a barley leaf, and observing the attraction of conidia using a digital microscope. Measurements were made using 20 single colonies on barley leaves.

In the second experiment, the number of conidia attracted to a charged plate from a barley seedling (placed 2 cm from the charged plate) was counted for a range of surface charges (0·5–9·0 nC). Ten insulator plates were placed on the conveyer (Fig. 2) and each plate was polarized for 30 s at the spore-collection site. The conidia attracted to the plates were observed using an objective zoom lens MX-5030RZII (×250) on a high-fidelity digital microscope KH-2700 (Hirox), according to the method described by Matsuda *et al*. (2005).

Consecutive collection of total conidia released

Experiments were carried out to estimate the total number of conidia released for a single colony over a 21-day period from inoculation. A barley seedling with a single colony on a leaf was placed in the electrostatic collection apparatus (Fig. 2) in an incubation room (20°C, continuous illumination of 56·1 mol m^{-2} s^{-1} with fluorescent lamps, RH 45–55%). The collection apparatus was operated continuously for 21 days. Each insulator plate was charged (7·0 nC) for 10 min in front of the leaf before being replaced by the next insulator plate (plate change took 3 s). A total of 3024 plates were exposed during each experiment (every 10 min for 21 days). The number of conidia deposited on each plate was counted every 6 h after collection using the

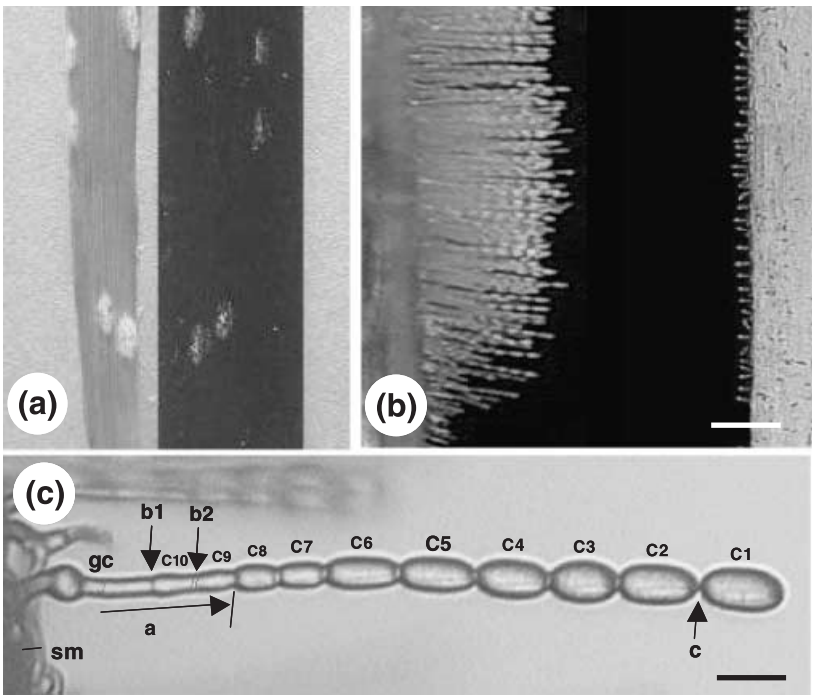

Figure 3 Macroscopic and microscopic views of conidia electrostatically attracted to the insulator from conidiophores of a 10-day-old powdery mildew colony on a barley leaf. (a) Conidia transferred to the insulator plate (right) at sites corresponding to colonies on a leaf (left). The transparent insulator plate was placed on a black sheet so the conidia were easier to see. (b) Digital micrograph showing attraction of abstricted conidia from conidiophores to the insulator plate. The insulator plate carrying a charge of 1·0 nC was placed 600 μm from the apex of the conidiophore. Bar = 200 μm. (c) A conidiophore with a developing chain of 10 conidial cells (C1–C10) and a generative cell (gc) on somatic mycelia (sm) on the leaf surface. Conidial cells were successively higher as the slender portion of the generative cell 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. Conidium C1 is seen just before abstriction and complete constriction of the septum (arrow c). Bar = 20 μm.

high-fidelity digital microscope. The number of conidia collected per hour was estimated by pooling the counts for each 10-min interval. This experiment was repeated six times.

The viability of conidia collected was tested in another, similar experiment. A seedling containing eight colonies was placed in the collecting apparatus, and the apparatus was operated as above. The 10th, 100th, 500th, 1500th, 2000th and 2500th insulator plate was removed immediately after collection, placed in a humid box (RH 95–99%) and incubated at 20°C for 10 h. The proportion of germinated conidia was estimated using the high-fidelity digital microscope.

Digital microscopy

The conidiophores of a colony on a barley leaf and the conidia attracted to the insulator plate were viewed using either the objective zoom lens MX-5030RZII (×250) or MX-2525CS (×1000) of the digital microscope. The zoom lens was set to the side of the leaf and the plate (Fig. 1c). Digitized images of the conidia were obtained with a 1/2″ Interline Transfer CCD camera and produced on a computer with Adobe PHOTOSHOP software (ver. 5·0) according to the method described by Oichi *et al.* (2004).

Results

Optimized electrostatic conditions for conidium attraction

Conidia were electrostatically attracted to the negatively polarized insulator plate (Fig. 3a,b). The digital microscopic observation revealed that conidiophores released the first mature conidia from the apex when the generative cells developed 10 conidial cells, and that the release of apical conidia occurred when the septum between the apical and second apical cells became fully constricted (Fig. 3c). Immature conidia (septa incompletely constricted) were not removed even when the surface charge on the insulator plate was 9·0 nC.

The charge on the insulator plate was directly proportional to the voltage on the conductor plate (Fig. 4a), and the electrostatic force to attract the conidia varied in proportion to the distance from the conidiophores (Fig. 4b). Because of structural restrictions, the plant had to be 2 cm from the collector plates. Test showed that a charge of 7·0 nC or higher was needed to attract conidia to the plate at this separation distance.

Viability of attracted conidia

The average germination rates (and standard deviation) of the conidia from eight colonies were 96·5 (±0·8); 99·5

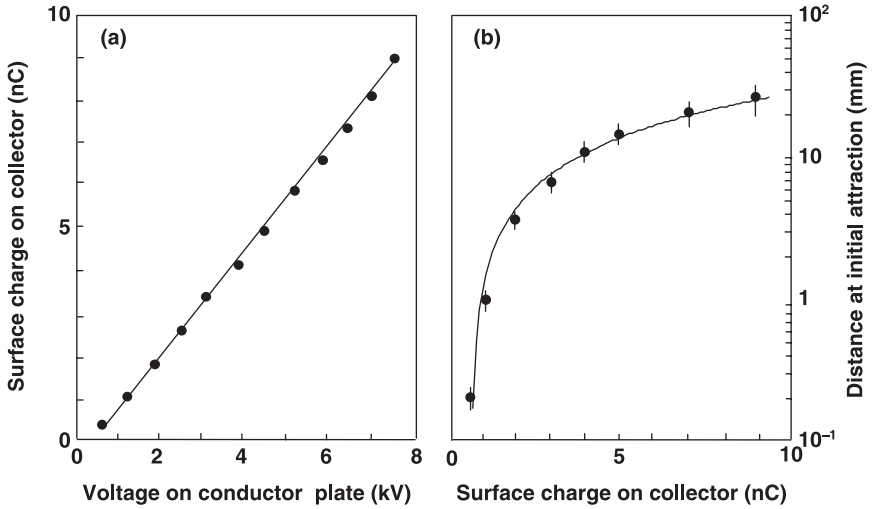

Figure 4 Optimization of electrostatic conditions to attract conidia released from colonies of *Blumeria graminis* f.sp. *hordei* on barley leaves. (a) Relationship between conductor plate voltage and charge induced on the surface of the insulator plate. The conductor plate was placed 2 cm from the insulator plate. (b) Relationship between the surface charge of the insulator plate and the largest separation distance that attracted conidia. Twenty colonies were tested at each voltage. Data are means with standard deviations.

(±0·1); 95·5 (±0·2); 95·0 (±0·3); 96·2 (±0·4); and 98·3 (±0·8)% on the 10th, 100th, 500th, 1500th, 2000th and 2500th insulator plates, respectively.

Estimation of total conidia released from individual colonies

Mature conidia were released from individual conidiophores all day long (Fig. 5). The release of conidia from these conidiophores did not appear to be synchronous. To express the total number of conidia released, the number of conidia in each set of six consecutive plates was plotted as the total conidia collected in 1 h (Fig. 6). Conidia were first collected 5 days after inoculation and continued to be collected for *c*. 3 weeks for all fungal colonies, although their area varied among the colonies tested. The expansion of colonies ceased 14–15 days after inoculation, but mature conidia continuously seceded from the colonies for another week. The colony area, duration of conidial secession and total conidia released by individual colonies through their life are summarized in Table 1. Between 6×10^4 and 16×10^4 total conidia were released per colony, with an average 12×10^4 conidia released in a life span of *c*. 460 h.

Table 1 Growth of individual colonies of *Blumeria graminis* f.sp. *hordei* on barley leaves for the duration of conidiation by conidiophores, assessed by direct counting of conidia continuously trapped onto electrostatically activated insulator plates

Colony[a]	Colony area (mm^2)	Duration of conidial secession (h)	Total conidia collected
a	29·8	468·0	150 003
b	28·5	465·3	115 093
c	23·2	466·0	60 291
d	29·7	466·0	70 955
e	50·0	465·3	144 733
f	66·4	467·7	161 883
Means	37·9	466·4	117 160

[a]Refer to Fig. 5 for individual colonies (a–f).

Discussion

The most important part of the present work was the use of an electrostatic force to collect the conidia released from conidiophores. Leach (1976) described that violently projected, wind-dispersed fungal spores become electrically charged at the instant of release. McCartney *et al*. (1982) calculated the actual surface charge as conidia impacted into charged cylinders, showing that the low-magnitude charge carried by spores is insufficient to influence their deposition on natural faces. Preliminary work for the present study revealed that conidia of barley powdery mildew are attracted to both negatively and positively polarized insulator probes (unpublished data). It is likely that the conidia were effectively polarized, with an opposite 'image charge' induced on the side facing the charged probe (Mizuno & Washizu, 1995; Halliday *et al*., 2002; Griffith, 2004). These opposing charges created an electrostatic force between the conidia and the probe.

When the voltage in the conductor was large enough, the electrical field generated caused the air to ionize and discharged the insulator (data not shown). In the present study, the apparatus worked only when the applied conductor voltage did not cause discharge (spark). Under the voltage conditions used here, the dielectrically polarized insulator plate did not detach immature conidia or affect the germination potential of the conidia collected. Evidently, the generative cells of the conidiophores produced viable progeny conidia even when exposed to an electrostatic force throughout their lifetime.

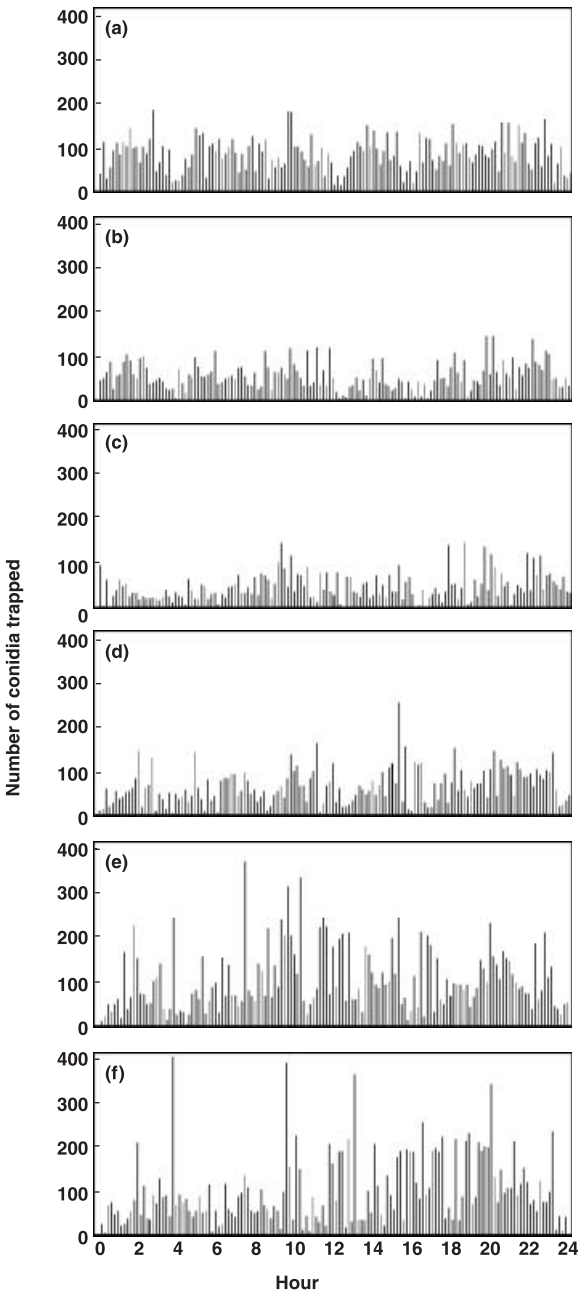

Figure 5 Number of mature conidia trapped in 10-min periods from a single colony of *Blumeria graminis* f.sp. *hordei* on six (a–f) barley leaves in 24 h, 14 days after inoculation, using an automatic spore collector.

Although abstricted conidia were easily separated from the insulator plate soon after depolarization, they became sticky from rapid secretion of substance(s) within 3 min on the polarized plate (data not shown). This secretion of substances by nongerminated powdery mildew conidia immediately after they attach to a leaf or artificial surface is typical behaviour (Carver *et al*., 1995, 1999; Nielsen *et al*., 2000; Wright *et al*., 2002), providing further evidence that the collection method did not affect the pre-infection behaviour of conidia on the host surface.

Hirata (1967) observed chemically fixed samples of powdery mildewed barley leaves collected at various stages after inoculation, and reported that a single colony produces up to 2×10^5 spores in its lifetime. In contrast, the present study successfully achieved consecutive monitoring of conidial production by individual living conidiophores on host leaves. Although direct comparison between the data is difficult, results from the present study (between 6×10^4 and $1{\cdot}6 \times 10^5$ conidia per colony) are lower than those shown by Hirata. It may be that some environmental factors are crucial in determining the production of conidia by the pathogen. In particular, the light intensity used in the present study was very low, and continuous weak light illumination without a dark period may result in lower sources of nutrients for the fungus because of limited photosynthesis.

This is the first known report of the direct determination of the duration of conidial secession, and a precise count of the total conidia that seceded from living individual colonies during their lifetime. This method is a useful tool for quantitative analysis of the conidiogenesis of powdery mildews in general, and experiments are planned to apply the present system to the examination of conidial production under natural conditions.

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References

Agrios GN, 1988. *Plant Pathology*. New York: Academic Press.

Aylor DE, 1990. The role of intermittent wind in the dispersal of fungal pathogens. *Annual Review of Phytopathology* **28**, 73–92.

Brown JKM, Hovmøller MS, 2002. Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease. *Science* **297**, 537–41.

Carver TLW, Thomas BJ, Ingerson-Morris SM, 1995. The surface of *Erysiphe graminis* and the production of extracellular material at the fungus–host interface during germling and colony development. *Canadian Journal of Botany* **73**, 272–87.

Carver TLW, Kunoh H, Thomas BJ, Nicholson RL, 1999. Release and visualization of the extracellular matrix of conidia of *Blumeria graminis*. *Mycological Research* **103**, 547–60.

Griffith WT, 2004. Electrostatic phenomena. In: Bruflodt D, Loehr BS, eds. *The Physics of Everyday Phenomena: A Conceptual Introduction to Physics*. New York, USA: McGraw-Hill, 232–52.

Halliday D, Resnick R, Walker J, 2002. Electric charge. In: Johnson S, Ford E, eds. *Fundamentals of Physics*. New York, USA: John Wiley & Sons, 505–19.

Hirata K, 1967. Notes on haustoria, hyphae and conidia of the powdery mildew fungus of barley, *Eryisphe graminis* f.sp. *hordei*. *Memoirs of the Faculty of Agriculture, Nigata University* **6**, 205–59.

Jarvis WR, Gubler WD, Grove GG, 2002. Epidemiology of powdery mildews in agricultural pathosystem. In: Bélanger

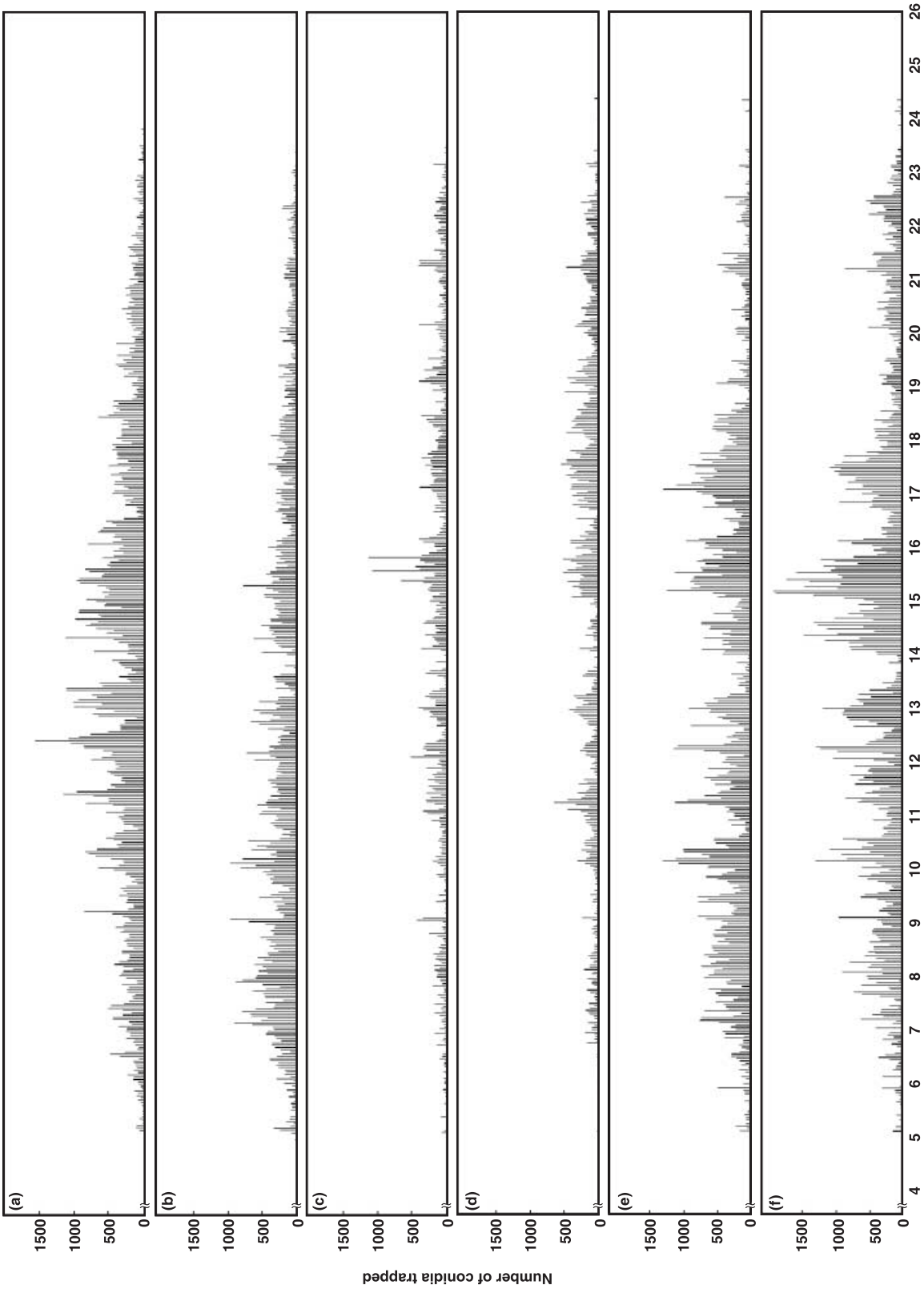

Figure 6 Number of mature conidia trapped in 1-h periods from a single colony of *Blumeria graminis* f.sp. *hordei* on six (a–f) barley leaves during the entire period of conidial secession using an automatic spore collector.

RR, Bushnell WR, Dik AJ, Carver TLW, eds. *The Powdery Mildews, A Comprehensive Treatise*. St Paul, MN, USA: APS Press, 169–99.

Leach CM, 1976. An electrostatic theory to explain violent spore liberation by *Drechslera turcica* and other fungi. *Mycologia* **68**, 63–86.

Matsuda Y, Sameshima T, Moriura N, Inoue K, Nonomura T, Kakutani K, Nishimura H, Kusakari S, Takamatsu S, Toyoda H, 2005. Identification of individual powdery mildew fungi infecting leaves and direct detection of gene expression by single conidium PCR. *Phytopathology* **95**, 1137–43.

McCartney HA, Bainbridge A, Legg BJ, 1982. Electric charge and the deposition of spores of barley mildew *Erysiphe graminis*. *Atmospheric Environment* **16**, 1133–43.

Mizuno A, Washizu M, 1995. Biomedical engineering. In: Chang JS, Kelley AJ, Crowley JM, eds. *Handbook of Electrostatic Processes*. New York, USA: Marcel Dekker, 653–86.

Nielsen KA, Nicholson RL, Carver TLW, Kunoh H, Oliver RP, 2000. First touch: an immediate response to surface recognition in conidia of *Blumeria graminis*. *Physiological and Molecular Plant Pathology* **56**, 63–70.

Oichi W, Matsuda Y, Sameshima T, Nonomura T, Kakutani K, Nishimura H, Kusakari S, Toyoda H, 2004. Consecutive monitoring for conidiogenesis by *Oidium neolycopersici* on tomato leaves with a high-fidelity digital microscope. *Journal of General Plant Pathology* **70**, 318–21.

Toyoda H, Matsuda Y, Shoji R, Ouchi S, 1987. A microinjection technique for conidia of *Erysiphe graminis* f.sp. *hordei*. *Phytopathology* **77**, 815–8.

Wright AJ, Thomas BJ, Kunoh H, Nicholson RL, Carver TLW, 2002. Influences of substrata and interface geometry on the release of extracellular material by *Blumeria graminis* conidia. *Physiological and Molecular Plant Pathology* **61**, 163–78.