Active Sporangium Discharge by *Peronospora destructor*

Charles M. Leach

Professor, Department of Botany and Plant Pathology, Oregon State University, Corvallis 97331.
Research was partially supported by Grant 59-2411-0-461-0 awarded by the Science and Education Administration of the United States Department of Agriculture. Published as Technical Paper 5953 of the Oregon Agricultural Experiment Station.
Accepted for publication 23 November 1981.

ABSTRACT

Leach, C. M. 1982. Active sporangium discharge by *Peronospora destructor*. Phytopathology 72:881-885.

Sporangium discharge by *Peronospora destructor* on infected onion leaves was photographed and visually observed using special illumination. The sporangia, whether liberated spontaneously or triggered by vibration (a single light tap) consistently were propelled along essentially parallel trajectories perpendicular to leaf surfaces irrespective of the orientation of the leaf surface to gravity. These results could not be explained on the basis of the generally accepted hygroscopic-twisting mechanism and an alternative electrostatic mechanism is proposed. An electrostatic mechanism was indicated by the occurrence of a highly organized pattern of sporangium discharge perpendicular to leaf surfaces, positively charged sporangia, and the determination that modifications of leaf voltages significantly influenced discharge velocity. Velocities of sporangia liberated from leaves at 200V(+) DC were 129% greater than for those discharged from leaves without supplemental voltage applied and velocities for sporangia liberated from leaves at 800V(+) were 80–82% greater than those for leaves at 200V. Active liberation of sporangia by *P. destructor* is similar to the active conidium release reported for *Drechslera turcica*.

Sporangia of several downy mildew pathogens are known to be forcibly discharged into the air (1,10,12). Nearly 100 years ago, Anton de Bary (1) observed that slight changes of humidity caused sporangiophores of *Peronospora* spp., as well as other fungi, to twist. He believed that this twisting caused the spores to be "thrown in every direction." Ingold (3) was not fully convinced that the twisting of sporangiophores of *Peronospora parasitica* effected spore discharge. Thirty years later, however, Ingold (4) reversed his earlier opinion and cited studies of Pinckard (10) on the tobacco downy mildew pathogen, *P. tabacina*, in support of a hygroscopic mechanism for spore discharge. Pinckard described active sporangium discharge by *P. tabacina* and other *Peronospora* species. Although he observed the twisting of sporangiophores in response to humidity changes, he did not state unequivocally that this motion caused spores to be thrown from the sporangiophores.

During studies on the relationship of environmental factors to spore discharge by the downy mildew pathogen of onions, *P. destructor* Berk. (C. M. Leach, P. D. Hildebrand, and J. C. Sutton, *unpublished*), we found that this fungus behaved remarkably similar to the imperfect fungus *Drechslera turcica* (6). Sporangia of *P. destructor* were mainly discharged in response to a lowering of the humidity from saturation, though some were also liberated when the humidity was returned to saturation. Likewise, exposure of these same two fungi to red-infrared radiation had a profound effect in both triggering and enhancing spore discharge. Leaf vibration also affected spore discharge of *P. destructor* (Fig. 1B) and *D. turcica* (7) and for both fungi this was dependent on humidity levels and the presence or absence of red-infrared radiation. The behavioral similarity of these two fungi suggests a common mechanism for active spore discharge. Since an electrostatic mechanism has been proposed to explain active discharge of conidia by *D. turcica* (8), a similar mechanism was investigated for *P. destructor*. To accomplish this objective, flights of discharged spores were followed photographically under various conditions, including the artificial manipulation of onion leaf voltages.

MATERIALS AND METHODS

Sporulating plants. Onion plants (*Allium cepa* L.) were infected systemically by inoculating "sets" with a sporangial suspension of *P. destructor* following a procedure described by Hildebrand and Sutton (2). The onions were grown in silica sand in 10-cm-diameter clay pots and were watered periodically with Hoagland's solution. Moisture in the sand was kept relatively uniform by means of cotton wicks that extended from within the pots to a water supply below. Plants were illuminated 14 hr/day under fluorescent lamps (four 40 W cool-white and four 40 W warm-white; 112 W/m^2) with a 19.5 C day and 17.5 C night regime.

A modification of a procedure recommended by P. D. Hildebrand (*personal communication*, 1980) was used to induce profuse sporulation. When the plants were 2–4 wk old, pieces of leaves 10–12 cm long were removed during the light portion of the cycle and gently attached to a plastic holder (27 × 110 mm). These were placed in moist chambers at 100% RH (measured with a HM111 Humidity Indicator; Weathermeasure Corp., Sacramento, CA 95841). The moist chambers (40 × 180 mm Pyrex glass tubes containing moistened filter paper) were sealed at both ends with rubber stoppers and then returned to a growth chamber to complete the 14-hr light period, followed by 10 hr dark, at the end of which sporulation was usually profuse. Subsequent transferring of specimens to the Tyndall apparatus for study (8) was done in a dimly lit room with specimens shaded with a lightproof cloth.

Photographing sporangium discharge. Single pieces of sporulating leaves approximately 2 cm long were removed gently with electrically insulated scissors and forceps and placed immediately into the Tyndall illumination apparatus (8) where discharge was observed visually or photographically. The apparatus used in earlier studies (8) was slightly modified by incorporating a fiber-optics illuminator as a light source ("Fiber-lite," manufactured by Dolan-Jenner Industries, Inc., Woburn, MA 01801) in place of the original microscope lamp. Light intensity was 1,800 W/m^2 when the specimen was placed in the Tyndall illumination apparatus, then increased to 8,200 W/m^2 during observation, or photography. For convenience, sporangium discharge was photographed with the focal plane horizontal to gravity (Figs. 1–5), however, active spore release was always perpendicular to the leaf surface irrespective of leaf orientation.

Three different durations of exposure were used to photograph actively discharged sporangia. To photograph trajectories of sporangia as they were initially liberated, exposure time was controlled manually (~0.5 to 1 sec). The shutter was first opened (cable release), and then almost immediately the specimen holder was given a single light tap with a light metal rod to initiate a pulse of synchronized spore discharge. The shutter was closed immediately after this tap. Figs. 1–3, except Fig. 1A, were

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

0031-949X/82/07088105/$03.00/0

photographed in this manner. To photograph the trajectories of spontaneously discharged sporangia (ie, no tap involved) numerous 1-sec exposures were made randomly while sporangia were being spontaneously shot into the air (Fig. 1A). To determine discharge velocities, spore release was synchronized by a single light tap of the specimen holder (8) and immediately tripping the camera shutter while set for a 0.125-sec exposure. Using this procedure, the distance traveled by sporangia during the 0.125-sec interval could be determined. Average velocities were determined from 50 measurements of in-focus trajectories. Velocities of spontaneously released sporangia were averaged from 15–25 measurements because of the inherent difficulty of photographing randomly released sporangia. Accuracy of the camera shutter at 0.125 sec was determined by means of a storage oscilloscope (8).

In the complete study, 600 photographs were obtained. Figs. 1–5 are examples of these photographs. In a typical experiment, the specimen was placed on the specimen holder, illuminated; and as sporangia began to discharge spontaneously, a rapid sequence of 10–15 exposures was made using the camera's motor drive. Then using the same specimen, the remainder of the 36-exposure film was used for a sequence of 10–15 exposures of synchronized sporangium liberation using a single tap per frame. This multi-exposure procedure was used both to photograph trajectories and to determine velocities. It also was used in velocity determinations when leaf potentials were artifically manipulated.

In these latter experiments, the specimen was adjusted to a certain voltage; eg, 200V(+), using a high voltage regulator, then a sequence of vibrationally released sporangia was photographed. After 10–15 exposures at a single voltage, the voltage was increased (eg, to 800V) using the same specimen and another 10–15 exposures taken. In this manner, various combinations and orders of leaf voltages were studied (Fig. 5).

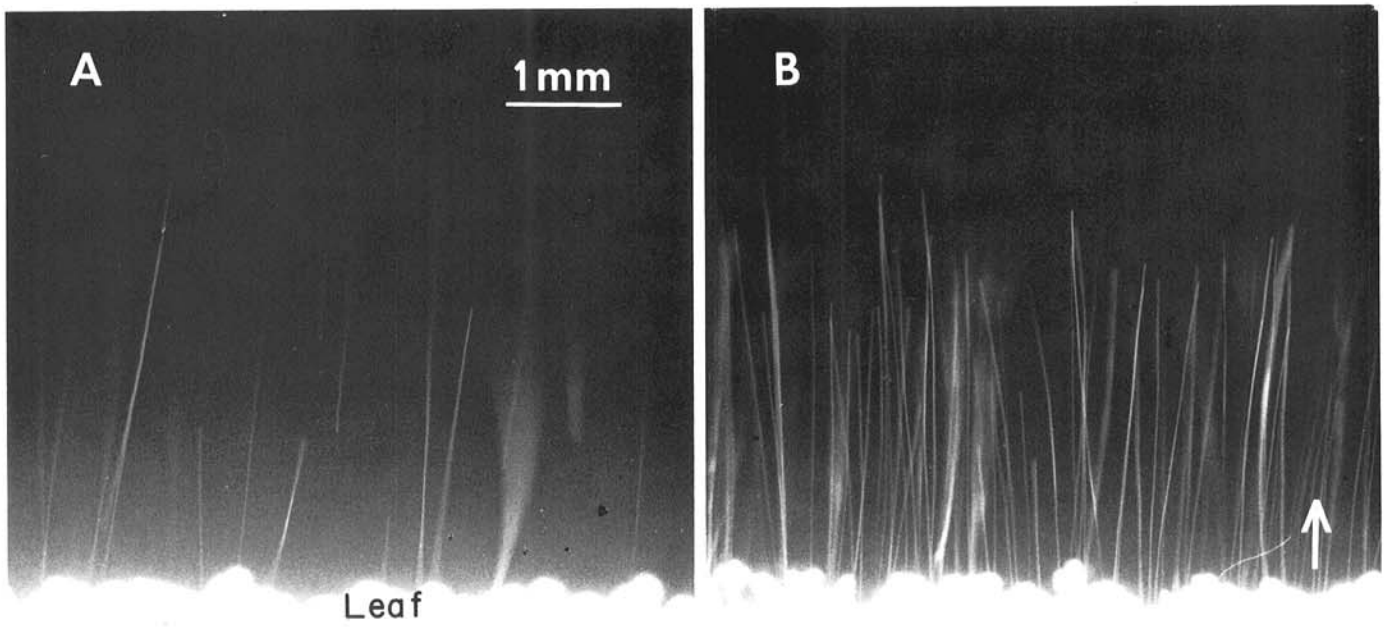

Fig. 1. Trajectories of actively discharged sporangia of *Peronospora destructor*. **A,** Sporangia liberated spontaneously. **B,** Same specimen a few seconds later in which discharge was synchronized by vibration (a single, light tap). Arrow indicates direction of flight; time exposures are approximately 0.5 sec.

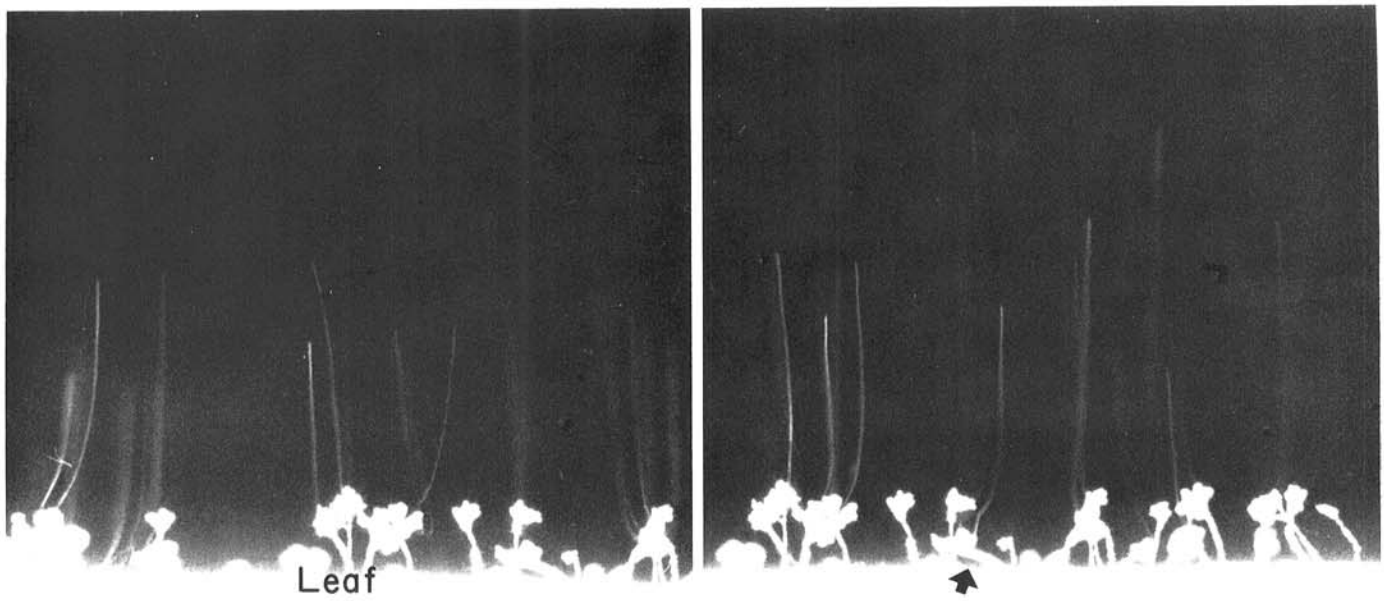

Fig. 2. Trajectories of actively discharged sporangia of *Peronospora destructor* showing their takeoff from individual sporangiophores. Two successive photographs are shown that include many of the same sporophores; the trajectory marked by an arrow is discussed in the text.

Camera, lenses, and film. Descriptions of camera, lenses, films, etc. are covered in an earlier article (8) and therefore, only summarized here. A single-lens reflex camera (Olympus OM-1, Olympus Optical Co., Ltd., Shibuya-ku, Tokyo, Japan) with lens mounted on an adjustable bellows was part of the Tyndall apparatus (8). Figs. 1–3 were photographed with a 50 mm, 3.5 lens at f 5.6 (Olympus Zuiko "Auto macro" lens) with manual time exposures of approximately 0.5–1.0 sec. Figs. 4 and 5 were photographed with a 50 mm, 1.8 lens (Olympus Zuiko "Auto-S") at f 8.0 and 0.125 sec. Kodak Tri-X Pan film, (ASA 400) was used almost exclusively. A high-speed color film (Kodak Kodacolor II, ASA 400) was used in some experiments and Fig. 2 is an enlargement from one of these negatives. The Tri-X film was developed for 5 min at 21 C in Acufine developer. (Acufine, Inc., Chicago, IL 60611).

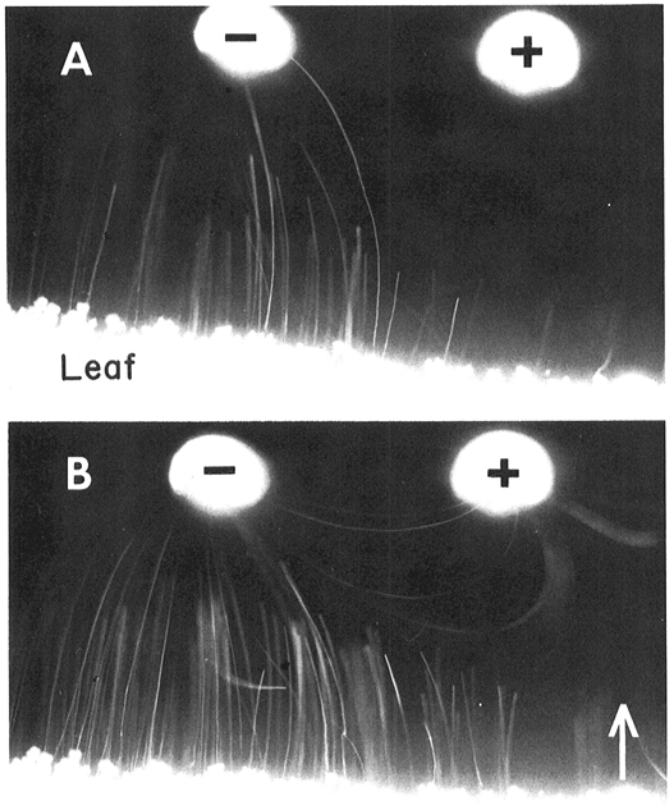

Fig. 3. Vibrational liberation of *Peronospora destructor* sporangia (two examples) near charged needle electrodes. The two electrodes were 5.5 mm apart and at 400 V DC; exposure was approximately 0.5 sec.

Electrical details. In most experiments, the sporulating leaf piece was attached by means of a small amount of "Vaseline" petroleum jelly to an electrically nonconductive holder. In experiments in which leaf potentials were manipulated artificially to determine their effect on sporangium discharge, a brass specimen holder was used with the sporulating leaf piece attached by means of a small amount of uncured silver epoxy cement ("Epo tek H31D," manufactured by Epoxy Technology, Inc., Billerica, MA 01821). This uncured cement is pastelike, sticky, and a good electrical conductor that hardens only at fairly high temperatures. The brass specimen holder was connected unipolarly to a high-voltage DC supply (Model 245 High Voltage Supply, manufactured by Keithley Instruments, Cleveland, OH 44139) having a maximum amperage of 10 ma.

To determine whether sporangia were charged when liberated, two needle electrodes at 400 V DC were positioned near the specimen within the Tyndall illumination apparatus (8). The tips of parallel electrodes 5.5 mm apart can be seen in Fig. 3.

RESULTS

Spontaneous liberation. Whenever sporulating onion leaves were transferred from darkness (moist chamber at RH 100%) to the Tyndall apparatus (RH 40–60%, uncontrolled) and exposed to light, at first no active sporangium discharge was evident. However, within 1–2 min sporangia were observed being actively discharged into the air in considerable numbers in trajectories approximately perpendicular to the leaf surface (Fig. 1A). The distance that sporangia were propelled actively varied from specimen to specimen and ranged from approximately a few millimeters to about 10 mm, after which they floated away randomly on air currents. The velocity of spontaneously discharged sporangia (Fig. 4A) in two specimens averaged 11.6 and 16.6 mm/sec, respectively.

Even slight air movements caused spore trajectories to deviate from vertical (Figs. 4 and 5B). To reduce these effects, experiments were conducted in a room having little air movement; in addition, a cloth draft screen was fastened around the specimen platform of the apparatus.

Vibrational release. Earlier studies of *P. destructor* (C.M. Leach, P. D. Hildebrand, and J. C. Sutton, *unpublished*) showed that under suitable conditions of humidity and exposure to red-infrared

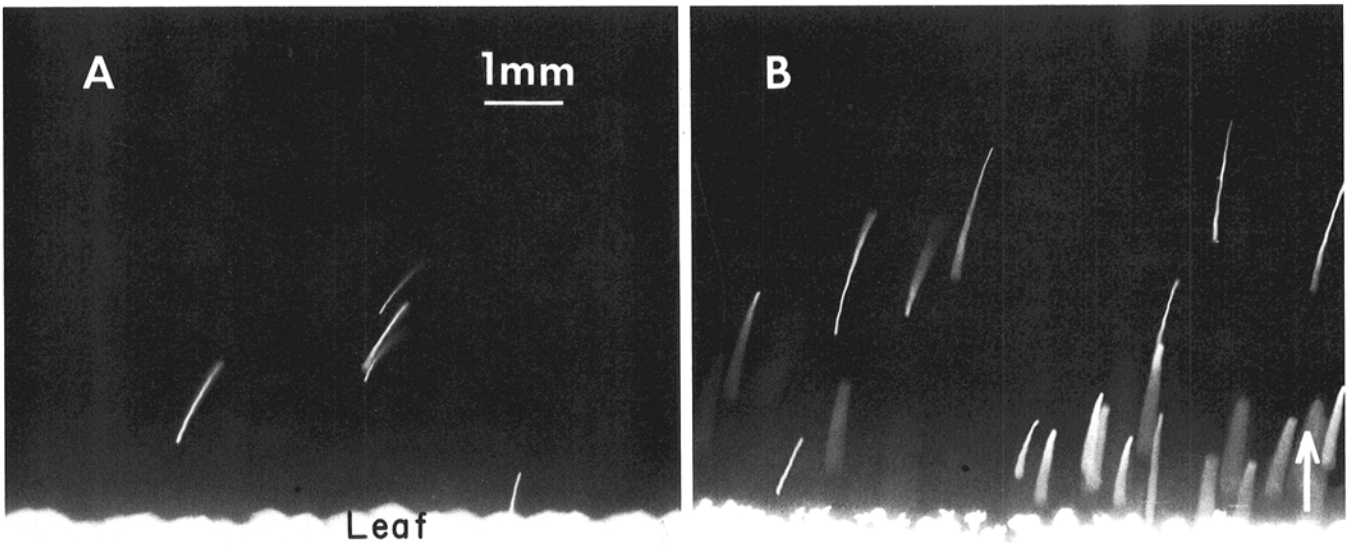

Fig. 4. Flights of *Peronospora destructor* sporangia discharged spontaneously **A,** compared to those liberated in response to vibration **B,** using the same specimen. Velocities were calculated from the distances traveled during the 0.125-sec photographic exposures.

light, slight vibration of sporulating onion leaves resulted in significant sporangial discharge. Photography of this phenomenon by using Tyndall illumination revealed that a single gentle tap consistently caused the synchronized release of large numbers of sporangia (Fig. 1). The trajectories of vibrationally discharged sporangia were always perpendicular to the leaf surface (Fig. 1B) and essentially identical to those of sporangia discharged spontaneously (Fig. 1A). Furthermore, the velocities of vibrationally released sporangia were not significantly different from those discharged spontaneously (Fig. 4).

Charge on sporangia. The parallel trajectories of both spontaneously and vibrationally discharged spores can be logically explained on the basis of an electrostatic mechanism similar to that proposed for *D. turcica* (8). This postulated mechanism involves the repulsion of unipolarly charged sporangia from a charged surface of the same polarity. To determine whether spores were charged when liberated, a pair of oppositely charged needle electrodes were placed several centimeters from sporulating onion leaves (Fig. 3). In three experiments, both spontaneously and vibrationally discharged sporangia were attracted to the negatively charged electrode; ie, the sporangia were positively charged. Active discharge of sporangia adjacent to the positively charged electrode (Fig. 3A) apparently was inhibited.

Effect of manipulating leaf voltages. If active sporangium discharge of *P. destructor* involves an electrostatic mechanism, any modification of leaf voltages should influence the velocity at which sporangia are discharged. To test this hypothesis, leaves were connected to a high voltage DC supply and velocities of vibrationally liberated sporangia were determined for different positive voltages. Voltage combinations (each combination on a single leaf) used were: unregulated leaf, followed by 200V(+), followed by 800V(+); 200V(+), followed by 800V(+), followed by 200V(+); 800V(+), followed by 200V(+), followed by unregulated; and unregulated leaf, followed by 800V(+), followed by unregulated. Voltages were selected on the basis of previous studies on *D. turcica* (8) and on bean leaves (9). A similar, but less extensive, study was conducted with negatively charged leaves.

Velocity of sporangium discharge, whether spontaneous or

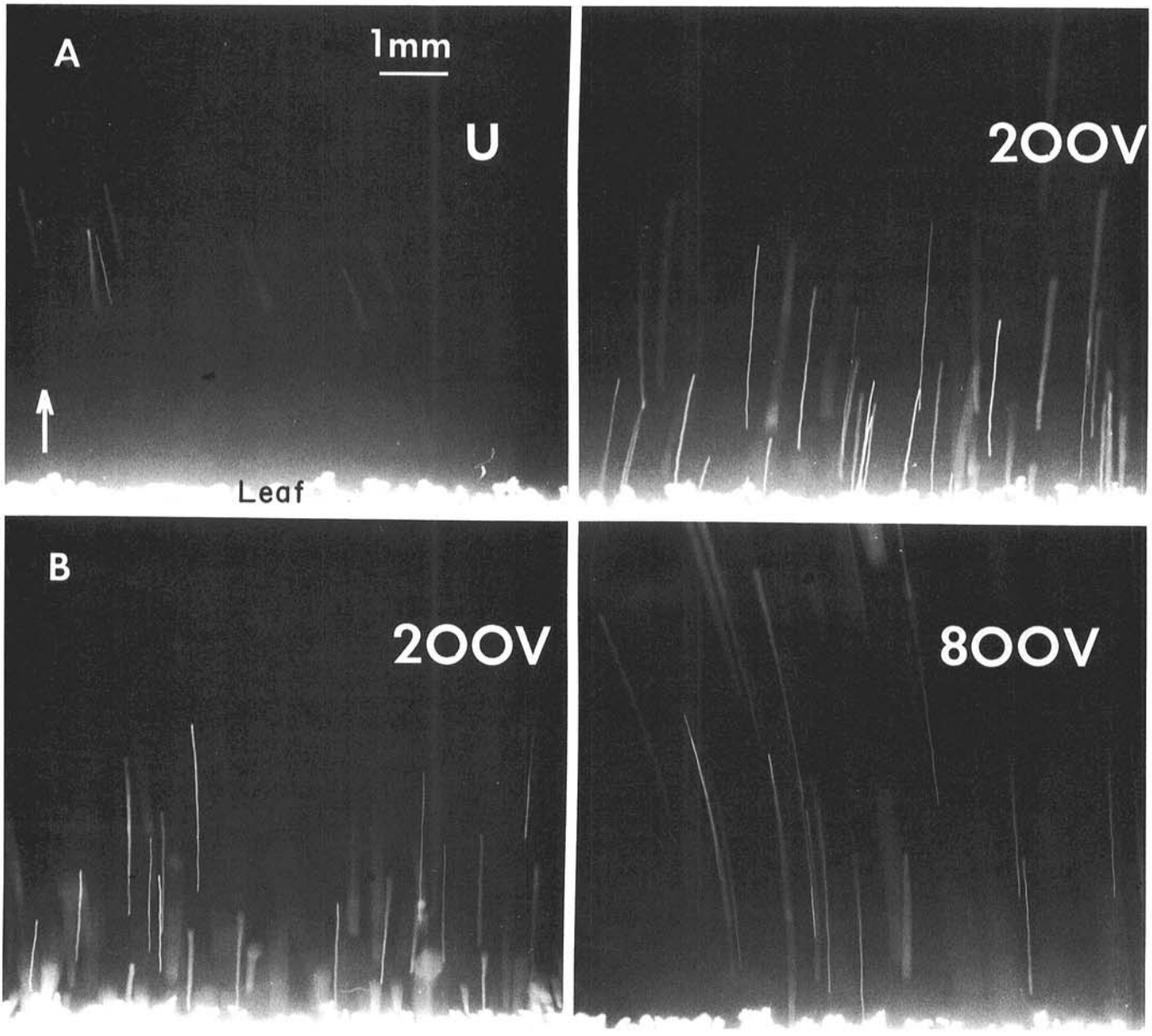

Fig. 5. The influence of leaf voltages on velocity of sporangium discharge from sporangiophores of *Peronospora destructor*. **A,** Flights of sporangia discharged from an unregulated leaf whose voltage was unknown (U) compared to the same leaf a few seconds later regulated at 200V(+)DC. **B,** Flights of sporangia from another leaf initially regulated at 200 V and then increased to 800 V. Discharge was vibrationally synchronized for A and B; exposure was a standard 0.125 sec; velocities were calculated from distances sporangia traveled in 0.125 sec.

triggered by vibrations, consistently related directly to the magnitude of leaf potentials. The greater the voltage, the greater the release velocities. Figure 5 is representative of these experiments. In Fig. 5A, it is apparent that the 0.125-sec spore flights are shorter for the unregulated leaf in which the voltage was unknown (left leaf) than for spores from the same leaf a few seconds later when the potential was increased to 200V(+)DC (right photograph). In Fig. 5B (another leaf) in which the effect of 200V on discharge velocity is compared to 800V, it is evident that the increase of leaf voltage was accompanied by a marked increase in discharge velocity. In two experiments in which leaf potentials were raised from 200V to 800V, the velocity (average of 50 measurements per voltage level) increased from 12.5 to 22.7 mm/sec (an 82% increase) and from 13.4 to 24 mm/sec (an 80% increase), respectively. In another experiment in which an unregulated leaf of unknown potential was adjusted to 200V, velocity of discharge increased from 7.9 to 18.2 mm/sec (a 129% increase). Respective standard deviations for the preceding velocities were 12.5 ± 2.6 mm/sec, 22.7 ± 6.4 mm/sec, 13.4 ± 4.3 mm/sec, 24 ± 2.5 mm/sec, 7.9 ± 0.9 mm/sec, and 18.2 ± 3.0 mm/sec.

In other experiments to determine if discharge velocities of spontaneously released sporangia differed from those vibrationally released when the same leaf was stabilized at the same voltage (50 measurements per treatment), it was found that velocities were not significantly different. This held true for unregulated leaves as well as for leaves held at 200V(+) and 800V(+).

From these experiments, it was concluded that velocity of sporangium discharge is directly dependent on magnitude of leaf voltages (the greater the voltage the greater the velocity of discharge), and that when leaves are kept at the same potential, the velocity of discharge for spontaneously and vibrationally liberated sporangia is essentially the same.

DISCUSSION

DeBary (1) visually observed active discharge of sporangia by species of *Peronospora* and concluded that spores were thrown into the air as sporangiophores twisted in response to humidity changes. Ingold's illustration (4) suggests that sporangia are propelled along random trajectories, as would be expected if deBary's mechanism is valid. I also have observed the twisting and twirling of sporangiophores in response to humidity changes, but remain skeptical that this mechanical action is sufficient to propel sporangia into the air. Photographs of active discharge, whether spontaneous or triggered by vibrations, only show highly organized, parallel trajectories perpendicular to the leaf surface. These results do not support the hygroscopic mechanism proposed by deBary. The vertical propulsion of sporangia from onion leaves, coupled with the facts that the sporangia are charged and their velocities are influenced by size of leaf potentials, suggests the operation of an electrostatic mechanism similar to that proposed for *D. turcica* (5,8).

The examples of sporangium discharge shown in Fig. 2 can be rationally explained on the basis of an electrostatic mechanism in which leaf surface and sporangiophores are unipolarly charged. In these photographs, the flights of some sporangia immediately after takeoff are slightly angled from the vertical (in respect to the leaf surface) but then they change direction to the vertical. An interpretation of this behavior is that the sporangia first respond to the influence of a perturbed electrostatic field associated with the sporangiophore, then as they move further away they come under influence of the more uniform field associated with the leaf surface. Specific evidence of a perturbed field effect associated with a sporangiophore is marked by an arrow in Fig. 2. Here the flight of a single sporangium discharged from a sporangiophore laying on its side was initially almost vertical but then it was soon deflected by the local field associated with an overhanging sporangiophore. Once past the sporangiophore, the flight of the sporangium reverted to the vertical as it came under the influence of the leaf surface field. If active propulsion of sporangia of *P. destructor* involves an electrostatic mechanism, then it is axiomatic that onion leaves must become charged. The design of my electrostatic field mill (an instrument used to measure field strengths above a charged surface) was unsuitable for measuring onion leaves because of their narrowness. In other studies (9), however, I measured fields of 200–777 V cm^{-1} at 27 mm above the surface of bean leaves. On the basis of the sporangium discharge velocity of *P. destructor* in which unregulated onion leaves were compared to those artificially adjusted up to 800 V, it can be estimated that leaf potentials on unregulated leaves were about 100 V. Photographically, the flights of sporangia of *P. destructor* liberated spontaneously were identical to those released in response to vibrations (Fig. 1). It is likely that the effect of the vibrational force is to mechanically weaken or break the anatomical bond between the sporangium and sporangiophore, thereby allowing electrostatic repulsive forces to come into play. In other studies (C. M. Leach, P. B. Hildebrand, and J. C. Sutton, *unpublished*), it was demonstrated that sporangium liberation in response to vibration is highly dependent on the level of atmospheric moisture and the presence or absence of red-infrared radiation. Although both these factors can influence leaf surface charges (9) it is also possible that they affect the strength of the anatomical bond between sporangium and sporangiophore. On rare occasions, while visually following active discharge of sporangia, I observed sporangia to merely fall from sporangiophores in response to vibration. This suggests that there may be occasions when vibrational discharge of sporangia may lack an active mechanism because leaves are insufficiently charged.

The active discharge of sporangia of *P. destructor* is similar to the active liberation of conidia by *D. turcica* (6–8), and both fungi respond almost identically to changes of humidity and to exposure to red-infrared radiation. Evidence was published describing an electrostatic mechanism for *D. turcica* (5,8); evidence appears to be equally strong for an electrostatic mechanism for *P. destructor*. This mechanism does not appear to relate to any physiological process within the fungus, but rather to surface electrostatics (fungus and leaf) governed by atmospheric-surface water relations. Although the origin of these surface charges still has to be explained, it may be similar to the electrical charging of fabrics that occurs at different humidities (11) or it might involve bound water electrets.

LITERATURE CITED

1. DeBary, A. 1887. Comparative Morphology and Biology of the Fungi, Mycetozoa and Bacteria (Translation by H. E. F. Garnsey and I. F. Balfour). Clarendon Press, Oxford, England. 525 pp.
2. Hildebrand, P. D. and Sutton, J. C. 1980. Maintenance of *Peronospora destructor* in onion sets. Canadian J. Plant Pathology 2:239-240.
3. Ingold, C. T. 1939. Spore Discharge in Land Plants. Clarendon Press, Oxford, England. 178 pp.
4. Ingold, C. T. 1971. Fungus Spores—Their Liberation and Dispersal. Clarendon Press, Oxford, England. 302 pp.
5. Leach, C. M. 1976. An electrostatic theory to explain violent spore liberation by *Drechslera turcica* and other fungi. Mycologia 68:63-86.
6. Leach, C. M. 1980. Influence of humidity and red-infrared radiation on violent spore release by *Drechslera turcica*—Additional evidence. Phytopathology 70:192-196.
7. Leach, C. M. 1980. Vibrational releases of conidia by *Drechslera maydis* and *D. turcica* related to humidity and red-infrared radiation. Phytopathology 70:196-200.
8. Leach, C. M. 1980. Evidence for an electrostatic mechanism in spore discharge by *Drechslera turcica*. Phytopathology 70:206-213.
9. Leach, C. M. 1981. Spore release studies: Influence of humidity and red-infrared radiation on leaf surface charges. (Abstr.) Phytopathology 71:235.
10. Pinckard, J. A. 1942. The mechanism of spore dispersal in *Peronospora tabacina* and certain other downy mildew fungi. Phytopathology 32:505-511.
11. Sereda, P. J., and Feldman, R. F. 1964. Electrostatic charging of fabrics at various humidities. J. Textile Inst., Trans. 55:288-298.
12. Weston, W. J. 1923. Production and dispersal of conidia in the Philippine Sclerospora of maize. J. Agric. Res. 23:239-278.