Influence of Humidity and Red-Infrared Radiation on Spore Discharge by *Drechslera turcica*—Additional Evidence

C. M. Leach

Professor, Department of Botany and Plant Pathology, Oregon State University, Corvallis 97331.
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ABSTRACT

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The influence of atmospheric humidity and red-infrared radiation on release of conidia from maize leaf lesions was studied in a series of experiments using a special "spore release apparatus." Temperature and air velocity were kept constant in all experiments. A low air velocity (0.5 m/sec) was selected to minimize the possibility of "wind" removal. Irradiation of sporulating maize leaf lesions with red-infrared radiation (IR) greatly increased spore release at lowered relative humidities (RH), but not when the air was saturated. Numbers of spores liberated from irradiated specimens always greatly exceeded those released in darkness under the same conditions. Even short exposures (1 to 2 min) of IR at constant low relative humidity caused massive release of conidia. A water filter (20 cm deep), used to remove heat, had little effect on spore release. When visible wavelengths were eliminated by filters, the IR alone (> 800 nm) triggered spore release. Experiments confirmed earlier reports that spore release may be initiated by either lowering or raising the atmospheric humidity. When the humidity was lowered and raised in a cyclical manner, a characteristic bimodal pattern of spore liberation occurred. The first major peak of spore release always coincided with humidity reduction and a second peak resulted from the raising of the humidity. The number of spores released by these humidity changes was higher when the changes occurred at fairly low humidities and was less near saturation. Though spore release triggered by humidity changes in darkness was less than in specimens exposed to red-infrared radiation, when the humidity was repeatedly cycled (ie, lowered and raised) in darkness, the number of spores released progressively increased for each successive cycle.

Release of conidia from maize leaves infected with *Drechslera turcica* (Pass.) Subram. and Jain (*Helminthosporium turcicum* Pass.) may result from strong winds, rain showers, violent discharge related to changes in atmospheric humidity (5), and exposure to red-infrared radiation (2). Violent discharge of conidia was first reported by Meredith (6) and later confirmed by Leach (2). Two theories have been postulated to explain the mechanism of violent release: one involves mechanical stresses induced in conidium and conidiophore by evaporation of cellular water (6), the other involves electrostatic forces (3).

The objective of the research described in this article was to further clarify the relationship of atmospheric moisture and red-infrared radiation (IR) to spore liberation by *D. turcica* under precisely controlled conditions.

MATERIALS AND METHODS

Preparation of specimens. Northern leaf blight lesions on naturally infected maize (*Zea mays* L.) were collected, dried in a plant press, and then stored at −20 C until needed. Single leaf lesions from the stored material were trimmed to ~100 × 20 mm and attached with rubber bands to a plastic holder on a piece of filter paper (Whatman no. 3). Specimens were soaked for 10 min in distilled water, shaken to remove excess water, and placed in clear plastic boxes (32 × 150 × 270 mm). The boxes were then placed in an incubator and subjected to a cycle of alternating temperatures (25 C, 12 hr; 20 C, 12 hr) and near-ultraviolet light (NUV, 12 hr; dark, 12 hr). The NUV source was a single 20-W BLB fluorescent "Black Light" lamp, placed 12–13 cm above the specimen (intensity 160 $\mu W/cm^2$). Length of incubation required for profuse sporulation varied from 4 to 7 days.

Spore release apparatus. A specially designed spore release apparatus (4) was used in all experiments. With it, the effects of humidity changes and exposure to red-infrared radiation on spore release could be determined precisely while keeping air temperature and air flow constant. In each experiment specimens were placed in the cylindrical Pyrex glass specimen chamber in which the air flow over the specimen was adjusted to 0.5 m/sec and temperatures were kept constant (refer to Figs. 1–3 for temperatures). Air velocity purposely was kept low to minimize the possibility of "wind" removal of spores. Air temperature (± 0.25 C) was monitored continuously with thermocouples in four locations. Recordings from only two of these locations are included in Figs. 1–3. Relative humidity (RH) was controlled precisely by combining saturated and dry air streams and could be lowered from saturation to 20%, or similarly raised, within 2 min (4). Because spores were effectively released during these rapid humidity changes, no attempt was made to simulate the slower changes that occur in nature. The relative humidity was monitored with thermocouple psychrometers (4) for air entering and leaving the specimen chamber so that possible effects of IR on humidity could be observed. Though IR radiation did affect the relative humidity, only the relative humidity records for the air entering the specimen chamber are included with the results (Figs. 1–3). Both RH and temperature were recorded continuously with a multichannel recorder ("Multi-point," PM 8325, Philips, The Netherlands) at a chart speed of 30 cm/hr.

Release of spores was monitored continuously with a specially designed precision spore trap capable of detecting the numbers of spores released per 1.1 min (4). Spores impinged onto Vaseline-coated slides moving past a slit orifice (1 × 32 mm) at 1 mm/1.1 min. The numbers of spores deposited for the complete slide were determined microscopically by systematically counting across the slides in 1-mm intervals using an eyepiece reticule for accuracy. At high deposition rates (> 500 spores/1.1 min) it was impractical to count every spore, and random counts were taken across the slide from which totals were calculated.

Red-infrared radiation. A 250-W unfiltered infrared lamp (Sylvania, USA) was placed in a special lamp housing 43.2 cm below the specimen (4). The unfiltered intensity at the specimen was 3,695 $\mu W/cm^2$; with a water filter, 20 cm deep, the intensity was 264 $\mu W/cm^2$, and with a Kodak 87C infrared filter, 1,960 $\mu W/cm^2$. No

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attempt was made at this time to use constant intensities for the filtered and unfiltered IR. The unfiltered Sylvania IR lamp emits strongly in the red-infrared spectrum, the water filter absorbed much of the IR and transmitted wavelengths < 950nm and a very small amount of radiation between 1,000 and 1,125 nm; the Kodak 87C infrared filter absorbed all visible radiation and transmitted only radiation > 800 nm. Intensity of radiation in the same plane as the specimen, was measured with a compensated thermopile (Model E20, Kipp und Zonen, The Netherlands) coupled with a sensitive galvanometer (Model A70, Kipp und Zonen).

EXPERIMENTS AND RESULTS

Experiments were conducted on sporulating leaf lesions to determine the interrelationships of atmospheric humidity and IR radiation to spore release. The results of seven selected experiments are presented (Figs. 1–3).

Spore release in darkness. Conidia consistently were liberated in darkness in response to either lowering or raising the humidity, but never under constant saturation (Figs. 1–3). The few spores trapped during periods of saturation should be considered "background" contamination resulting from the random release of loose spores adhering to tubing of the spore release apparatus. Liberation of spores increased almost instantaneously when the RH was lowered. The sharp peak of spore liberation triggered by lowering of the RH was usually followed by a rapid decline in numbers of spores released.

Raising the relative humidity up to and including saturation, also triggered spore liberation and was evident in most experiments conducted in darkness (Figs. 1–3). Raising the relative humidity caused almost instantaneous release of spores followed by a rapid decline as the air became saturated.

Repeated humidity cycles in darkness. Repeated lowering and raising of the relative humidity in darkness consistently resulted in a characteristic bimodal pattern of spore release (Figs. 1–3). The spore release pattern accompanying the first humidity cycle (Fig. 3A) shows the typical bimodal pattern seen in many experiments. A succession of repeated humidity cycles in darkness consistently resulted in repeated patterns of spore release, indicating that all available spores were not liberated at one time. This is evident in the first three humidity cycles of Fig. 1A, and the first four cycles of Fig. 3A. In experiments involving repeated humidity cycles in darkness (Figs. 1A and 2A) there was a gradual increase in total number of spores liberated with each successive cycle. This reponse was consistent in numerous experiments.

Humidity level and spore release in darkness. The number of spores released on either raising or lowering the humidity correlated with the size of the humidity change; ie, lowering the relative humidity from 100 to 85% triggered far less spore discharge than decreasing it from 100 to 45%. To determine this relationship more precisely, in one experiment the RH was lowered and raised in darkness in discrete steps (Fig. 2A). Negligible release of spores occurred during a first humidity cycle in which the RH was lowered to a constant 54% and then returned to saturation. During the second cycle of stepwise reductions, spores were not liberated in appreciable numbers until the final lowering from 54 to 27%, ie,

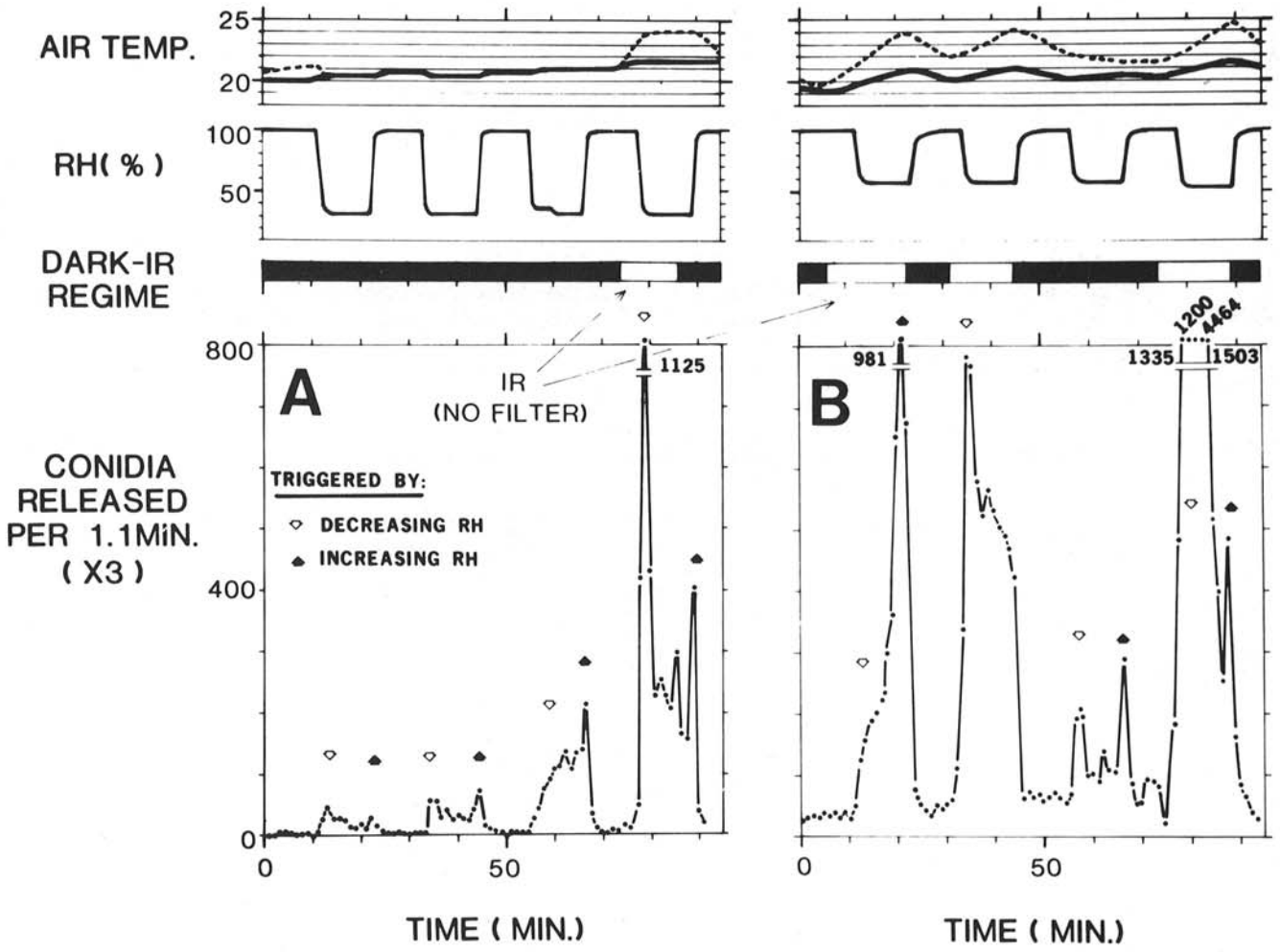

Fig. 1. A comparison of spore release by *Drechslera turcicum* induced by raising and lowering the relative humidity (RH) in darkness versus exposure of specimens to unfiltered red-infrared (IR) radiation. **A)** Liberation of conidia in response to three cycles of lowering and raising humidity in darkness followed by a final cycle in which the specimen was exposed to IR. **B)** Specimen was exposed to IR during the first two humidity cycles, left in darkness during the third humidity cycle, and finally exposed to IR during the fourth cycle. Temperatures are for air entering the specimen chamber (solid line) and leaving the chamber (dotted line). Air velocity at the specimen was constant at 0.5 m/sec.

only decreasing the humidity in the lower range caused significant spore liberation. Likewise, when the relative humidity was returned to saturation in a stepwise manner in darkness (Fig. 2A), the first increase from 27 to 78% caused liberation of conidia, but the increase from 78% to saturation had no effect.

Effect of red-infrared radiation. Exposure of specimens to IR, particularly in the lower RH ranges (Figs. 2B and C), greatly enhanced spore release. Liberation of conidia from irradiated specimens far exceeded that in darkness. The effects of IR are shown in Fig. 1. In the first experiment (Fig. 1A), the first three humidity cycles in darkness resulted in a gradual increase in numbers of conidia discharged for each successive cycle even

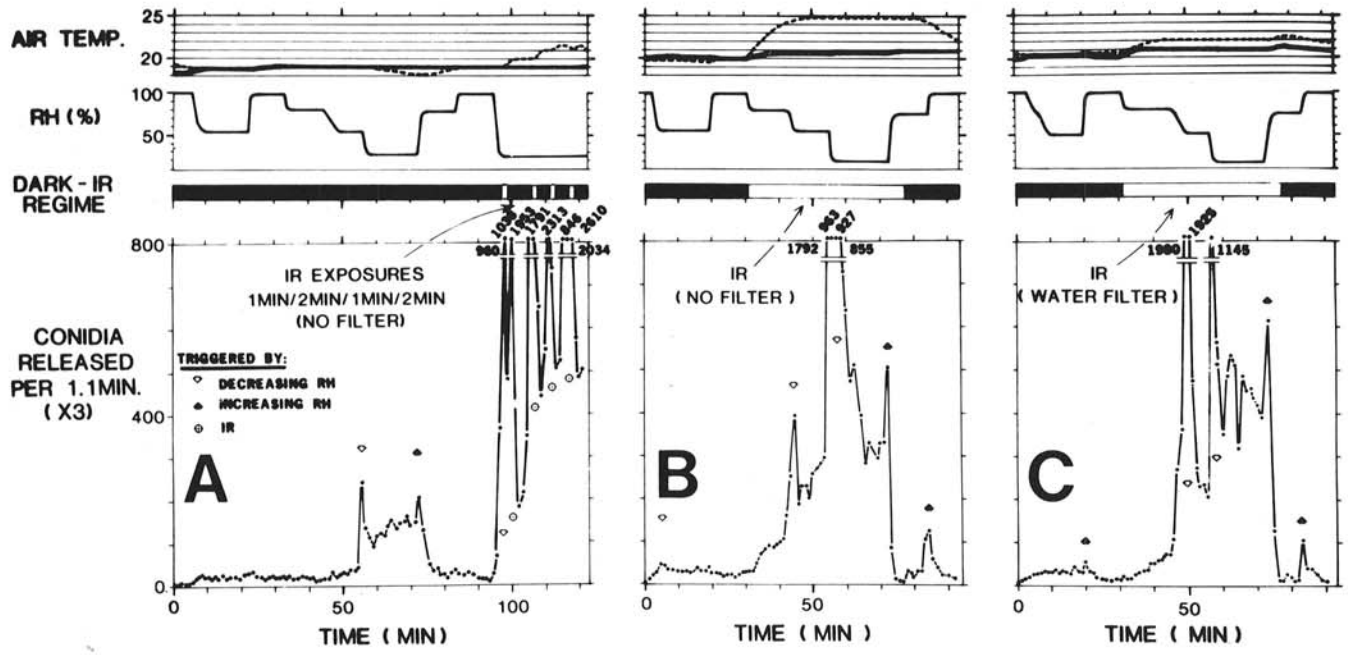

Fig. 2. The influence of relative humidity (RH) on spore release by *Drechslera turcicum* from specimens in darkness versus specimens exposed to red-infrared radiation (IR). **A)** Effect of stepwise reduction of humidity, followed by the stepwise increase in darkness, followed by short exposures to IR at low RH. **B)** Effect of similiar stepwise reduction and increase of humidity while specimen was exposed to unfiltered IR. **C)** Effect of similiar stepwise reduction and increase of humidity while specimen was exposed to IR transmitted through a water filter (20 cm deep) to reduce heating effects. Temperatures and air velocity same as in Fig. 1.

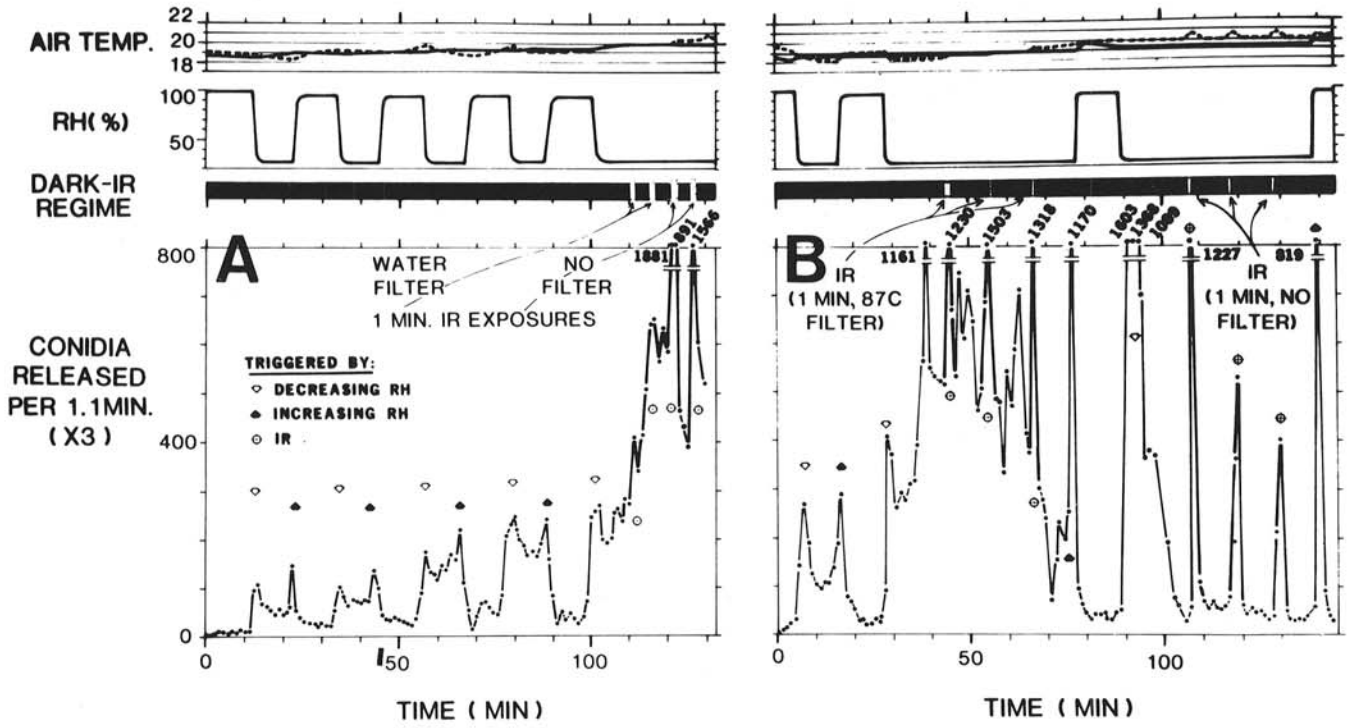

Fig. 3. The effect of short exposures (1 min) to red-infrared (IR) radiation on spore release by *Drechslera turcica*. **A)** Specimen exposed to four humidity cycles in darkness before being exposed to filtered (20 cm water) and unfiltered IR radiation during fifth cycle. **B)** A single relative humidity cycle in darkness, followed by two cycles at low relative humidity during which the specimen was exposed to filtered and unfiltered IR radiation (87C filter transmits IR > 800 nm). Temperature and air velocity same as in Fig. 1.

though the total liberation per cycle was relatively small. During the period of saturation preceding the fourth relative humidity cycle, the specimen was exposed to unfiltered IR and this resulted in a massive release of conidia when the RH was lowered to 32%. This liberation was typically bimodal with one release coinciding with the lowering and another with the raising of the RH. A subsidiary peak occurred after 86 min and coincided with switching off of the IR, and another larger peak followed when the specimen was returned to saturation (89 min). This slight increase in release of spores coinciding with the end of the IR exposure was observed on several occasions. In another experiment (Fig. 1B), the specimen was exposed to IR during the first two humidity cycles (minimal RH 57 and 57.5%, respectively), and these were followed by a humidity cycle in darkness (minimum RH 56.5%), and then another cycle with IR (minimum RH 51%). Relatively massive release of spores occurred during the IR regimes compared to the small liberation in darkness.

Spore liberation was greatly increased by exposure of specimens to IR radiation at lowered humidities but not at saturation. An exposure to IR radiation (Fig. 1A) which began during a period of saturation (74 min) caused no liberation of conidia. Only when the RH was lowered (78 min) did spore release begin. In the experiment shown in Fig. 1B, each of three IR exposures was purposely begun during periods of saturation. None of these caused liberation of spores until the humidity was lowered. Consistently, IR radiation stimulated spore release at lowered RH but not at saturation.

Humidity level and IR release. Two experiments were conducted to determine the interrelationship of spore release triggered by changes in humidity level and IR; in one, unfiltered IR (Fig. 2B) was used and in the other a 20-cm water filter reduced the effects of heat (Fig. 2C). In the experiment with unfiltered IR, the humidity initially was lowered to 49% in darkness which resulted in negligible spore release (Fig. 2B). After saturation was restored, the IR lamp was switched on and the RH was lowered in discrete steps (100, 79, 50, and 17% RH) and then raised in steps (17,75, and 100% RH). In the filtered IR experiment (Fig. 2C), a similar stepwise decrease and increase in relative humidities was followed.

Exposure to unfiltered IR (Fig. 2B) while lowering the relative humidity from 100 to 79% triggered only a minor spore release; lowering it from 79 to 50% caused a marked spore liberation, and the final reduction from 50 to 17% caused a massive release of conidia. Elevation of the relative humidity from 17 to 73% in this same experiment triggered a significant liberation of conidia which far exceeded that resulting from raising the relative humidity from 73% to saturation. Water-filtered IR (Fig. 2C) caused a similar pattern of spore release except that there was a more massive liberation on lowering the relative humidity in the intermediate range (79 to 51%).

The influence of red-infrared radiation on spore release is dependent on level of atmospheric humidity and is most pronounced at lower humidities. At higher humidities the IR effect on spore release is much reduced and at saturation there is none.

Effect of brief IR exposures at low humidities. In one experiment, to determine the effect of brief exposures of specimens to IR, following various RH changes in darkness (Fig. 2A), the RH was finally lowered to 24% (lowered at 95 min) and kept constant. The specimen was then subjected to two 1-min and two 2-min exposures to unfiltered IR. Five major peaks of spore release were measured during this period. The first was triggered by decreasing the RH to 24%, then each of the succeeding peaks resulted from the IR exposures. Liberation of spores in response to the 1-min exposures was massive; however, the 2-min exposures caused even greater releases. During this experiment the IR caused a noticeable increase in air temperature and, therefore, another experiment (Fig. 3A) was conducted to determine if reducing this heat would influence spore release. Following four RH cycles in darkness, during the fifth cycle the specimen was subjected to two 1-min IR exposures with a water filter (20 cm deep), followed by two 1-min exposures without a filter. IR with and without the filter caused increased liberation of conidia (Fig. 3A); however, liberation triggered by unfiltered IR was much greater than that for the filtered IR. Because the IR intensities were not at all comparable for the unfiltered (3,695 $\mu W/cm^2$) and filtered (264 $\mu W/cm^2$) treatments, the differences may have been merely a dosage response.

Another experiment in this series (Fig. 3B) compared the effect of unfiltered broad-band red-infrared radiation with infrared radiation lacking visible wavelengths. A Kodak 87C filter (transmits only wavelengths $>$ 800 nm, approximately) was used to absorb visible radiation. During the second RH cycle the specimen was subjected to three 1-min exposures to unfiltered IR (exposures at 45, 55.6, and 66.8 min). Each caused a massive release of conidia indicating that infrared radiation alone will trigger spore release. Three exposures to unfiltered IR during the next relative humidity cycle (107, 117, and 128 min) also triggered major release of spores, although these were generally smaller than for the filtered IR. This reduction may have resulted from fewer spores being available. There was a single major spore liberation at 39 min (Fig. 3B) that coincided neither with a humidity change nor an exposure to IR. Results of later studies (C. M. Leach, *unpublished*), make it seem likely that this may have resulted from vibration of the specimen chamber caused by inserting the Kodak filter. Vibrational release will be discussed in another article.

DISCUSSION

In this article I have explored one small facet of the complex interrelationships among the factors that affect plant disease, the influence of environmental factors on spore liberation by *D. turcica*. Ingold (1) states in the preface of his notable book on spore liberation and dispersal: "...we are now in an age when the study of mechanisms is nearing an end, and emphasis will probably be on extension of the knowledge of the quantitative evaluation of dispersal in the overall ecological picture of fungi in field situations." Although his assessment may be correct, I believe that there is still much of significance to be learned about spore liberation by foliar plant pathogens that could be relevant to plant pathology.

Results of numerous spore trapping studies (7) have revealed that liberation of conidia by dry-spored Fungi Imperfecti under natural conditions coincides with the lowering of atmospheric humidity that occurs at dawn. Proving a direct relationship between humidity changes and spore liberation is difficult under natural conditions because of so many other variables. Few precise laboratory studies demonstrating the relationship between atmospheric humidity and spore release have been conducted, probably because of the technical difficulties involved in controlling and measuring humidity in a dynamic system. The "spore release apparatus" developed for these studies (4) enabled me to analyze spore release under precisely controlled conditions. The results of experiments conducted in this apparatus show that atmospheric humidity is a very important factor in the violent discharge of conidia by *D. turcica*, and that it can be a limiting factor. Spore liberation results from both lowering and raising the atmospheric humidity; for *D. turcica* the magnitude of the response is always greatest when the changes occur at lower humidities.

I previously reported that cyclical lowering and raising of the humidity under controlled conditions causes a bimodal pattern of spore release (3), with the first major release coinciding with the lowering of atmospheric humidity, followed by a second release when the humidity is restored to saturation, or at least to a higher level. Palmerley and Benedict (8) have disputed these findings, particularly my conclusion that increasing atmospheric humidities trigger spore release (2). Results reported herein again demonstrate the existence of this phenomenon in the laboratory.

The profound effect of red-infrared radiation on spore release was reported earlier for *D. turcica* (2) and the research presented in this article confirms this finding. Varying the atmospheric humidity in darkness caused spore liberation to range from negligible to significant, but when the same specimens were exposed to red-infrared radiation at lowered atmospheric humidities, there was always massive release of spores which far exceeded that in darkness. Red-infrared radiation is an important component of

solar radiation; therefore, a similar response would be expected under natural conditions.

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