

Influence of Humidity, Red-Infrared Radiation, and Vibration on Spore Discharge by *Pyricularia oryzae*

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ABSTRACT

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Under controlled conditions in which air temperature and air velocity (0.5 m/sec) were kept constant, spore liberation from diseased rice culms was triggered by vibration and by increasing and decreasing relative humidities. Increasing relative humidity consistently triggered greater spore release than did decreasing relative humidity, and small increases near saturation were more effective than were increases at lower RH. Spore release when relative humidity was lowered from and returned to saturation exhibited a characteristic bimodal distribution: a peak associated with decreasing RH and a peak associated with increasing relative humidity.

Spore discharge by *P. oryzae*, a fungus that liberates its conidia at night, was little influenced by exposure to red-infrared radiation (IR). Effects of vibration on spore liberation ranged from negligible to massive: release was greatest at reduced relative humidities and least at saturation; however, even at saturation appreciable numbers of spores were discharged. Red-infrared radiation had little influence on vibrational release of conidia. The violent nature of spore discharge by *P. oryzae* was confirmed visually by using special illumination.

Pyricularia oryzae Cav., a fungus that causes an important foliar disease of rice known as "blast," normally discharges its conidia at night with maximal release approximately between 0100-0300 hr (2,21,22,23,25). Spores release also may be triggered during the day by rain showers (20). Meredith (16) suggested that liberation of conidia by *P. oryzae* may involve an active discharge process and Ingold (5) suggested possible mechanisms. I have observed violent discharge of conidia by *P. oryzae* under special illumination (C. M. Leach, unpublished).

Most dry-spored Fungi Imperfecti, unlike *P. oryzae*, commence liberating conidia during the morning (3,17); some like *P. oryzae* (20), also discharge spores in response to brief rain showers (7,14,23). Humidity changes are particularly important in spore discharge by numerous fungi (5,17); however, the precise relationship of humidity to spore release has not been determined either for *P. oryzae* or is it well understood for most other fungi. Recent studies on *Drechslera turcica* (8,12,13) and *D. maydis* (13) have revealed that liberation of conidia can be triggered by both lowering and raising the relative humidity (RH), also that exposure of these fungi to red-infrared radiation greatly increases spore discharge (8,12,13).

Various theories have been advanced to explain the mechanism of violent discharge of conidia (6,17). I have proposed an electrostatic mechanism for *D. turcica* (9,10) that also should apply to all dry-spored foliar pathogens, including *P. oryzae*. If this hypothesis is valid, then why does *P. oryzae* discharge its spores at night whereas most other fungi do so during the morning? The purpose of the present research was to attempt to answer this question by analyzing the relationship of atmospheric humidity, red-infrared radiation (IR), and vibration to spore liberation by *P. oryzae* under controlled conditions.

MATERIALS AND METHODS

Preparation of specimens. Infected, dried rice culms (obtained from Hozumi Suzuki, Rice Research Division, Tohoku Agricultural Experiment Station, Japan) were stored in sealed plastic bags at 5 C until needed. Four to six culms (each ~7 cm

long) were placed on a rectangular piece of filter paper (No. 3 Whatman) and attached with rubber bands to a plastic holder (7 × 28 × 110 mm). Specimens were soaked for 10 min in distilled water and transferred to a moist chamber (clear plastic box, 32 × 50 × 270 mm). Profuse sporulation was induced by incubating specimens under a cycling regime of temperature and near-ultraviolet (NUV) radiation (25 C day, 20 C night; 12 hr NUV, 12 hr darkness). Specimens were placed under a single "Black Light" fluorescent lamp (distance, 12-13 cm; 20 W BLB Sylvania; 160 μW/cm²). Incubation time ranged from 5 to 12 days.

Spore release apparatus. A previously described (11) spore release apparatus designed to control air velocity, relative humidity, and temperature was used in all experiments. Sporulating rice culms were placed in the cylindrical (4 × 30 cm) Pyrex glass specimen chamber of the apparatus. Air velocity was constant (0.5 m/sec at the specimen) and purposely kept low to minimize "wind" removal of conidia. Air temperature was kept relatively constant at 19-20 C (refer to Figs. 1-4 for precise temperatures). Air temperature was monitored at four locations within the apparatus by means of thermocouples (± 0.25 C), but for simplicity recordings for only two locations are included with the results (Figs. 1-4). Relative humidity was precisely controlled by combining saturated and dry air streams (11). With this arrangement it was possible to cycle the relative humidity from 100% to 20% and back to saturation in ~2 min humidity. Again, for simplicity, only the relative humidity of air entering the chamber is included (Figs. 1-4). During all experiments temperatures and relative humidities were recorded continuously with a multichannel recorder operated at a chart speed of 30 cm/hr (Multi-point, PM 8325; Phillips, The Netherlands). Air velocity in the specimen chamber was measured with a hot-wire anemometer (Alnor Type 8500 thermo-anemometer; Alnor Instrument Co., Chicago, IL 60610). Released spores were monitored with a precision spore trap (11) in which the air stream from the spore release apparatus was directed via a 1 × 32-mm orifice onto Vaseline-coated glass slides (11 × 75 mm) that moved past the orifice at 1 mm/1.1 min. Conidia deposited on each slide were mounted in water under coverslips and counted by the systematic microscopic examination of the complete slide surface.

Red-infrared radiation. The source of red-infrared radiation was a 250 W infrared lamp (Sylvania, Lamp Division, Danvers, MA 01923) located in a lamp housing positioned 43.2 cm below the

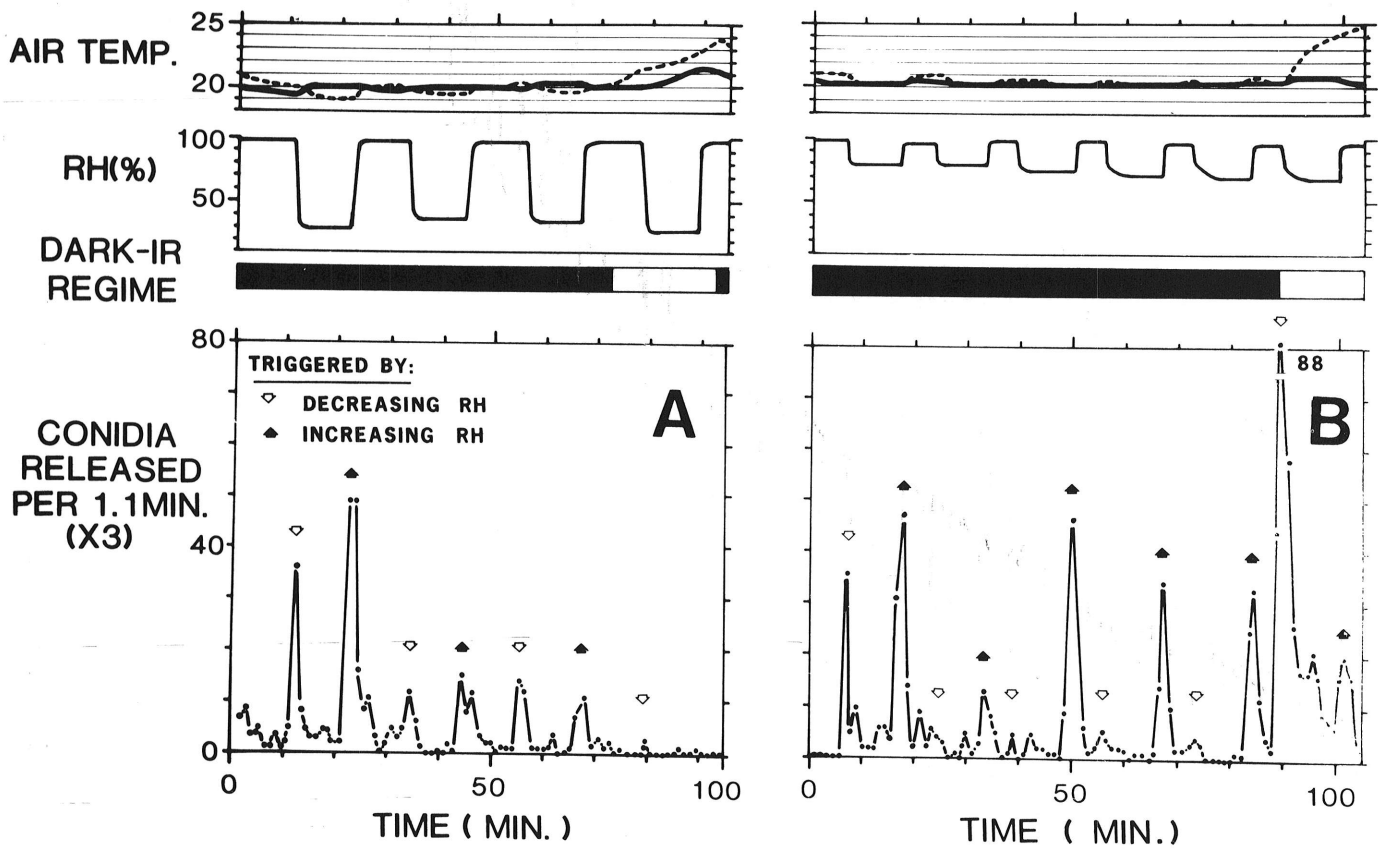


Fig. 1. Liberation of conidia by *Pyricularia oryzae* in response to changes of relative humidity (RH) and exposure to red-infrared radiation (IR). A, Relative humidity was cycled between 100 and 28% minimum. B, Relative humidity was cycled between 100 and 68% minimum. (Air velocity at specimen, 0.5 m/sec. Temperatures are for air entering specimen chamber, a solid line, and leaving chamber, a dotted line.)

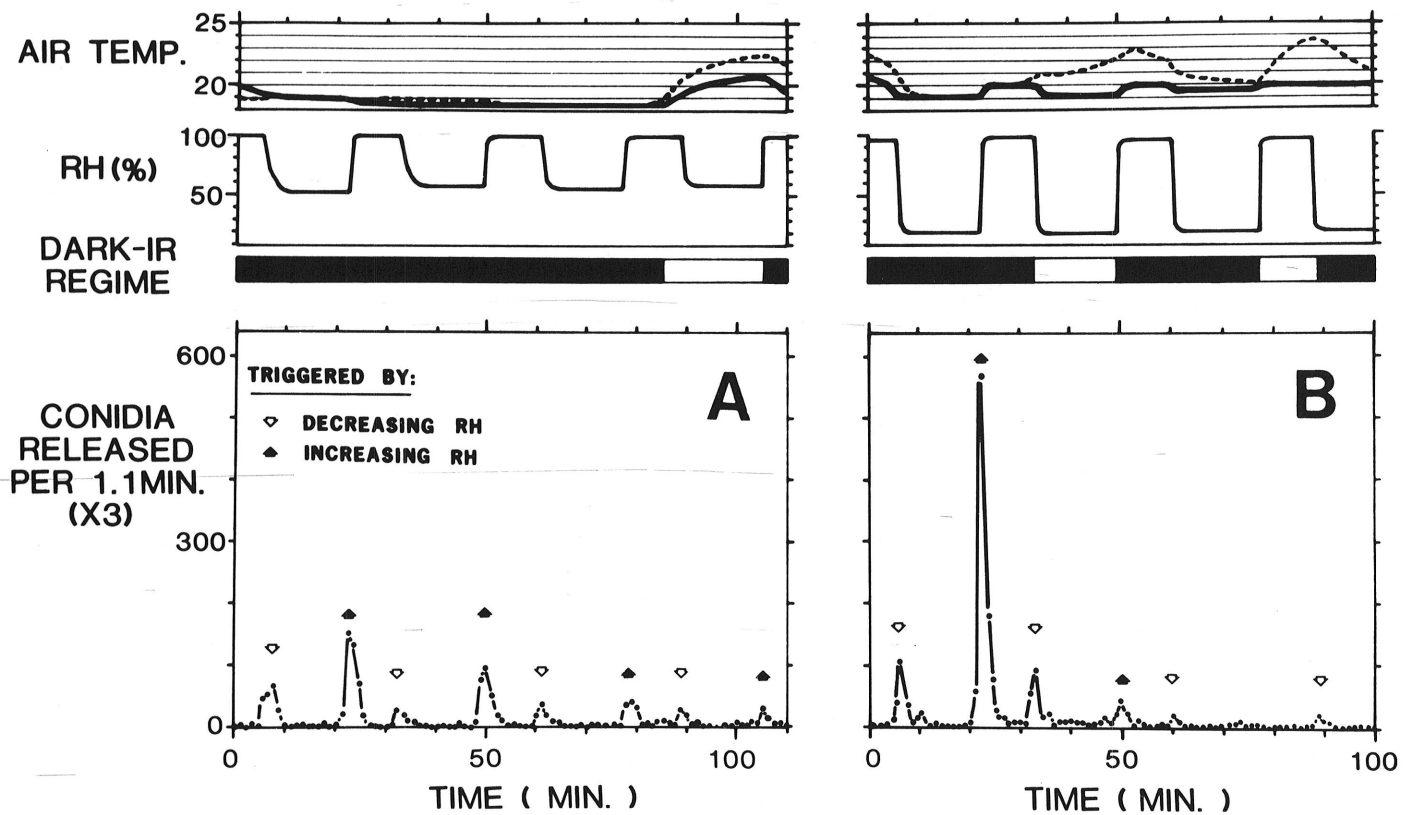


Fig. 2. Spore liberation by *Pyricularia oryzae* in response to changes of relative humidity (RH) and exposure to red-infrared radiation (IR). A, Minimum RH, 51%; B, Minimum RH, 19%. (Air velocity and temperatures same as Fig. 1).

specimen (11). Intensity of the unfiltered IR at the specimen was $3,695 \mu\text{W}/\text{cm}^2$. The lamp emitted a broad band of radiation, extending from the red through the infrared spectrum. Intensities were measured with a compensated thermopile (Model F20; Kipp und Zonen, The Netherlands) and a galvanometer (Model A70; Kipp und Zonen).

Vibrations. An arbitrary but standardized procedure was used to vibrate the specimens. In each instance vibration (Figs. 1-4) was produced by dropping a 50 g weight from a height of 5 cm onto a piece of hard cardboard placed on top of the glass specimen chamber. No attempt was made to determine the vibrational force

necessary to release conidia, but visual observation under special illumination (C. M. Leach, *unpublished*) revealed that even extremely light tapping of the specimen chamber triggered discharge.

RESULTS

Humidity changes in darkness In three experiments specimens were subjected to series of repeated humidity cycles (lowering and raising relative humidity) in darkness. The RH changes were large in one experiment (Fig. 1A, 100 to 28%), smaller in another (Fig.

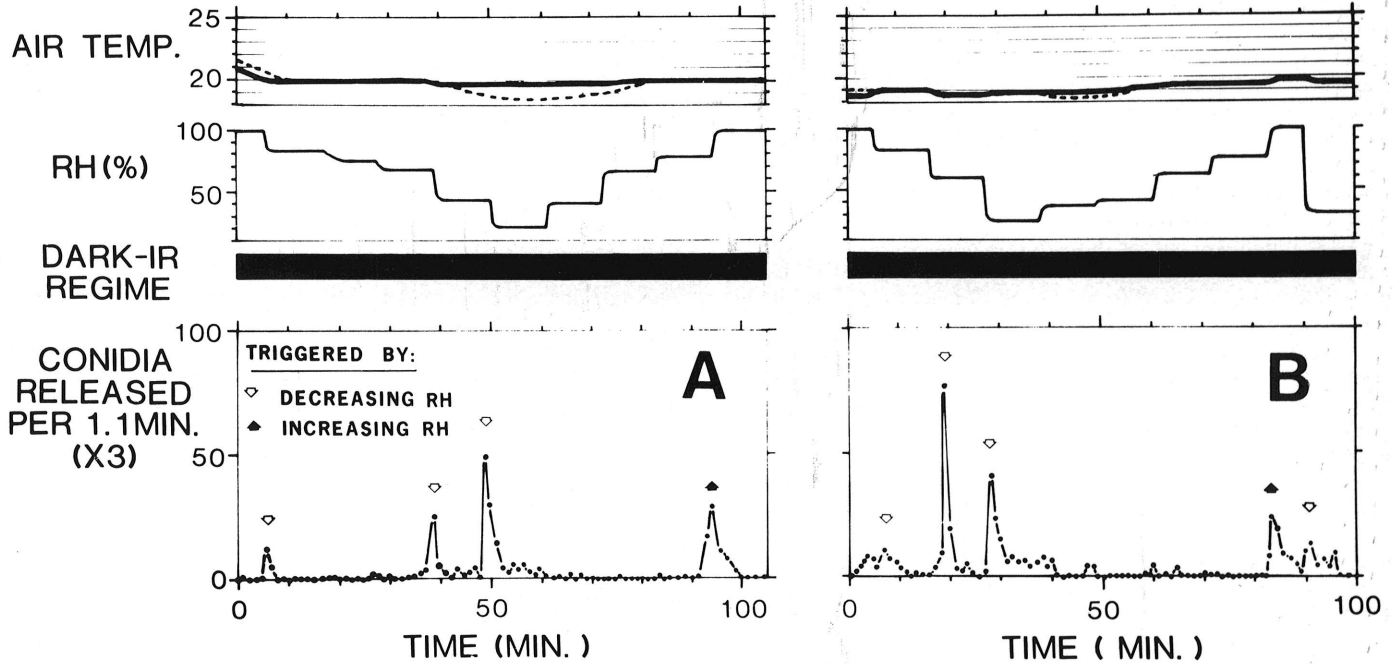


Fig. 3. Conidium liberation by *Pyricularia oryzae* in response to different relative humidity (RH) levels. A, Relative humidity was lowered and raised in discrete steps with the minimum, 21%; B, Similar, minimum RH, 24%. (Air velocity and temperatures same as Fig. 1).

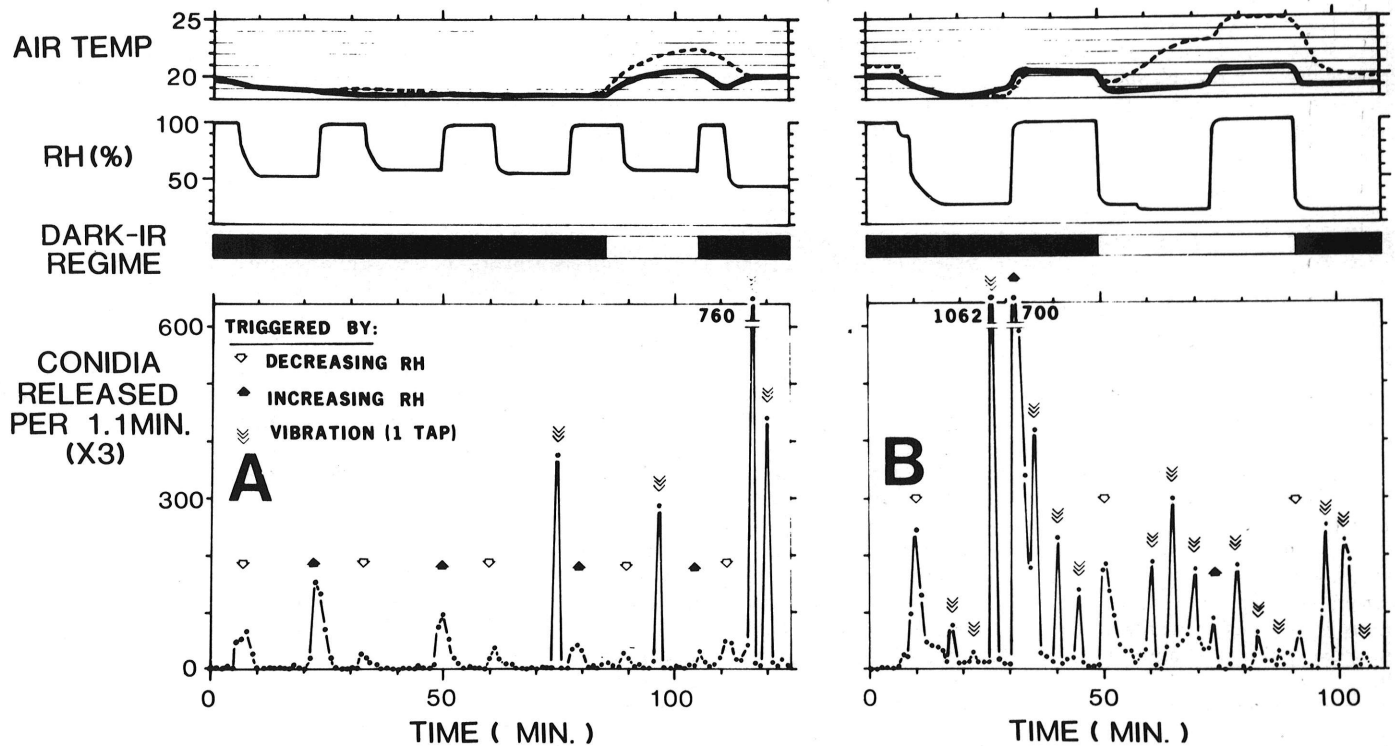


Fig. 4. Vibrational release of conidia by *Pyricularia oryzae* related to relative humidity (RH) and exposure to red-infrared radiation (IR). A, Minimum RH during the first four cycles was 51%, and during the last cycle, 44%. B, Minimum RH, 19%. (Air velocity and temperatures same as Fig. 1).

1B, 100 to 68%), and intermediate in a third (Fig. 2A, 100 to 51%). In all three experiments rates of conidia liberation exhibited a bimodal pattern. Conidia were first released in response to lowering and then to raising the relative humidity. In each experiment spore release was greatest when the humidity was increased. When the relative humidity was cycled over a fairly wide range (Fig. 1A, 100 to 28%), most spores were liberated during the first humidity cycle; with each succeeding cycle there was a marked decrease in numbers of spores liberated. However, when the humidity was cycled over a much smaller range (Fig. 1B, 100 to 68%), the numbers of spores released per cycle remained approximately the same.

To determine more precisely the level of humidity most effective in triggering liberation of conidia, two similar experiments were conducted in darkness in which the RH was lowered and raised stepwise from 100 to 82%, 82 to 74%, 74 to 67%, 67 to 43%, and 43 to 21%, in one experiment (Fig. 3A), and from 100 to 82%, 82 to 49%, and 49 to 24% in the other (Fig. 3B). In both experiments the first small decrease in relative humidity triggered only a small liberation of conidia. Maximal liberation of spores occurred only for relative humidity reductions at the lower humidity levels. With stepwise humidity increase, few conidia were released at the lower ranges; only when it was increased from 78 to 100% in one experiment (Fig. 3A) and from 76 to 100% in another (Fig. 3B) was there a significant liberation of conidia.

I concluded that *P. oryzae* liberates conidia in darkness in response to both decreasing and increasing humidity; however, raising the RH, particularly near saturation, is the most effective.

Effect of red-infrared radiation. Sporulating rice culms were exposed to IR at saturation (Fig. 1A, first 5 min of exposure), at an intermediate RH (Figs. 1B and 2A) and at a low RH (Figs. 1A and 2B). Only in a single experiment (Fig. 1B) in which the specimen was exposed to a rather small RH cycle (100 to 68%), was there any evidence of an IR enhancement of spore liberation. In this experiment there was a significant increase in air temperature (Fig. 1B) but this also was true of other experiments (Figs. 1A, 2 and 4) in which no IR effect was observed. We concluded that IR does not have the profound effect on spore liberation reported for other fungi. However, the enhancement that did occur in one experiment suggests the need for further study.

Effect of vibration. Sporulating rice culm specimens were vibrated in darkness as well as exposed to IR radiation while being held at various levels of RH. Vibrations caused significant release of conidia even though spore liberation also was dependent upon the relative humidity (Fig. 4). Liberation of conidia was most abundant when specimens were vibrated at low RH and least at saturation (Fig. 4). However, even at saturation the numbers of spores liberated by vibration equalled or exceeded by those liberated in response to humidity changes alone. There was no indication that IR radiation enhanced spore release by vibration (Fig. 4).

DISCUSSION

Pyricularia oryzae, the cause of rice blast liberates conidia nocturnally when the atmospheric humidity is high (20,26). This nocturnal liberation of spores is unique among dry-spored Fungi Imperfecti; most liberate their spores during daylight hours (3,22). Why the difference? My studies on *Drechslera turcica* (8,12-14) and on *D. maydis* (13) have shown that some spore liberation can be triggered solely by changes in atmospheric humidity. Exposure of *D. turcica* to IR also will trigger spore release at constant RH as well as enhance release caused by the humidity changes (8,12). The biomodal response of *P. oryzae* was similar to that of the *Drechslera* spp. except that the *Drechslera* spp. tend to respond more to decreasing RH (8,12), while *P. oryzae* responds more to increasing RH, particularly near saturation. In *Drechslera* spp. humidity changes at low RHs, whether decreasing or increasing, were more effective than those near saturation. This was also true for *P. oryzae* for decreasing RHs, but not for increasing RHs where changes near saturation were most effective. Another major difference was *P. oryzae*'s almost complete lack of response to IR radiation, except in one experiment (Fig. 1B). In contrast,

the *Drechslera* spp. are profoundly influenced by exposure to IR radiation (8,13). Suzuki (24) reported that light suppresses spore release, but in my experiments there was no evidence of any suppression by IR radiation.

I have proposed that the violent spore discharge of dry-spored Fungi Imperfecti involves an electrostatic mechanism (9,10) which results from charges associated with surface-atmospheric moisture changes. Although *P. oryzae* liberates its conidia nocturnally, its response to humidity changes is essentially similar to those reported for *D. turcica* (8,12) and *D. maydis* (13). The major difference between the *Drechslera* spp. and *P. oryzae*, their response to IR radiation, suggests that the influence of IR is probably not a photoelectric, but rather another effect that has still to be explained. IR radiation, for example, may influence the strength of the bond between conidium and conidiophore. Past studies with water filters (20 cm deep) indicate that it is not merely a temperature effect (10).

On the basis of results from my laboratory studies, it is possible to forecast that, under natural conditions, conidia of *P. oryzae* should be liberated in greatest numbers at night or in the late evening as the atmospheric humidity increases toward saturation. The results of the spore trapping studies by Suzuki (25,26) and Ou et al (20) indicate that this indeed happens. Ou et al (20) stated that greatest liberation of conidia takes place at night and is associated with dew formation. Dew may be important in nature, but I have demonstrated that humidity increases alone trigger spore liberation by *P. oryzae* (Figs. 1-4). My electrical studies, (9) which have included measurement of leaf surface charges (C. M. Leach, unpublished), show significant electrical changes related to dew formation and these may well be involved in the liberation that Ou et al (20) associated with dew formation. *P. oryzae*'s ability to liberate conidia in response to both increasing and decreasing humidities (Figs. 1-3) suggests that under some conditions conidia may be liberated during the morning in response to the decreasing humidity.

To fully understand spore liberation, the conditions that favor spore formation must also be understood. Spore development of many dry-spored Fungi Imperfecti occurs at night because it is favored by high atmospheric humidities and is inhibited by blue light. Thus for *Drechslera* spp., maximal numbers of spores are normally present on leaf lesions at dawn (15) when many are likely to be released in response to the lowering of humidity that accompanies sunrise. *Pyricularia oryzae* in contrast, thrives in the humid habitat of the rice paddy where spore production is favored by conditions during the daylight hours. Suzuki (25) and Barksdale and Asi (2) reported that both continuous darkness and continuous exposure to light inhibit spore release. Sporulation is favored by 6-8 hr of darkness followed by light. Others also have indicated the importance of light in stimulating sporulation (1,18,19,27) although some isolates sporulate in darkness (1). Exposure to light, particularly near-ultraviolet light, increases the number of conidia produced and hastens maturity; thus, one would expect maximal numbers of mature conidia to be present on lesions in the late afternoon. Suzuki (26) reported that maximal numbers of conidia are present on lesions between 1800 and 1900 hr. With most spores present during the evening, and spore release by *P. oryzae* favored by increasing humidities (Figs. 1-3), particularly near saturation, it becomes understandable why *P. oryzae* liberates its spores at night in contrast to the daytime liberation of conidia by *D. turcica* (12).

Rain showers also cause the liberation of conidia by *P. oryzae* (20), although under some conditions rain also may remove spores from the air (24). Other foliar pathogens also release their spores in rain showers (7,14,23), and this may be a form of vibrational release (4,13). Vibrational release of conidia by *P. oryzae* can be demonstrated in the laboratory (Fig. 4), and it seems likely that this same phenomenon would occur in nature in response to rain and wind buffeting of plants.

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