Sporangium Discharge by *Peronospora destructor:* Influence of Humidity, Red-Infrared Radiation, and Vibration

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ABSTRACT

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The effects of relative humidity (RH), red-infrared radiation (IR), and vibration on sporangium discharge from onion leaves infected with *Peronospora destructor* were analyzed under precisely controlled conditions. Airflow (0.5 m/sec) and temperature (17–18 C) were kept constant. Many sporangia were discharged as the RH was reduced; reductions below ~59% RH were most effective. Increasing the atmospheric RH from a lower level to a higher level, or to saturation, also triggered sporangium liberation, although the numbers of sporangia released were always less than for comparable reductions of RH. No sporangia were liberated in darkness when the air was saturated (RH 100%). Brief exposures to IR (eg, 1 min) induced sporangium discharge with maximum release occurring at reduced RH. When the air was saturated, only a few spores were liberated by irradiation. Continuous exposure to IR during complete RH cycles (ie, lowering and then raising RH) enhanced total spore release compared to similar RH changes in darkness. Vibration of onion leaves also triggered sporangium discharge. The number of spores liberated by vibration was influenced by both RH and IR. Greatest discharge occurred when leaves were vibrated while exposed to IR at reduced RH; far fewer sporangia were released when leaves exposed to IR were vibrated in a saturated airstream. No sporangia were liberated from vibrated leaves placed in darkness in a saturated airstream.

Additional key words: downy mildew.

Liberation of inoculum is an important event in epidemics of foliar plant diseases. There is much information on weather factors that govern spore release (1,5,9,20), but, with few exceptions, precise relationships of weather and the mechanism of active spore discharge by fungal pathogens are poorly understood. Epidemics of downy mildew occur in onion crops in many parts of the world, but little is known about the effects of weather variables on the discharge of spores (sporangia) by the downy mildew pathogen, *Peronospora destructor* (Berk.) Casp. Yarwood (23) recognized that spores are produced only at night when humidity is near saturation and observed maximum discharge under glasshouse conditions a few hours after the spores appeared to be morphologically mature (0600 hours, California). Hildebrand and Sutton (7) reported that spore dispersal from onions in the field in Ontario, Canada, increased abruptly at 0700 hours EST and peaked between 0800 and 0900 hours. Peak dispersal coincided with declining relative humidity (RH) and the drying of leaves. Most spores were trapped when wind speed exceeded 0.2 m/sec.

Studies on other species of *Peronospora* and of *Pseudoperonospora* have revealed important effects of RH changes (particularly declining RHs) and of mechanical shock in spore discharge. *Peronospora trifoliorum* failed to discharge sporangia in a saturated atmosphere in the laboratory (4). Sporangia of *P. tabacina* and *Pseudoperonospora humuli* were discharged as RH decreased (3,21). Cruickshank (3) suggested that mechanical shock also may be involved in sporangium liberation in *P. tabacina.* Transient discharge of *P. humuli* sporangia was related to onset of rain but only when the RH was well below saturation (21). Although RH changes, onset of rain, and mechanical shocks may affect sporangium discharge in species of *Peronospora* and *Pseudoperonospora,* relationships of these factors and discharge have not been analyzed precisely.

Sporangia of *P. destructor* are discharged actively (Fig. 1) by a mechanism (18) that is similar to that reported for conidium discharge by *Drechslera turcica* (16). Because red-infrared radiation (IR), vibration, and RH all markedly influence release of conidia by *D. turcica* (13,14,19), the present study was designed to measure the effects of these factors on sporangium release by *P. destructor.*

MATERIALS AND METHODS

Preparation of specimens. Onion sets were inoculated by hypodermic needle with a suspension of sporangia of *P. destructor* by following the procedures of Hildebrand and Sutton (6). Sets were then planted in silica sand in unglazed clay pots (9 cm diameter), which were irrigated periodically with Hoagland's solution. A cotton wick was placed in the sand in each pot and dipped into water below to maintain a supply of moisture. Onions were grown under fluorescent lamps (4 × 40 W warm-white and 4 × 40 W cool-white; 112 W/m^2) with a 14-hr day and 10-hr night cycle. Corresponding day and night temperatures were 19.5 and 17.5 C. When the plants were 2–3 wk old and showing symptoms of systemic disease, a 10-cm segment of leaf was removed from each of two plants. The segments were attached to a plastic holder (27 × 110 mm) with rubber bands and suspended in a horizontal, inverted position along the central axis of a cylindrical glass chamber (4 × 18 cm). A strip of moistened filter paper was placed in the chambers and the ends of the chamber were sealed with rubber stoppers. RH was measured with a humidity meter (model HM 111, Weather Measure Corp., Sacramento, CA 95841). The specimens and chambers were returned to the growth cabinet to complete a 14-hr light cycle. After the next dark cycle (10-hr), sporulation was abundant on most specimens. For each experiment, one chamber was chosen from among several on the basis of abundance of sporulation. To start an experiment, the stoppers and filter paper were quickly removed from the chamber, which was then inserted

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into a "spore release apparatus" (12). Use of interchangeable specimen chambers minimized disturbance of the sporulating leaf surfaces.

Spore release apparatus. Sporulating onion leaves were placed in a specially designed apparatus (12) and subjected to precisely controlled changes of RH, exposed to IR, or vibrated while the air speed (0.5 m/sec) and temperature were held constant. Air speed was measured periodically with an anemometer (Type 3500 Thermo-Anemometer; Alnor Instrument Co., Niles, IL 60648); air temperature was recorded continuously with two thermocouples positioned in the airstream, one located in the air entering the specimen chamber, the other in the air leaving the chamber. Temperature of the air entering the chamber varied slightly from experiment to experiment (17.25–18.5 C) but was constant during each experiment (±0.25 C). RH was controlled precisely by mixing saturated and dry airstreams and could be lowered from saturation to about 20%, or similarly raised, within 2 min (12). RH was monitored continuously with two thermocouple psychrometers (12) located at the entrance and exit of the specimen chamber. Psychrometers and thermocouples were connected to a multichannel recorder (Multipoint, model PM 8235; Philips, The Netherlands) operated at a chart speed of 30 cm/hr.

Release of spores was monitored continuously with a specially designed precision spore trap (C.M. Leach, *unpublished*) capable of accurately detecting the numbers of spores released per 1.1 min (12). Within the spore trap, sporangia impinged onto Vaseline-coated (petroleum jelly) microscope slides moving past a slit orifice (1×32 mm) at 1 mm/1.1 min. Numbers of spores deposited during an experiment were determined microscopically by systematically counting across the slides at 1-mm intervals with an eyepiece reticle. At high deposition rates (>500 spores/1.1 min), it was impractical to count every spore; counts were randomly made across the slides from which totals were estimated.

Red-infrared radiation. A 250-W, unfiltered, infrared lamp (Sylvania) was placed in a housing 43.2 cm below the specimen chamber (12). Radiation intensity at the specimen was 3,695 $\mu W/cm^2$ as measured by a compensated thermopile and galvanometer (12). Although the effects of IR on air temperature and RH were known from previous studies (13), their influence on air temperatures was assessed again by monitoring the temperature of air entering and leaving the specimen chamber. Air flowing into the chamber (18.7 C constant) was exposed to IR for either 2 or 10 min at two different RH levels (RH 77 and 100%). Exposure for 2 min at the two RH levels caused a small increase in air temperature (0.20–0.25 C) and a resultant small change in RH. Exposure for 10 min increased the air temperature by 1.6 C at RH 100% and 2.0 C at RH 77%. The humidity change induced by IR was negligible at saturation, but 5% for RH 77%.

RESULTS

RH and spore liberation. Earlier field studies on onion downy mildew indicated the importance of RH changes as well as the possible role of light on spore release (7). Studies were initiated to analyze these relationships under controlled conditions. RH was lowered from saturation to 36% in two repeated cycles in darkness, except for one short exposure to IR (Fig. 2). No sporangia were liberated at the onset when leaves were subjected to a flow of saturated air (RH 100%) in darkness; but when the RH was lowered (10 min) to 77%, sporangia were released immediately (12 min). When RH was lowered further to 36% (15 min), massive liberation occurred for several minutes; but thereafter, few additional sporangia were detected during the remainder of this period of low RH. This indicated the importance of a humidity change rather than low RH in triggering sporangium discharge and that the change from 100 to 77% RH was less effective as a trigger than the change from 77 to 36% RH. Liberation of sporangia also occurred when the RH was increased from 36% back to saturation (25 min), although fewer spores were trapped compared to those trapped during the preceding RH reduction. This low incidence of spores was not due to a lack of available sporangia; when the RH was lowered a second time (35 min), many sporangia were released and more were liberated when the RH was again returned to saturation (50 min). A short (2 min) exposure to IR (40–42 min) during the second humidity cycle triggered a major release of spores. Spore release was negligible whenever the air was saturated or after it had reached a constant reduced level.

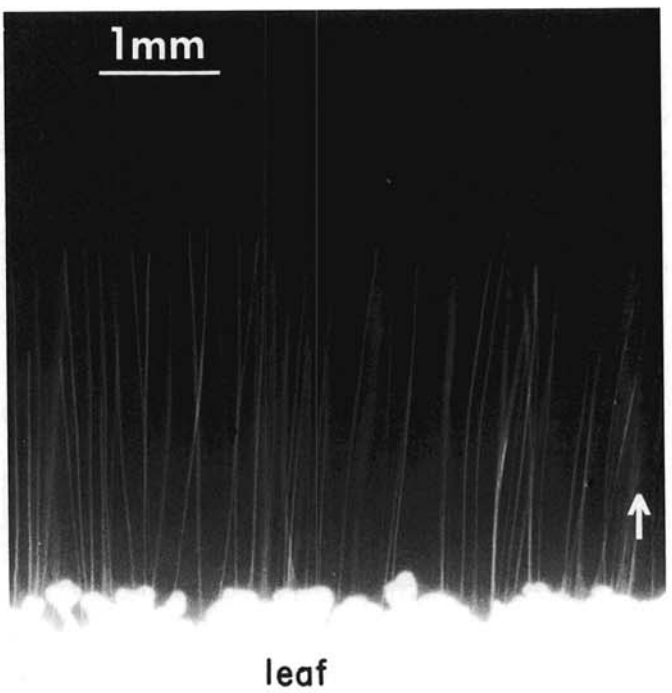

Fig. 1. Trajectories of actively discharged sporangia of *Peronospora destructor*. Release was synchronized by lightly tapping the onion leaf (time exposure; arrow indicates the direction of flight, which was at right angles to gravity).

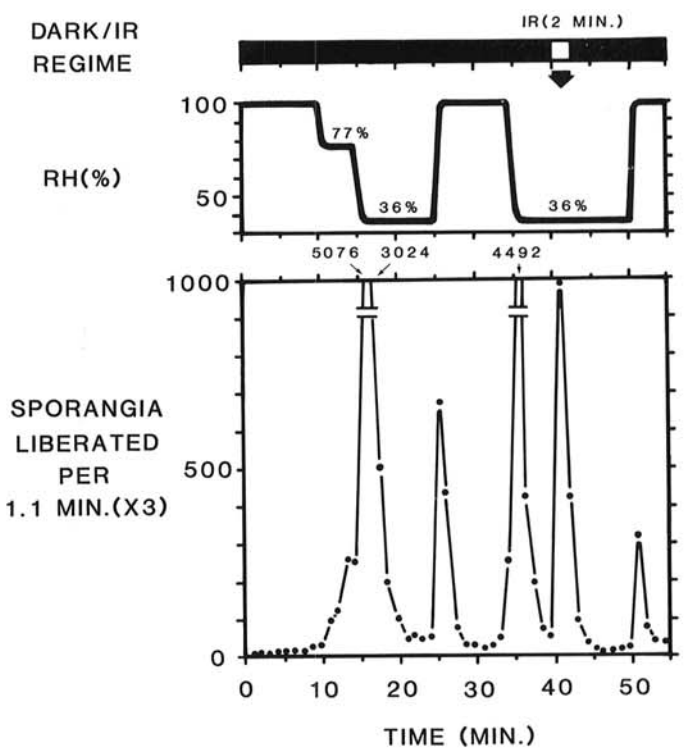

Fig. 2. The effects of relative humidity (RH) and red-infrared radiation (IR) on spore discharge by *Peronospora destructor* (air speed, 0.5 m/sec; air temperature range 17.25–17.75 C).

To analyze effectiveness of changes of RH at different humidity levels on sporangium discharge in darkness, RH was lowered and raised in a stepwise manner (Fig. 3) and then finally lowered from saturation to 20% (90 min) to determine if all sporangia had been discharged. Almost any humidity change triggered liberation of spores, although some changes were more effective than others. Lowering of RH in higher ranges (100 to 73%, 73 to 59%) was less effective than in the lower ranges (59 to 39.5% and 39.5 to 25%). Greatest release resulted from the reduction from 59 to 39.5%. A reduction in the lowest RH range (25 to 20%) caused the release of relatively few spores. As the RH was later increased (beginning at 58 min), each stepwise increase caused a small number of spores to be liberated. Numerous spores were liberated during the final reduction of RH (90 min), indicating that not all spores had been liberated during the preceding humidity changes.

IR and sporangium liberation. The initial experiment (Fig. 2) revealed a pronounced effect of a short exposure to IR applied during constant reduced RH (36%). The isolated influence of IR and its possible interaction with RH levels were further examined by irradiating specimens with unfiltered IR in a saturated airstream or at 45% RH (Fig. 4). At each RH, sequences of three identical 1-min exposures were applied and a 10-min exposure was given during a final period of low RH (90–100 min). The series of 1-min exposures in a saturated airstream (15, 20, 25, 70, 75, and 80 min) triggered spore liberation, although the releases were relatively small. In the first sequence of three exposures (15–25 min), the number of spores increased progressively with each successive exposure, suggesting carry-over effect from one exposure to the next. However, this progressive increase was not evident in the second sequence of IR exposure conducted in a saturated airstream (71, 76, and 81 min). Exposure to IR when the RH was decreased to 45% (at 40, 45, and 50 min) triggered a much greater response than similar exposures at 100% RH. In general, numbers of spores liberated in response to IR only were less than those triggered by lowering the RH in darkness; ie, at 30, 55, and 90 min. In this experiment (Fig. 4), the effect of a longer (10 min) exposure to IR as the RH was being lowered (90 min) could not be separated from that attributable to the change in RH. However, the enhancement of sporangium discharge by longer exposures was evident in other experiments both at saturation and reduced RHs, cf. Fig. 6C (35–57 min) at saturation, and Fig. 6B (60–77 min) and Fig. 6C (94–107 min) at reduced RH.

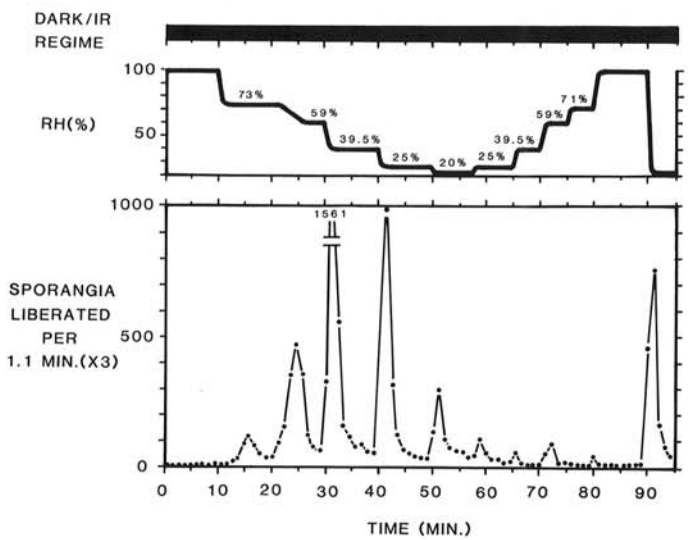

Fig. 3. The effects of stepwise lowering and raising the relative humidity (RH) in darkness on spore liberation by *Peronospora destructor* (air speed, 0.5 m/sec; air temperature range 18–18.5 C).

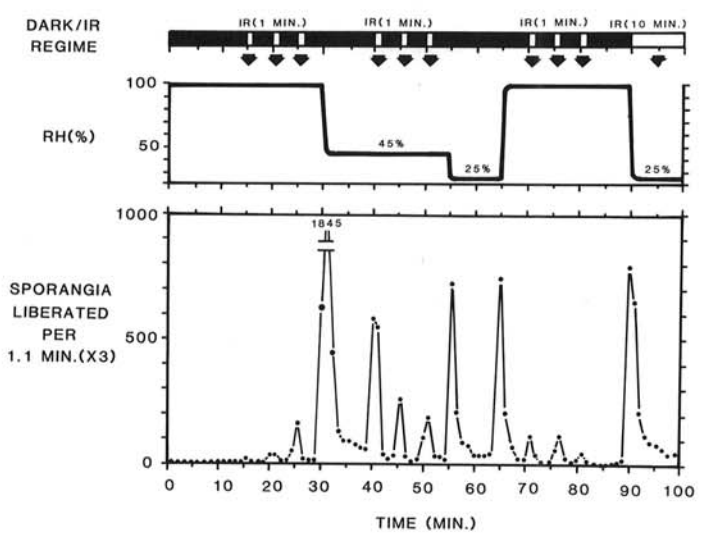

Fig. 4. The effects of short exposures (1–10 min) of red-infrared radiation (IR) at different relative humidities (RH) on spore discharge by *Peronospora destructor* (air speed, 0.5 m/sec; air temperature range 18.0–18.25 C).

Vibrational liberation of sporangia. Vibrational liberation of spores was measured in a series of experiments (Figs. 5 and 6) with a standardized but arbitrary vibration (20 g weight dropped from a height of 5 cm onto the specimen chamber) both in darkness and during exposure to IR and at different RHs. Spores were not liberated by vibration from the specimen in a saturated airstream in darkness (Fig. 5, taps at 10, 15, 20, 55, 60, and 65 min; Fig. 6C, taps at 15, 20, 25, and 30 min). However, many were released in saturated air in response to vibrations when onion leaves were also exposed to IR (Fig. 6 C, taps at 40, 45, 50, and 55 min). Vibrational discharge occurred in darkness whenever humidity was lowered (Fig. 5, taps at 35, 40, 45, 80, and 85 min; Fig. 6 B, taps at 20, 25, 30, 45, 50, and 55 min; Fig. 6 C, taps at 70, 75, and 80 min), but the numbers of discharged sporangia were always less than that solely attributable to lowering RH.

Exposure of sporulating onion leaves to IR consistently enhanced spore liberation attributable to vibration both in a saturated airstream and when RH was decreased (Figs. 5 and 6). Spores were not released in response to vibrations of the specimen maintained at 100% RH in darkness (Fig. 6 C, taps at 15, 20, 25, and 30 min); but as soon as this same specimen was exposed to IR while still at 100% RH, sporangia were discharged in response to vibrations (Fig. 6C, taps at 40, 45, 50, and 55 min). At lowered RHs, exposure to IR generally enhanced sporangium discharge triggered by vibration (Fig. 6A, taps at 20, 25, and 30 min; Fig. 6B, taps at 65, 70, and 76 min; and Fig. 6C, taps at 97 and 103 min). The significance of vibrational release, thus, appears to be dependent on

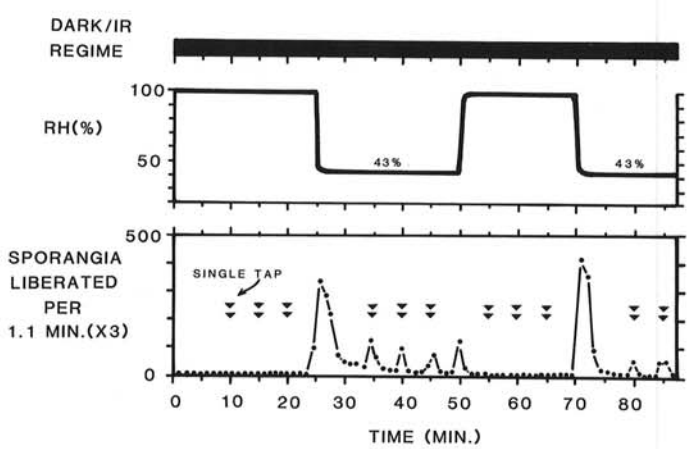

Fig. 5. Vibrational liberation of sporangia of *Peronospora destructor* from onion leaves in darkness at different relative humidities (standardized vibration was induced by dropping a 20-g weight 5 cm onto the specimen chamber; air speed, 0.5 m/sec; air temperature range 17.5–17.75 C).

environmental conditions, particularly RH, and the presence or absence of IR. The relative importance of vibrational release when compared to discharge solely associated with RH changes or exposure to IR is difficult to judge because of the arbitrary nature of the vibrational force.

DISCUSSION

The dynamics of most epidemics of foliar diseases are complex, and usually some facets are not well understood. In this study, we have attempted to determine the relationship of changes in atmospheric RH and exposure to IR to liberation ("takeoff") of *P. destructor* sporangia. Under certain conditions, the sporangia of this downy mildew fungus are forcibly discharged several millimeters into the air (18), very much like conidia of *D. turcica* (16). Previous studies (13–15) have demonstrated the importance of RH changes and IR to conidium discharge by *D. turcica* and several other foliar pathogens belonging to the Fungi Imperfecti. We have found that these factors are also important in the liberation of sporangia by *P. destructor*.

The liberation of sporangia by *P. destructor* in response to RH changes is bimodal with maximum discharge associated with decreasing RHs and a smaller response triggered by increasing RHs. *D. turcica* (13,14), *D. maydis* (14), and *Pyricularia oryzae* (15) behave similarly except that maximum discharge of conidia by *P. oryzae* is associated with increasing RHs. Spore discharge for all

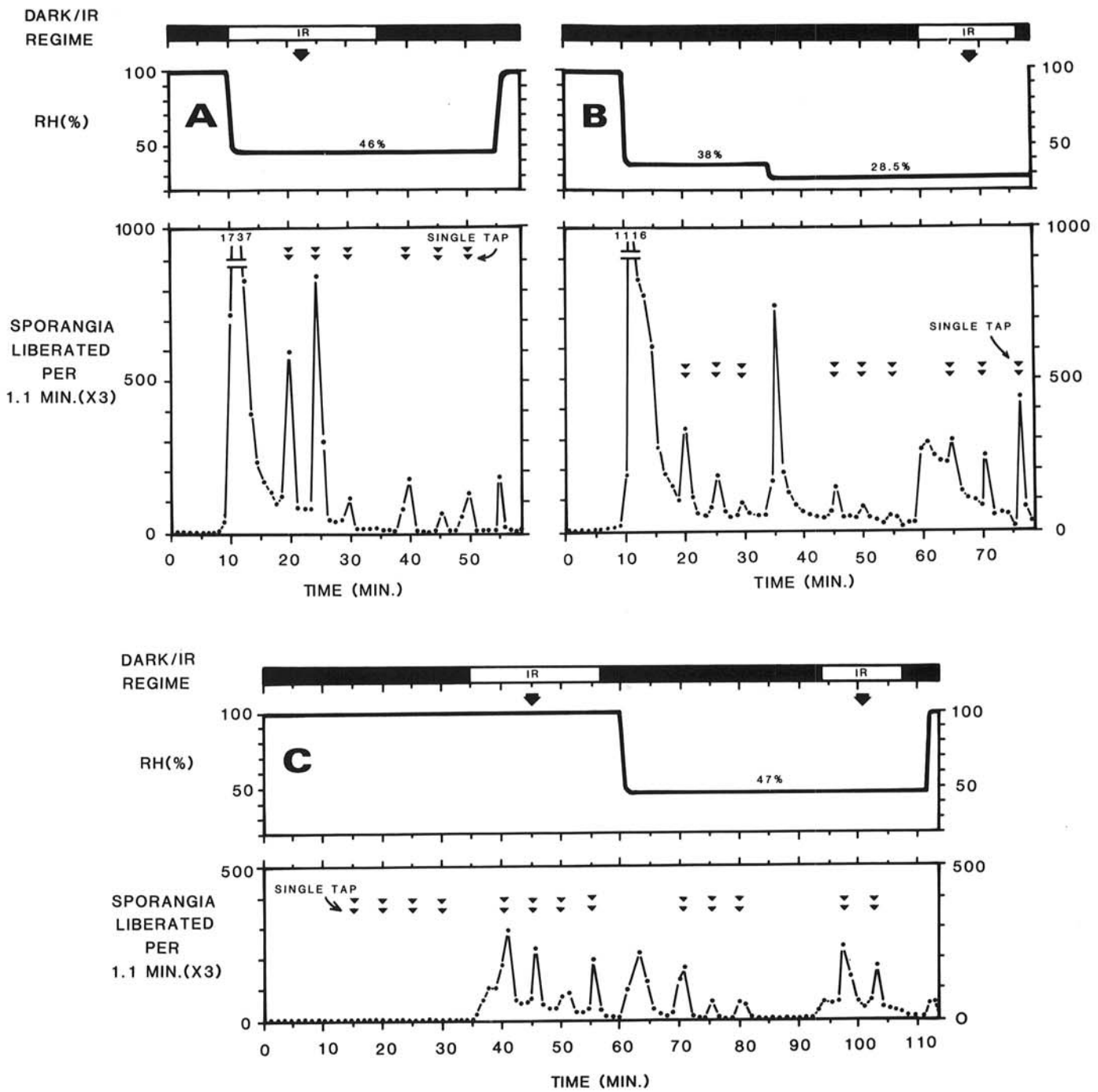

Fig. 6. The results of three experiments to determine the interaction of relative humidity (RH), exposure to red-infrared radiation (IR), and vibration on spore liberation by *Peronospora destructor*. **A,** Vibrational release from onion leaves at 46% RH in darkness and also exposed to IR. **B,** Vibrational liberation at low RH (38 and 28.5%) with and without exposure to IR. **C,** Vibrational liberation both at saturation and decreased RH with and without exposure to IR (air speed, 0.5 m/sec; air temperature range 17.5–18.0 C; tap intensity same as described in Fig. 5).

four fungi is most responsive to RH changes in the lower ranges (eg, less than RH 59% for *P. destructor*). The actual change in RH appears to be important in triggering discharge rather than low RH per se. Typically, as the RH is lowered there is an accompanying major release of spores, which gradually declines to near zero as the RH stabilizes at a lower level. Release triggered by increasing RHs follows a similar pattern.

In none of the fungi so far investigated (13–15) were all the spores present on a sporulating lesion discharged in response to a single humidity cycle or to a single exposure to IR. Thus, whenever humidity cycles or IR exposures were repeated, each repetition was normally accompanied by release of another batch from the remaining spores. In all of these studies, it must be emphasized that humidity cycles and IR exposure were brief in comparison to similar events that would occur under natural conditions. Therefore, one must be cautious in interpreting their significance under natural conditions. The actual role of RH changes in spore release has not been explained. One might assume that changes in atmospheric RH would influence the strength of the bond between spore and sporophore, yet the discharge of spores accompanying both decreasing as well as increasing RHs do not support this hypothesis. A recent discovery that RH changes significantly influenced electrical charges of leaf surfaces (17; C.M. Leach, *unpublished*) could explain the RH effects in relation to the electrostatic mechanism postulated to explain active spore discharge (16,18).

IR has a major effect on spore discharge by several of the Fungi Imperfecti (13,14), and we have found that this is also true for liberation of sporangia by *P. destructor*. Exposure of *P. destructor* to IR at reduced RHs was very effective in both triggering and enhancing spore discharge. This IR effect was even evident when onion leaves were exposed in a saturated airstream (100% RH), although the numbers of spores liberated were much reduced compared to similar exposures at reduced RHs. The role of IR in spore discharge is not understood. Although it is a simple matter to demonstrate that unfiltered IR can influence leaf surface temperatures, air temperature, and RH, it is more difficult to determine its effects within the "microclimate" of sporophores. We doubt, however, that the IR response can be explained solely on the basis of these more obvious effects. A less obvious hypothesis is that the IR directly affects the strength of the bond between spore and sporophore, perhaps through dehydration of cell wall components. Another possible explanation for the pronounced effect of IR on spore discharge is its influence on leave surface charges. Experimental studies on bean (*Phaseolus vulgaris*) and corn (*Zea mays*) leaves subjected to changes in RH and exposure to IR have revealed some profound and rapid changes in electrostatic fields (17; C.M. Leach, *unpublished*). These changes may be involved in the electrostatic mechanism postulated for active spore discharge in several fungi (11,16,18).

Onions grown under field conditions are normally subjected to much buffeting by wind and rain or the impact of water drops from overhead irrigation. Cruickshank (3) suggested that release of sporangia by *P. tabacina* might involve mechanical shock. Bainbridge and Legg (2) found that the flapping of barley leaves is sufficient to liberate conidia of *Erysiphe graminis*. Hirst and Stedman (8) have demonstrated the importance of the impact of raindrops on leaves in causing a "puff" type of liberation of dry spores, a form of liberation that differs from that caused by rain splash. "Rain tap and puff" liberation (8) is evident in the results of a number of field studies in which major release of conidia accompanied brief rain showers (10,19,22). We have demonstrated experimentally a "puff" type of spore discharge by *P. destructor* (Figs. 5 and 6) triggered by leaf vibrations but not under all conditions. Greatest release of spores occurred when leaves were vibrated at low RHs and this was further enhanced when leaves were exposed to IR. However, some release occurred even at saturation (100% RH) whenever leaves were irradiated with IR, but not in darkness. In general, vibrational liberation of sporangia by *P. destructor* was similar to that reported for *D. turcica, D. maydis* (14), and *P. oryzae* (15). Photographic studies of vibrationally discharged sporangia of *P. destructor* (18) and conidia of *D. turcica* (16) have revealed that this form of discharge is not merely the dislodgement of spores. Spores are actually actively propelled several millimeters along highly organized trajectories that are parallel and perpendicular to the leaf surface as is shown in Fig. 1.

Under highly controlled conditions, we have shown that humidity changes, IR, and vibration can have major effects on the liberation of sporangia by *P. destructor*. These results are in general agreement with field studies (7). Additional studies are now needed to relate these findings to the effects of wind and rain on spore liberation.

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