Spore Discharge by the Pecan Scab Pathogen, *Cladosporium caryigenum*

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ABSTRACT

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Conidial discharge by *Cladosporium caryigenum* from heavily diseased pecan leaf and nut shuck (involucre) tissue was studied under controlled exposure to relative humidity (RH), temperature, vegetative wetness (VW), and infrared radiation (IR). Spore release was minimal as RH decreased from near saturation to 40%, but further decrease stimulated considerable spore discharge which was enhanced by exposure to IR (>40 $\mu E \cdot m^{-2} \cdot sec^{-1}$). Sustained periods of constant RH <40% also favored spore release but were less favorable than RH that fluctuated above and below 40%. Spore release was stimulated by short (circa 1 min) IR exposures and brief (2-min) RH changes. Spore release was recorded only as the specimen dried off or when leaf wetness was maintained <15%. Vibration triggered spore release at low RH, especially when specimens were exposed to IR, but vibrational effects were considerably less effective than those reported previously for other hyphomycetes.

Additional key words: epidemiology, *Fusicladium effusum*.

Cladosporium caryigenum (Ell. et Lang.) Gottwald, comb. nov. (syn. *Fusicladium effusum* Wint.) (3) causes a severe foliar and nut disease of pecan, *Carya illinoensis* Koch. (2,10). The disease, which is known as pecan scab, is prevalent in most pecan-growing areas.

Few attempts have been made to explain the spore discharge mechanism of this pathogen. Valli (12) speculated that spores are either shaken loose from scab lesions on leaves, twigs, and shucks (involucre) and deposited on nearby susceptible tissues by wind or are spread by the dripping action of rain, dew, or fog from old lesions onto new tissues. Nolen (10) also believed that local dispersal was the result of rain splash and attributed long-distance dissemination to wind. Converse (1) demonstrated localized dispersal of conidia by the dripping action of water both in greenhouse and orchard experiments. To determine if wind removed spores in the laboratory, Converse directed air currents moving at ~4.47 m/sec (28 C and 60% relative humidity [RH]) over severely infected nuts and onto greased microscope slides. Spores of the pecan scab fungus did not readily detach from the conidiophores under these conditions.

Daily release of *C. caryigenum* spores in orchards has recently been related to periods of rapidly decreasing relative humidity and vegetative wetness (4). In orchards there was a diurnal periodicity of spore discharge with major release always occurring during daylight hours.

Spore discharge has been investigated for several other *Cladosporium* species. Spore release of *C. herbarum* was correlated with rising temperatures, daylight, and declining RH (11). Numbers of spores of *Cladosporium* sp. in the atmosphere were greatest during periods of low relative humidity (<60%) during daylight hours (6). In contrast to the preceding reports, which indicate spore release to be associated with decreasing RH, Harvey (5) demonstrated in wind tunnel experiments with six *Cladosporium* species (*C. herbarum, C. sphaerospermum, C. cladosporioides, C. elatum, C. resinae,* and *C. macrocarpum*) that spore catches were approximately eight times greater during "wet air" periods, when water droplets were atomized into the air stream, than during "dry air" periods. The stimulatory effects of red-infrared radiation on spore release have been recently demonstrated (7–9).

An apparatus was recently developed that enables precise and rapid changes in relative humidity while maintaining constant temperature and air flow has allowed much more critical investigation of spore discharge by many fungi (7–9).

The purpose of this study was to further clarify the relationship of environmental factors to spore discharge by *C. caryigenum* under precisely controlled conditions.

MATERIALS AND METHODS

An apparatus similar to that designed by Leach (7) was constructed and used in all experiments. The apparatus was located in a room where ambient air temperature was ~20 ± 2 C. Air temperature in the specimen chamber was measured by a single thermocouple located adjacent to the specimen. Relative humidity was measured by a wet-bulb thermocouple placed in the airflow at the entrance to the chamber. A vegetative wetness probe was located inside the specimen chamber in the airflow immediately in front of, but not obstructing, the specimen holder. The vegetative wetness probe consisted of a chemically treated printed circuit whose surface resistance changes with hydration. The probe was connected in series with two standard D-cell flashlight batteries and then to a three-pen strip chart recorder. Electrical signals from temperature and relative humidity probes were also recorded on the strip chart. Air velocity was set at 0.5 m/sec by the use of a thermoanemometer (Type 8500, Alnor Instrument Co., Niles, IL 60648) and held constant in all experiments. Light was supplied by a 250-W red-infrared bulb (General Electric). Spectral output of this bulb was ~60% in the red-IR range and ~40% throughout the remaining visable range. Light intensity was measured with a quantum radiometer-photometer (model Li 185, Li-Cor. Inc., Lincoln, NE 68504) equipped with a near-infrared sensor with a 70-nm band width centered at 790 nm. The sensor was placed inside the specimen chamber at the level of the specimen to calibrate intensity settings. To reduce adverse thermal effects, the light beam was directed at a mirror below the specimen chamber and reflected at a 90-degree angle onto the specimen after passing through a water filter 10 cm deep. Light intensity was controlled by a rheostat control. Full light intensity was maintained in all experiments except experiment 9 (Fig. 9B) in which it was increased by increments from 0 to 60 $\mu E \cdot m^{-2} \cdot s^{-1}$. Vibrations used to simulate

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mechanical disturbances on the phylloplane were instantaneous and standardized by dropping a 50-g weight from a height of 5 cm onto the specimen chamber, as described by Leach (7,8).

Temperature varied ± 1.5 C except during one experiment designed to determine the effect of temperature on spore release (Fig. 9A and B). Spore trap tapes of clear cellophane were coated with a base mixture of 10% polyvinyl alcohol in distilled water and dried for 24 hr. A second adhesive coat of Vaseline petroleum jelly plus 10% paraffin, thinned to a soft paste with toluene, was brushed onto the slide. The spore trap slide was gently heated on a hot plate until all brush streaks in the trapping surface disappeared, cooled, and placed in the spore trap. Spores released from the specimen exited the specimen chamber through a slit-aperture and were impacted on the spore-trapping surface. The spore trap consisted of a modified time clock and pulley arranged to draw the spore trap slide along a track in front of the aperture at a rate of 11.5 mm/hr. This provided a resolution of 1.6 min of collection time per microscope field width band across the slide when viewed at ×400 and 1.3 min when viewed at ×600. The exposed tapes were mounted in acid-fuchsin-lactophenol and made permanent by incorporation of 1% polyvinyl alcohol in the stain.

Heavily scabbed pecan nut shucks, leaves, and petioles from trees of cultivar Cherokee were collected in the morning and either used immediately or held in a moist vegetable crisper for 1–4 days to stimulate production of new conidia. Sporulating lesions were excised and affixed to a glass microscope slide with Parafilm and Teflon tape. Leaf lesions were ~2–4 mm in diameter. Sometimes pieces of nut shuck (involucre) tissue ~10 mm square were also used. A moistened piece of filter paper was placed between the specimen and the slide to deter rapid drying of the specimen during periods of low relative humidity. The specimen slide was attached (sporulating surface downward) to the specimen chamber holder with rubber bands.

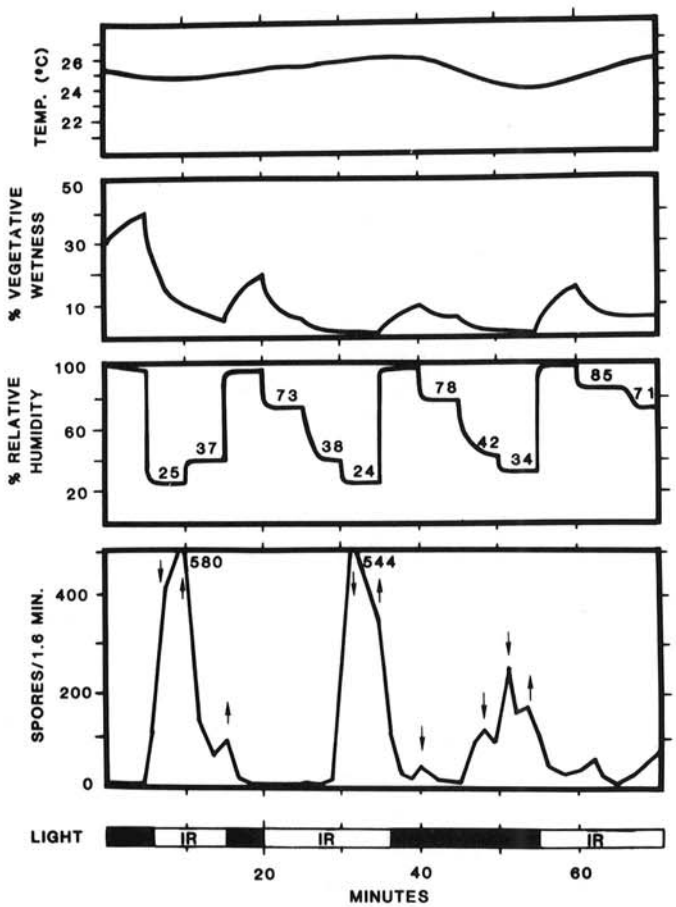

Fig. 1. Spore release of *Cladosporium caryigenum*. Comparison of rapid decrease in RH from saturation to 25% RH versus a gradual (stepwise) decrease in the presence of IR. Absence of IR is shown in the third decrease of RH. Arrows indicate spore liberation associated with decreasing or increasing RH.

RESULTS

In a series of laboratory experiments, the effects of relative humidity (RH), vegetative wetness (VW), and light period on spore release was measured.

The effect of relative humidity and red-infrared radiation on spore release. Results of preliminary experiments revealed that exposure of the specimen to repeated cycles of RH, light, and vibration during a 24-hr period did not exhaust its ability to discharge spores and only small variations in spore release from repeated identical conditions were detected. To insure consistent release, all experiments were conducted within a 2-hr period. The experiments discussed below were designed to demonstrate the reaction of *C. caryigenum* to various conditions and to allow comparisons of different sets of conditions within each experiment. The design of these experiments was based on the reactions of *C. caryigenum* in numerous preliminary experiments. In the first experiment (Fig. 1), the first two periods of decreasing RH were combined with infrared radiation (IR) followed by a third period of decreasing RH conducted in complete darkness. During the first two periods of decreasing RH, spores were released as the humidity was decreased in the presence of IR. In the second period, maximum spore release occurred only when the RH decreased from 38 to 24%. Little spore liberation was detected at RHs above 40%. In total darkness, spore release was less than half that observed in the light, with some liberation at 40% RH. Spore release was minimal when RH was near saturation both in the presence of IR and in darkness.

Peaks of spore release corresponding to "RH valleys" or periods of low RH were observed in all subsequent experiments (Figs. 2–6). Spore release was always greatest during exposure to IR irradiation and low RH (Figs. 2 and 3). Significant spore release was first recorded in darkness as RH declined from 42 to 26% RH (Fig. 2). During the following 10 min at 26% RH, release first diminished and then stabilized at a lower rate. During the second period of lowered RH in the presence of IR, the maximum discharge again corresponded to the 43 to 26% RH shift, although subsequent liberation continued at a somewhat higher rate than that recorded in darkness.

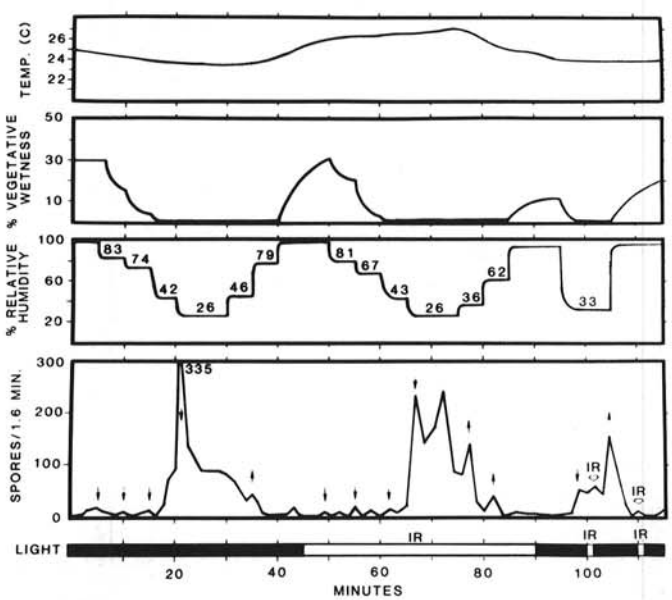

Fig. 2. Spore release by *Cladosporium caryigenum*. Comparison of slow (stepwise) decrease or increase in RH in presence of IR versus darkness. Black arrows indicate spore release associated with decreasing and increasing RH. White arrows indicate spore liberation corresponding to 1-min IR exposures.

To separate the triggering effects of RH shifts and IR irradiation, experiments (Figs. 3 and 4) were conducted in which the specimens were briefly exposed (1, 2, or 5 min) to IR while RH was held constant. These brief IR exposures were separated from RH changes by a minimum of 2.5 min, which in previous experiments was found to be sufficient time to separately identify the effects of IR and RH. Spore release was stimulated by decreasing RH; however, there was also a definite liberation associated with IR exposures of 1, 2, and 5 min (Fig. 4). Downward shifts of 97 to 45% RH (Fig. 3) and 90 to 50% RH (Fig. 4) in darkness also stimulated spore release, but only at reduced rates. The shortest IR exposures (1 min) usually stimulated release at all RH levels, although greater response was recorded at low RH (Fig. 4). Spore release corresponding to shifts from low to high RH were inconsistent. Generally a small peak of release was observed prior to the final decrease in spore liberation; however, such shifts occasionally depressed spore release.

Rapid changes of RH on spore release, comparable to those encountered in the orchard after summer rain showers by Gottwald and Bertrand (4) were also investigated (Figs. 5 and 6). One experiment (Fig. 5) involved RH changes of ~100-50-100-25% RH repeated in both light and darkness with each RH period (saturation or low RH) 5 min in duration. Discharge consistently corresponded to lowering RH to either ~50 or ~25% in light or darkness. Liberation at saturation was always negligible. Approximately 10 times as many conidia were released when RH was decreased from near saturation to ~25% as when it was

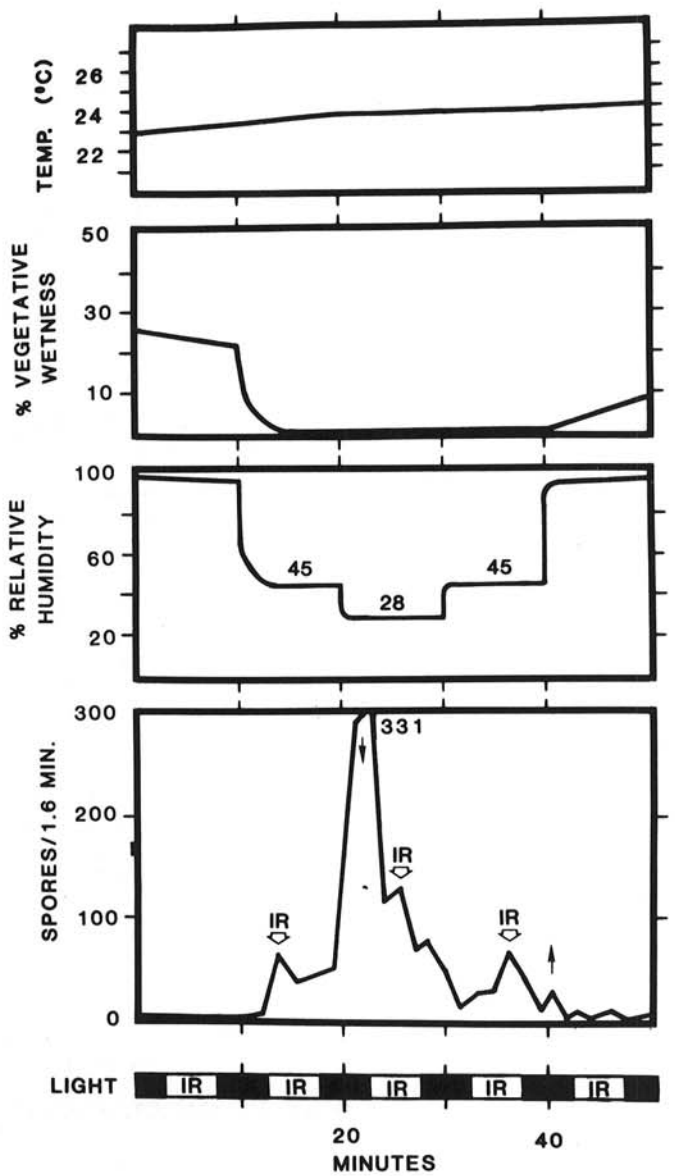

Fig. 3. Conidium discharge by *Cladosporium caryigenum*. Effects of changes in RH and IR separated by 2.5 min. Black arrows indicate spore release associated with decreasing and increasing RH. White arrows indicate spore liberation stimulated by 5-min IR exposures.

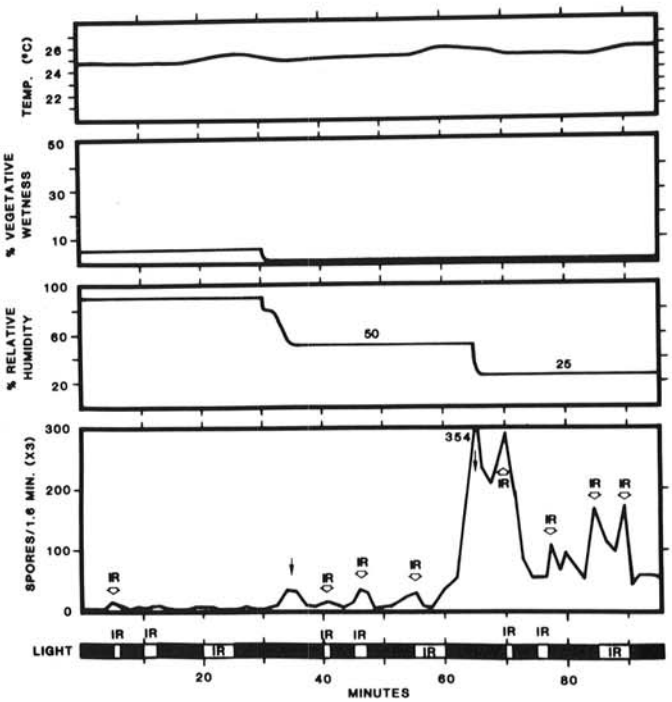

Fig. 4. Effect of length of IR exposure (1, 2, or 5 min) on spore discharge by *Cladosporium caryigenum* subjected to different atmospheric humidities. Black arrows indicate spore liberation associated with decreasing RH. White arrows indicate spore release stimulated by IR exposure.

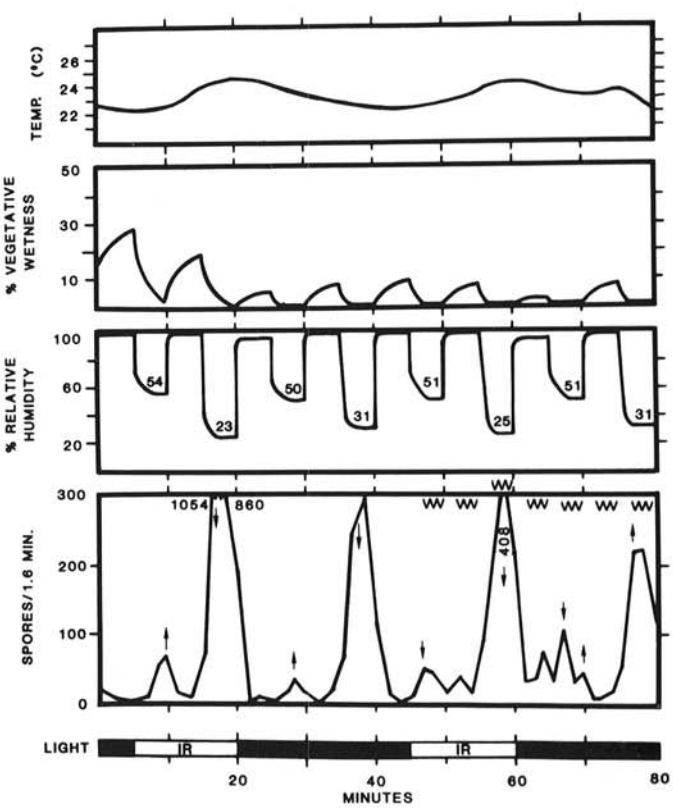

Fig. 5. Effect of brief humidity changes on spore release by *Cladosporium caryigenum* as related to response to IR and vibration. Arrows indicate spore liberation associated with decreasing and increasing RH. Zigzag line indicates points of multiple vibration separated by 1 min.

decreased only to ~50%. Exposure to IR during the humidity cycles increased spore discharge approximately threefold compared to that in darkness. In another experiment, very rapid RH changes from near saturation to 63–67% and to 28–39% RH for 1 min had no detectable effect on spore release (Fig. 6). However, rapid decreases from saturation to 20–23% RH, sustained for 2 min, induced considerable release of conidia in both light and darkness. Again, release in light was nearly three times that in darkness. Reversing this pattern, ie, rapid increases in RH from a low level to a higher level (20 to 38–40% and 20 to 98% RH) caused only minor reduction of spore release in both light and darkness when sustained for 1 min; however, when the rapid (2-min) changes from 20 to 98% RH were sustained in both light and darkness, spore release was greatly reduced (Fig. 6). The greatest spore liberation in all experiments involving brief RH changes occurred when RH was lowered from 98 to 25% in the presence of IR.

The effect of instantaneous vibration on spore release. This effect was investigated in three separate experiments (Figs. 5,7, and 8). Vibration at RH above 50% whether conducted in light or darkness had no effect on spore release in any of the experiments. Vibration in darkness at lower humidities (RH <50%) only mildly stimulated spore release (Figs. 7 and 8). However, several successive instantaneous vibrations during and preceding rapid decreases in RH had an inhibitory effect, slightly depressing the expected spore release (Fig. 5 [compare the second and fourth major peaks]). Vibration at low RH (<50%) in the presence of IR stimulated conidium release (Figs. 7 and 8). Several successive instantaneous vibrations during and following a rapid RH decrease appeared to decrease the expected peak of spore release.

Simulation of early morning drying conditions. In pecan orchards, vegetative wetness and relative humidity normally decrease as temperature and intensity of solar radiation increase. Simulation of early morning drying conditions in light and darkness was studied (Fig. 9A and B). Very few spores were released in darkness, but considerable numbers of them were liberated in light (Fig. 9B). The first release of conidia occurred as RH decreased from 41 to 22%. Spore release was further stimulated as IR intensities increased above 40 $\mu E \cdot m^2 \cdot s^{-1}$.

The effect of vegetative wetness on spore release. No attempt was made to directly control percent vegetative wetness (VW) in any of the experiments (Figs. 1–9). However, close measurement of this parameter was deemed necessary because of the importance attributed to vegetative wetness in relation to spore release by Gottwald and Bertrand (4). Vegetative wetness was directly related to RH and was therefore only incidental to spore release. RH near saturation generated by the apparatus increased vegetative wetness, while RH below saturation decreased wetness. Vegetative wetness stabilized at 0% when RH was 85% or below. Liberation of conidia occurred when VW was <15%, decreasing, or maintained at 0%.

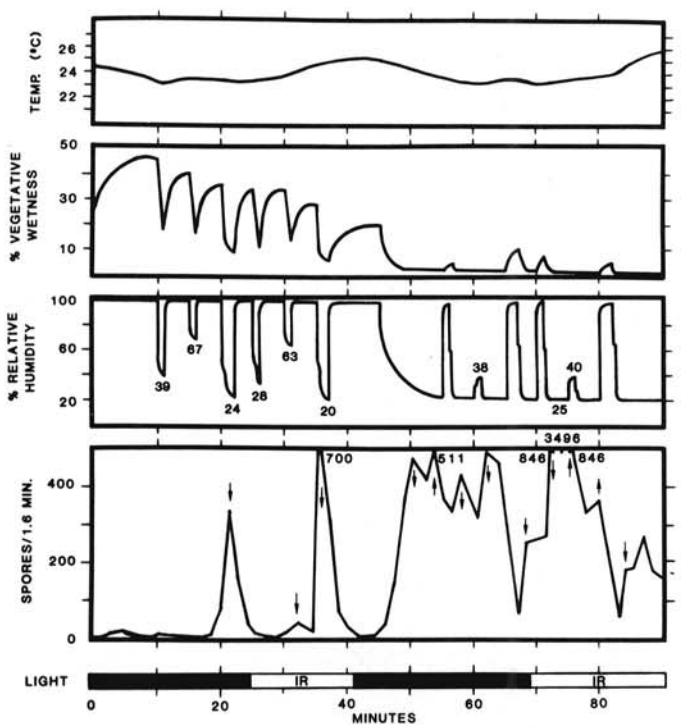

Fig. 6. Spore discharge by *Cladosporium caryigenum* in response to rapid decreases and increases in RH of short duration. Arrows indicate spore liberation in response to decreasing or increasing RH.

DISCUSSION

Greater airborne spore concentrations of *C. caryigenum* are found in pecan orchards during the morning when RH decreases, dew evaporates from the phylloplane, and temperature and intensity of solar radiation increase (4). Spores are also known to be liberated after rain showers. Results of the simulation of early morning drying conditions (Fig. 9B) duplicate those reported from the orchard by Gottwald and Bertrand (4). Rapidly decreasing RH was especially conducive to liberation of conidia. This effect was enhanced further by the presence of infrared radiation. Increasing RH and sustained levels above 40% were inhibitory to conidia discharge. IR had only slight stimulatory effect in this RH range (Figs. 1–6).

The effect of decreasing RH on spore release and its enhancement by IR has been demonstrated previously for two other genera of hyphomycetes (8,9). *Drechslera turcica* and *D. maydis* responded similarly to decreasing RH and exposure to IR. Rapid changes in VW and RH caused by intermittent rainfall and drying periods reported by Gottwald and Bertrand under orchard conditions (4) were simulated in the laboratory (Fig. 6). The tremendous spore discharge induced by rapid RH decreases combined with IR exposure under laboratory simulation is indicative of the large increases in aerial spore concentration experienced during drying periods between intermittent rain

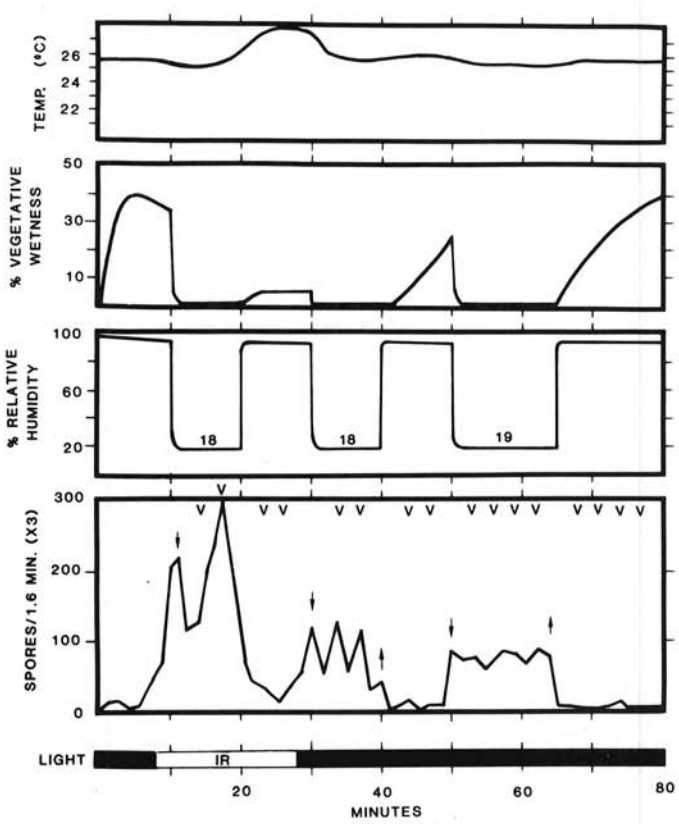

Fig. 7. Effect of vibration on spore discharge by *Cladosporium caryigenum* at low RH or near saturation in darkness or while exposed to IR. Arrows indicate spore liberation in response to decreasing and increasing RH. V = points of instantaneous vibration.

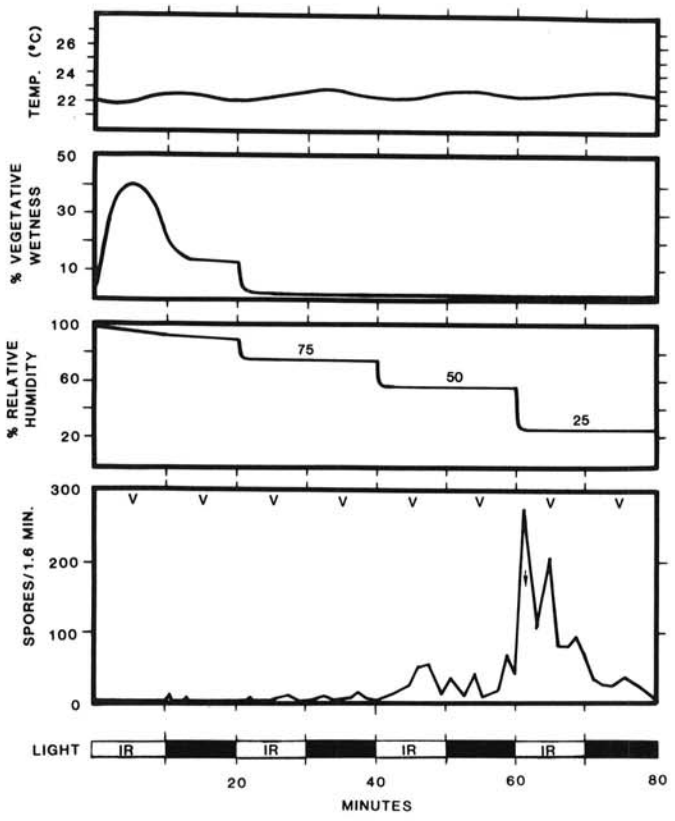

Fig. 8. Influence of vibration on spore release by *Cladosporium caryigenum* in IR and darkness at various RH levels. Arrow indicates spore liberation in response to decreasing RH. V = points of instantaneous vibration.

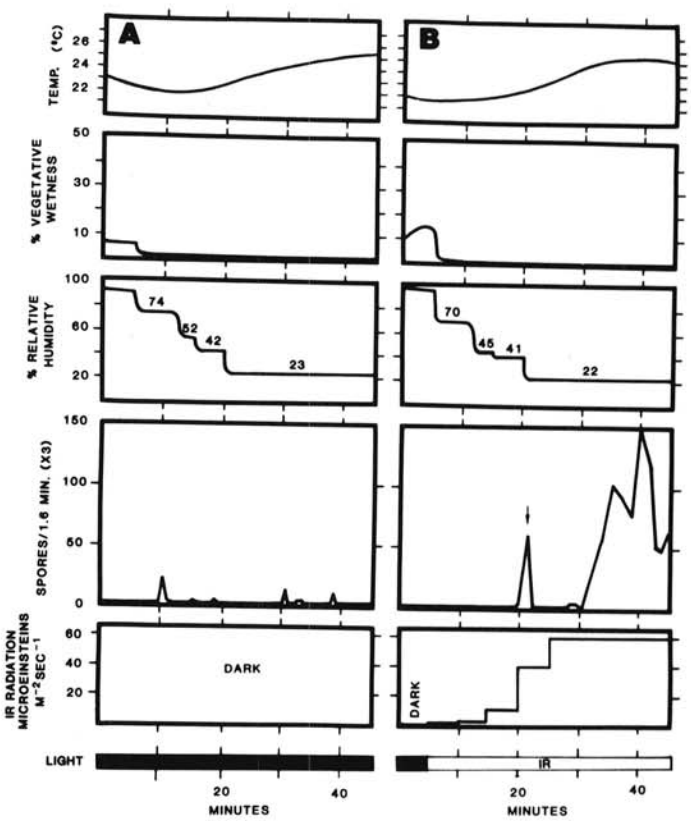

Fig. 9. Spore release by *Cladosporium caryigenum* during simulation of early morning orchard drying conditions of decreasing RH and VW and increasing temperatures. **A,** Experiment conducted in darkness. **B,** Same sample, but conducted in a regime of increasing IR intensities simulating increasing solar radiation. Arrow indicates spore liberation in response to decreasing RH.

showers.

Although spore release by *C. caryigenum* was sensitive to vibration, especially in the presence of IR, it was by no means as sensitive as that of *D. turcica, D. maydis,* or *Pyricularia oryzae.* Compared to those hyphomycetes, the reaction of *C. caryigenum* to vibration was much reduced. Reaction to vibration at RH near saturation was nil (Fig. 8). IR was found to stimulate the effect of vibration at low RH where the other fungi showed no such response (Figs. 7 and 8). However, a succession of closely repeated vibrations resulted in a disruption of the expected peak of spore release at low RH in both light and darkness (Fig. 5). All three hyphomycetes investigated by Leach were much more responsive to vibrational release (8,9).

The results of this study are consistent with field results for aerial spore concentrations of *C. caryigenum* by Gottwald and Bertrand (4) and those of Pady et al (11) and Hirst (6) for other *Cladosporium* species, which show significant increases in aerial spore concentrations following decreases in RH. Converse's conclusion that *C. caryigenum* was not air-dispersed was based on an experiment in which temperature, wind speed, and RH of 60% were constant (1). The present study indicates that release of spores of *C. caryigenum* above 40% RH is minimal and is stimulated when RH is <40% and is also stimulated by abrupt increases and decreases in RH. Similarly, Harvey's (5) conclusion, based on studies of several *Cladosporium* species, that "wet air" was more conducive to spore release than "dry air" was based on wind tunnel experiments in which wet air periods were generated by 30-sec atomizations of water droplets into the air stream at 15-min intervals. Such intermittent atomization of water would likely have caused rapid fluctuations in RH, with the consequent stimulation of spore discharge demonstrated by *C. caryigenum* in this study. Our results agree with and support the conclusions of other researchers who have studied spore release by *C. caryigenum* and other *Cladosporium* spp.

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