Factors Affecting Spore Liberation by *Cladosporium carpophilum*

T. R. Gottwald

Research plant pathologist, USDA-ARS, Southeastern Fruit and Tree Nut Research Laboratory, Byron, GA 31008.

Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the USDA or imply approval to the exclusion of other products that also may be suitable.

The author wishes to thank Raymond Pate and James Stucky for technical assistance and C. M. Leach for a most concise and helpful review of this manuscript.

Accepted for publication 19 May 1983.

ABSTRACT

Gottwald, T. R. 1983. Factors affecting spore liberation by *Cladosporium carpophilum*. Phytopathology 73:1500-1505.

Conidial discharge by the peach scab fungus, *Cladosporium carpophilum*, from heavily diseased peach fruits was studied under controlled relative humidity (RH), temperature, wind speed, and red-infrared radiation (IR). As RH decreased from near saturation to 40%, spore release was minimal, but further decreases stimulated considerable spore discharge. Sustained periods of constant RH <40% also favored spore release which was enhanced by exposure to IR (>40 $\mu E \cdot m^{-2} \cdot sec^{-1}$). Spore release was stimulated by short IR exposures (~2 min) and brief (2-min) RH changes. Spore release at low RH was increased slightly by vibration, created by dropping a 50-g weight 5 cm onto the specimen chamber, especially when specimens were exposed to IR.

Additional key words: epidemiology, *Prunus persica*.

Peach scab, caused by *Cladosporium carpophilum* Thum., is an important disease of peach (*Prunus persica* [L.] Batsch) in growing areas east of the Rocky Mountains in the USA. The pathogen causes circular olivaceous to black spots on fruit, twigs, and leaves of the host. Fruit lesions frequently coalesce to cover much of the fruit surface, seriously detracting from its appearance, quality, and market value. When infections are severe, cracking of the fruit often occurs, predisposing the fruit to brown rot caused by *Monilinia fructicola* (6).

Keitt (6) found that dry conidia of *C. carpophilum* were difficult to dislodge from their conidiophores, but were easily detached in free water, and stressed the importance of rain for liberation. Lawrence and Zehr (7) recently demonstrated aerial dissemination of *C. carpophilum* conidia in an orchard. Aerial spore concentrations during both dry and moist periods increased after the "calyx-split" stage when mean daily temperatures exceeded 15 C. Aerial release of the closely related *Cladosporium caryigenum* (Ell. et Lang.) Gottwald was related to periods of rapidly decreasing relative humidity (RH) and vegetative wetness, with major release always occurring during daylight hours (2). In *C. herbarum*, spore discharge was associated with rising temperatures, daylight, and declining RH (13). Hirst (5) reported that numbers of spores of *Cladosporium* sp. in the atmosphere were greatest during periods of low RH (<60%) during daylight hours (5). Rich and Waggoner (14) reported double daytime maxima in airborne spore concentration of *C. cucumerinum* and concluded that a light misting rain can wash spores from the leaf surface even though there is no vibration. The mist also scrubs spores from the air. Turbulent storms can jar and whip leaves and dislodge conidia efficiently. In contrast to the preceding reports, which indicate greater spore release to be associated with decreasing RH, wind tunnel tests by Harvey (4) demonstrated that *C. herbarum, C. sphaerospermum, C. cladosporioides, C. elatum, C. resinae*, and *C. macrocarpum* 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. In reviewing Harvey's report, Gottwald (1) pointed out that these greater spore catches were more likely due to the effects of fluctuating RH caused by intermittent inductions of saturated air into an air stream with otherwise moderate RH rather than to the "wet air" periods themselves.

Spore release of *C. caryigenum* on pecan under controlled conditions was related to fluctuating RH and sustained periods of RH <40%. This effect was enhanced by exposure to red-infrared irradiation (IR) and vibration (V) (1).

The Leach apparatus, which enables precise and rapid changes in RH while maintaining constant temperature and air flow, has been used recently to investigate the spore liberation systems of several other Hyphomycetes as well as Ascomycetes and an Oomycete (3,4,8,10–12).

The purpose of this study was to investigate spore discharge by *C. carpophilum* under precisely controlled laboratory conditions to clarify the effects of environmental factors on spore discharge and thereby gain additional insight into the epidemiology of this important peach pathogen.

MATERIALS AND METHODS

Infected peach cultivar Redglobe and unnamed seedling peach fruits were collected near Byron, GA, in June and July of 1981 and 1982. Experiments were conducted on the fruits either the day they were collected when this followed a rainy period conducive to sporulation, or after 2–6 days of incubation in a moist chamber when fruits were collected following a dry period. Infected portions of fruit (~20–30 mm in diameter) were excised and mounted on brass specimen holders with copper wire. The slide assembly was then suspended in the specimen chamber in an inverted position (Fig. 1).

Sporulating cultures of *C. carpophilum* were used in a few cases when nonsporulating lesions were found in the field. An isolate obtained from cultivar Redglobe fruit was transferred from an oatmeal agar culture to 2.5-cm-square pieces of sterile cellulose sponge infiltrated with oatmeal agar. The sponge cultures were incubated for ~30 days at 26 C under a 12-hr-light/dark regime 1050 $\mu E \cdot m^{-2} \cdot s^{-1}$ until a profusely sporulating fungal mat had developed. Sponge cultures provided the firm specimen substrate needed for easy mounting and were more resistant to drying than agar cultures. Sponge cultures were mounted on specimen holders and used immediately. Comparative tests revealed that sponge cultures reacted to all environmental conditions exactly the same as fruit lesions except more spores were released per event from the

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.

sponges.

Environmental factors (RH, temperature, air flow, and light) were precisely controlled in an apparatus similar to that described and used by Leach (1,9). The apparatus was located in an air-conditioned laboratory with an ambient air temperature of ~20 ± 2 C. Air temperature in the specimen chamber was measured by a copper-constantan thermocouple adjacent to the specimen. Another similar thermocouple, placed in the air flow at the entrance to the specimen chamber, was used as a dry bulb comparison for RH determinations. RH was measured continuously with a copper-constantan thermocouple psychrometer also located in the air stream at the entrance to the specimen chamber. Electrical signals from temperature and RH thermocouples were recorded on a three-pen strip chart recorder. Air velocity was set at 0.5 m/sec with the aid of a thermoanemometer (Type 8500, Alnor Instrument Co., Niles, IL 60648) and held constant in all experiments. 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. The light beam was directed at a mirror below the specimen chamber and reflected at a 90-degree angle onto the specimen. The infrared light source increased temperature at the level of the specimen by 0.5–0.6, 2.0–2.2, or 2.5–3.0 C for exposures of 10, 20, or 30 min, respectively. When the light was turned off, chamber temperature resumed its previous preradiation level within a few minutes. Light could be maintained at any desired intensity between 0 and 60 $\mu E \cdot m^{-2} \cdot s^{-1}$ with a rheostat. Vibrations used to simulate buffeting of the fruit by wind or rain were instantaneous and standardized by dropping an arbitrarily chosen 50-g weight from a height of 5 cm onto the specimen chambers.

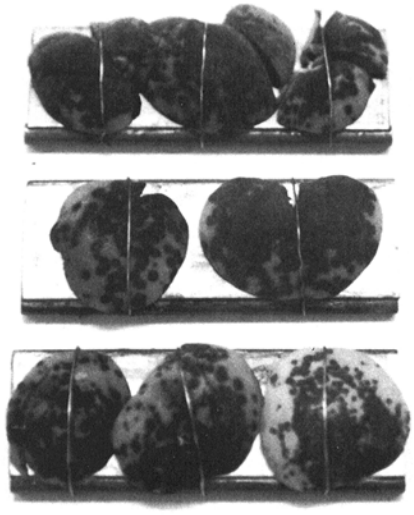

Fig. 1. Spore trap slides mounted with excised portions of *Cladosporium carpophilum* infected fruit. Diseased tissue is attached to the brass slide surface with copper wire. The slide assembly is then suspended in the specimen chamber in an inverted position.

The spore trap consisted of a modified clock and pulley arranged to draw a spore trap tape along a vertical track across a slit aperture (0.5 × 17 mm) at 11.5 mm/hr. Spores released from the specimen exited the specimen chamber through the slit-aperture and impacted on the spore-trapping surface of the tape. This mechanism provided a resolution of 1.6 min of collection time per microscope field band width across the slide at ×400. Clear cellophane spore trap tapes were coated with a base mixture of 10% polyvinyl alcohol in distilled water followed by a second adhesive coat of Vaseline petroleum jelly plus 10% paraffin. Exposed tapes

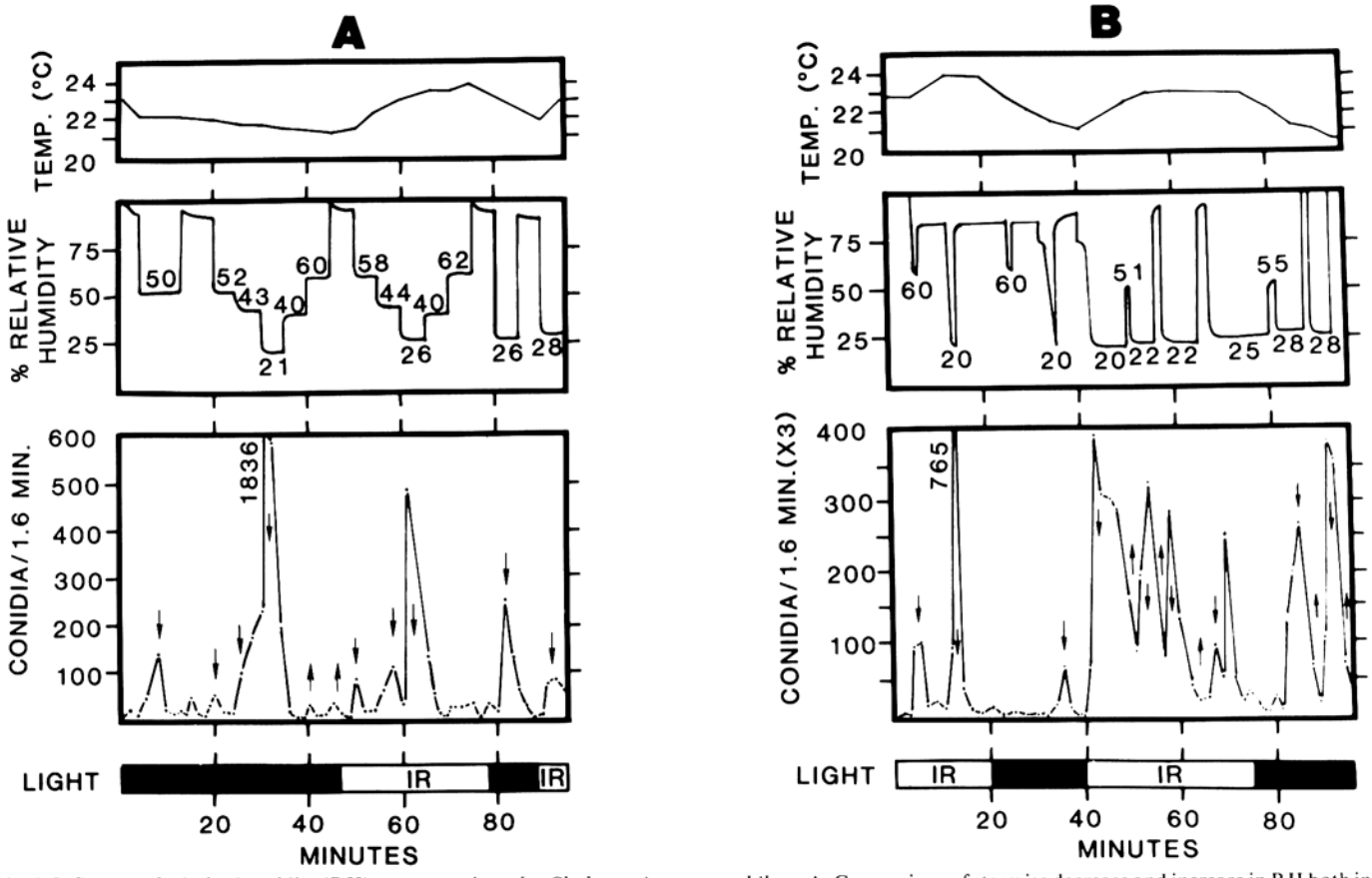

Fig. 2. Influence of relative humidity (RH) on spore release by *Cladosporium carpophilum*. **A,** Comparison of stepwise decrease and increase in RH both in darkness and with exposure to infrared irradiation. **B,** Spore release associated with rapid fluctuations of RH (1–2 min duration). Black arrows indicate peaks of spore release in response to decreasing (↓) or increasing (↑) RH.

were mounted in acid-fuchsin lactophenol and made permanent by incorporation of 1% polyvinyl alcohol in the stain.

Specimen exhaustion (decreasing response to repeated stimulus of the same type over time) occurred when specimens were used over a prolonged period or in more than one experiment. Therefore, new specimens were used in each experiment and experiments were limited to 140 min or less.

Fig. 3. Example of detached trichome with adhering hypha of *Cladosporium carpophilum* which were frequently seen on spore trap slides. Phase-contrast micrograph (×230).

RESULTS

The experiments described below were designed to demonstrate the effects of various environmental parameters on spore release by *C. carpophilum* and to allow comparison of different combinations of conditions within each experiment.

Although numerous experiments were conducted on *C. carpophilum*, only those that best demonstrate certain responses have been included. All unpresented experiments and data further substantiate the findings reported herein; however, due to variation in sporulation among the specimens used in these experiments, they did not reveal all effects as clearly.

Effects of RH and IR on spore release. In the first experiment (Fig. 2A), RH was initially decreased from near saturation to 50% RH and increased back to near saturation again. The next two cycles were stepwise decreases in RH from saturation to ~21 and 26% RH and back, first in darkness then in IR. The final two cycles were from saturation to ~27% RH, first in darkness then in IR. Spore release was great whenever the humidity was decreased below ~40% (Fig. 2A—30, 60, and 80 min). Whenever RH was increased from below 40% to above 40% RH, spore release quickly declined (35, 65, and 85 min). Each successive stepwise decrease in RH above 40% triggered minor spore release peaks. Stepwise increases in RH above 40% occasionally, but inconsistently, triggered momentary small releases of conidia, but this effect was somewhat inconsistent.

The second experiment demonstrates the effect of rapid fluctuations in RH of <3 min duration, both from saturation to lower RH and from low RH to some higher RH. The effect was demonstrated in darkness and in light (Fig. 2B). These rapid

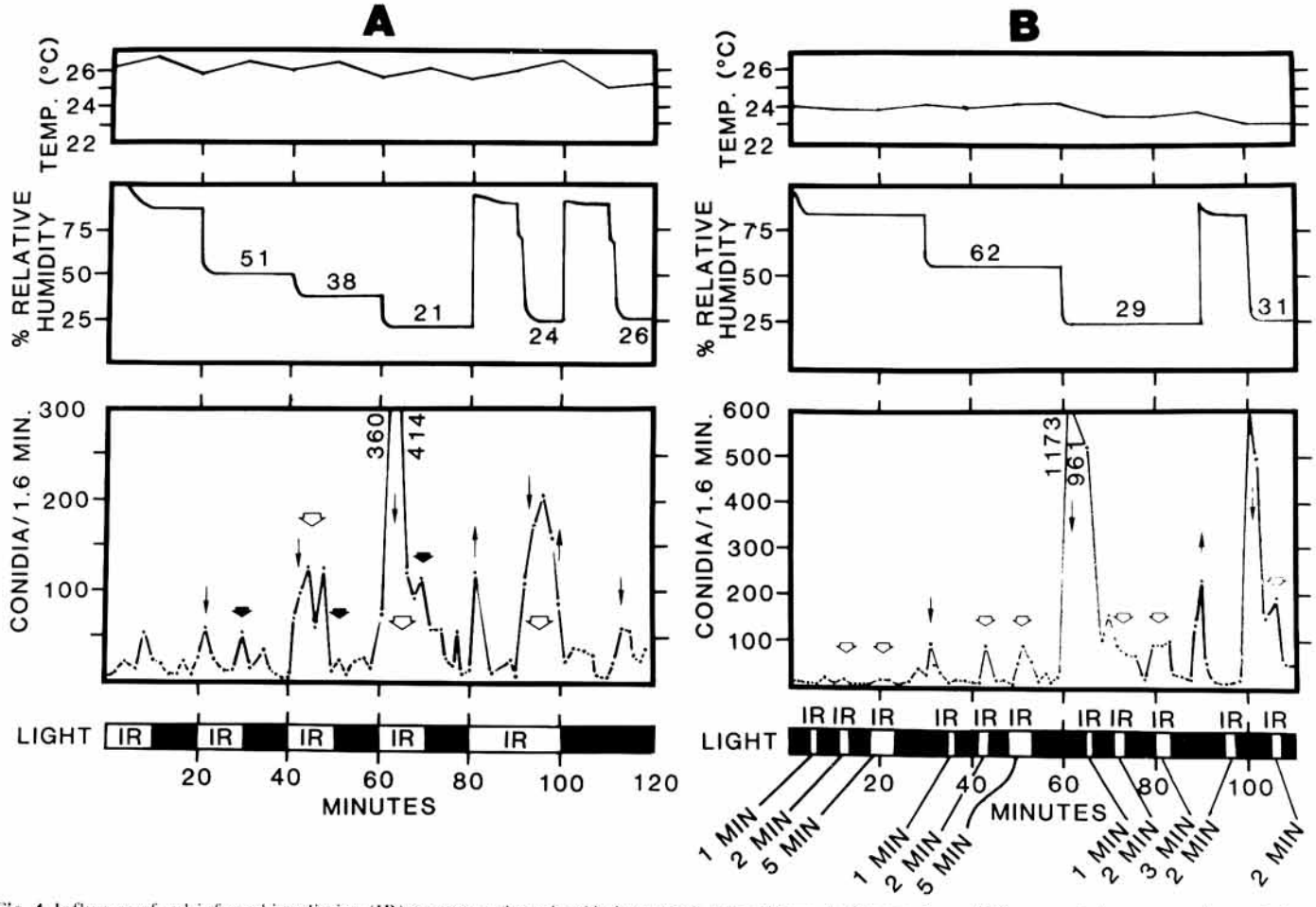

Fig. 4. Influence of red-infrared irradiation (IR) on spore release by *Cladosporium carpophilum*. **A,** Comparison of IR versus darkness at various relative humidity (RH) levels. **B,** Spore release associated with brief exposures to IR (1, 2, 3, or 5 min; at an intensity of 60 $\mu E \cdot m^{-2} \cdot s^{-1}$) at various RH levels. Large white arrows indicate spore release in response to IR. Large black arrows indicate spore release stimulated by the termination of an IR irradiation period. Small black arrows indicate peaks of spore release in response to decreasing (↓) or increasing (↑) RH.

changes consistently triggered spore release, although they were much more effective when the specimen was irradiated than in darkness (Fig. 2B—compare min 6 and 12 with min 26 and 34). Specimen exhaustion was not the cause of fewer conidia of *C. carpophilum* being released into the air stream during the dark period; when RH was again lowered in the presence of IR (min 40–45), many more spores were released. Lowering RH from saturation to 20% was far more effective in liberating conidia than lowering it to only 60% RH. When the reverse fluctuation of RH was made, ie, from a maintained low RH of 20–28% to some higher RH, each increase in RH caused a major reduction of spore release, but spore release quickly resumed when the RH was again lowered (Fig. 2B— 50, 56, 65, 80, 87, and 93 min). Rapid fluctuation in RH tended to dislodge numerous trichomes from the fruit surface as well as spores. Spores and mycelium were often attached to these structures (Fig. 3). Numbers of trichomes dislodged increased the greater the change in RH.

Consistently in both experiments (Fig. 2A and B), a reduction in RH caused an initial peak of conidia release which then lessened when the RH was sustained at a lower level. For further release to occur, an additional stimulus was needed (Fig. 4).

Effects of infrared radiation. Following the observations of the effects of IR exposure on spore release in the first two experiments (Fig. 2A and B), the effect of IR was investigated more extensively (Fig. 4A and B). In the first IR experiment, exposures were made at several different constant-RH levels ranging from 100 to 21% RH as shown in Fig. 4A. IR had little effect on spore liberation at sustained RH levels of 83 and 51%, but had a considerable effect at 38 and 21% RH (Fig. 4A—40–50, 60–70, 90–100 min, white arrows). In three cases, there were minor peaks of spore release at the termination of an IR period (Fig. 4A— min 30, 50, and 70, large black arrows).

To investigate the effect of short duration IR on conidia liberation, specimens were irradiated with IR for 1-, 2-, 3-, or 5-min intervals at various RH levels (Fig. 4B). Virtually no conidia were released into the air stream when irradiated at high RH (Fig. 4B—min 5–6, 12–14, and 19–24); however, moderate spore catches were recorded from 2-, 3-, and 5-min irradiations at 62, 29, and 31% RH (Fig. 4B, white arrows). Irradiation of the specimen for 1-min periods at RH near saturation and at 62% did not elicit additional spore discharge; however, the effect of 1 min of exposure at 29% RH was masked by a spore peak caused by decreasing RH from 62 to 29%. This demonstrated that *C. carpophilum* required a minimum of 2 min of irradiation to react by liberating conidia at higher RH.

Effects of vibration on spore release. Under natural conditions, plants are continually buffeted by wind and rain. This buffeting was simulated by artificially vibrating specimens in another series of experiments (Fig. 5A and B) in which both RH and exposure to IR was varied.

Although instantaneous vibration triggered spore liberation at nearly all values of RH, the results were not consistent at all RH values. Generally, release was greatest at low RH (<30%) and was further enhanced by exposure to IR, at 60 $\mu E \cdot m^{-2} \cdot s^{-1}$ (Fig. 5A—120 min, and B—75 min). At higher RH, IR did not affect vibrational release (Table 1).

Simulation of morning drying. Typically at sunrise during the growing season in peach orchards in the southeastern USA, air temperature increases, dew evaporates, solar radiation increases, and RH decreases. Experiments were designed to simulate these morning drying conditions using the same specimen in both light (IR) and darkness to allow direct comparisons between peaks (Fig. 6A and B). In darkness, only a single relatively small peak of spore release occurred during a decrease in RH from 32 to 23% (Fig.

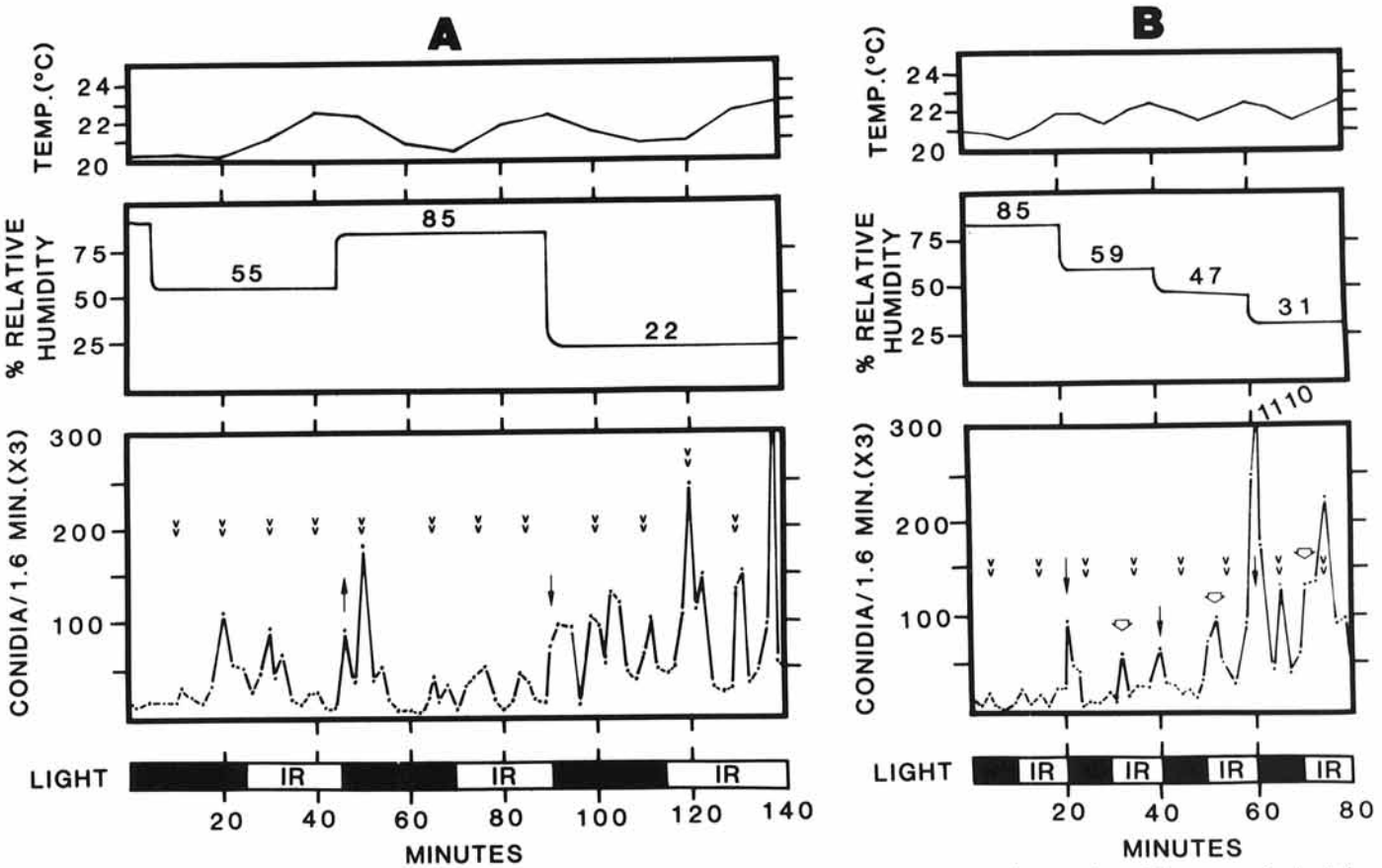

Fig. 5. The effect of vibration on spore discharge by *Cladosporium carpophilum*. **A,** Vibration applied to the specimen at three different sustained relative humidity (RH) levels in darkness and in red-infrared irradiation (IR). **B,** Demonstration of increasing effectiveness of vibration at successively lower RH levels both in darkness and exposure to IR. Double "Vs" over peaks indicate the point of instantaneous vibration created by dropping a 50-g weight 5 cm onto the specimen chamber. Large white arrows indicate peaks of spore liberation attributable to IR stimuli. Small black arrows (↓) indicate spore release peaks caused by decreases in RH.

6A— min 25). When the same specimen was exposed to IR, the first major peak of spore release was recorded when the RH was lowered from 55 to 32% simultaneously with an increase in IR from 20 to 30 $\mu E \cdot m^{-2} \cdot s^{-1}$ (Fig. 6B; min 15). Subsequent peaks of spore release corresponded to further incremental increases in IR and a final decrease in RH from 32 to 22% (Fig. 6B—min 20, 25, and 30).

DISCUSSION

In peach orchards in the southeastern United States, airborne conidia of *C. carpophilum* could be trapped during both rainy and dry conditions. Numbers of airborne spores also corresponded to the amount of sporulation occurring on peach scab lesions (7). These findings do not support Keitt's conclusion (6) that rain-splash is the most important agent in spore liberation by *C. carpophilum*.

Although dispersal of the conidia of *C. carpophilum* by splashing rain undoubtedly occurs, the findings of this study indicate the presence of an aerial spore release system dependent on fluctuations of humidity, particularly decreasing RH. Fluctuations in RH appear to trigger aerial spore dispersal by *C. carpophilum*, which is enhanced somewhat by light (IR) and to a small extent by vibration. Indeed the vibration caused by raindrops striking leaves and fruit may be more important than splash dispersal in inducing spore release.

The effect of decreasing RH on spore release and its enhancement by IR and vibration has recently been demonstrated by Leach for several species of plant pathogenic fungi (8–12). Gottwald found that two entomogenous fungal pathogens of the pecan weevil, *Beauveria bassiana* and *Metarhizium anisopliae*, also responded to decreasing RH and IR in much the same way as *C. carpophilum* by releasing conidia into the air stream (3). By the use of a simple Tyndall apparatus, Leach has observed violent spore dispersal of *Cladosporium fulvum* in response to decreasing RH and IR (8).

In a more recent study with *C. caryigenum*, a fungus closely related to *C. carpophilum* both morphologically and pathologically, Gottwald demonstrated that decreasing RH and IR triggered spore release responses nearly identical to those observed here with *C. carpophilum* (4). In both cases, rapidly fluctuating RH, especially when RH dipped below 40%, increased aerial spore concentrations. Both fungi also reacted to abrupt increases in RH by releasing spores. The effects of IR on spore liberation of both species was also nearly identical. Changes in RH had more effect on spore release when specimens were irradiated with IR, although the effect was more pronounced upon *C. caryigenum*. *C. caryigenum* was also stimulated to release spores by as little as 1 min of exposure to IR (60 $\mu E \cdot m^{-2} \cdot s^{-1}$), while at least 2-min exposures were required to effectively increase spore release by *C. carpophilum*. Both fungi reacted to brief 1-min fluctuations in RH by releasing large numbers of conidia into the air stream. The mycelium of *C. carpophilum* has previously been described attached to the trichomes on peach fruit surfaces (6). The removal and dispersal of entire trichomes with intact mycelium and spores sheared by changes in RH, however, may afford another previously unreported means for inoculum dispersal (Fig. 3).

The response to vibration by both *Cladosporium* species was somewhat weaker than that reported for other hyphomycetes

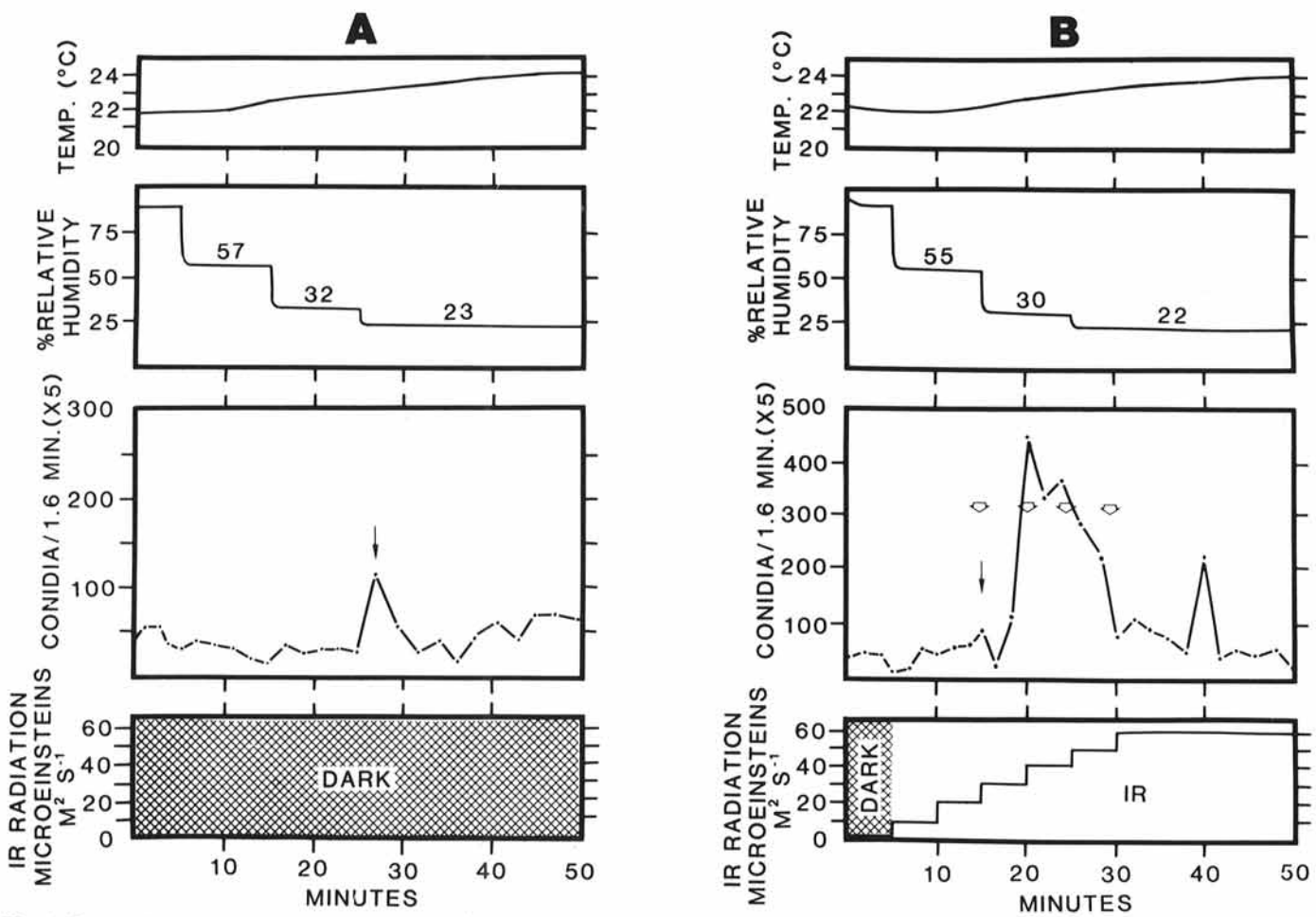

Fig. 6. Spore release by *Cladosporium carpophilum* during simulation of early morning drying conditions of decreasing relative humidity (RH) and increasing temperature. **A,** RH lowered in darkness, then in **B,** using the same specimen, RH lowered, accompanied by increasing red-infrared irradiation (IR) intensity. Large white arrows indicate spore release corresponding with increases of IR. Small black arrows (↓) indicate release in response to decreasing shifts in RH.

TABLE 1. Summary of spore release responses by *Cladosporium carpophilum* to various combinations of conditions

Conditions			
Timing	RH	IR/Dark	Response (%)[a]
Sustained[b]	High	IR	5.9
		Dark	5.2
	Med	IR	15.8
		Dark	5.7
	Low	IR	44.1
		Dark	8.6
Momentary decrease from saturation to:	Med	IR	13.3
		Dark	0.4
	Low	IR	100.0
		Dark	8.2
Momentary increase from low RH to:	Med	IR	11.0
		Dark	1.6
	Saturation	IR	6.7
		Dark	1.2
RH shift from saturation to:	Med	IR	4.7
		Dark	6.0
	Low	IR	50.5
		Dark	30.0
RH shift from med RH to:	Low	IR	75.5
		Dark	75.0
	Saturation	IR	1.9
		Dark	2.2
RH shift from low RH to:	Med	IR	1.5
		Dark	1.0
	Saturation	IR	24.2
		Dark	10.1
Brief IR	Saturation		0.8
	Med		5.3
	Low		10.6
Vibration	Saturation	IR	8.0
		Dark	21.3
	Med	IR	10.0
		Dark	10.8
	Low	IR	44.0
		Dark	24.0

[a] Response (%) was measured as number of spores released due to an event/max spore release peak recorded during that experiment. Response (%) shown is the mean response arrived at by averaging the response to an event over several experiments in which that event occurred.

[b] High RH = relative humidity held near saturation >85%; Med RH = relative humidity levels of 38 to 62%; Low RH = relative humidity <30%; IR = Red-infrared light at 60 $\mu E \cdot m^{-2} \cdot s^{-1}$; and Brief IR = Irradiation of 60 $\mu E \cdot m^{-2} \cdot s^{-1}$ for 3 min or less. Vibration was produced by dropping a 50-g weight 5 cm on top of specimen chamber.

compared to their response to RH changes (3,9–11). Both *C. carpophilum* and *C. caryigenum* responded more to vibrations at low RH and responded hardly at all at RHs near saturation.

Gottwald demonstrated, under both orchard and laboratory conditions, that *C. caryigenum* possessed a diurnal periodicity of spore release (1,2). In dry weather, greatest discharge occurred when morning drying conditions of decreasing RH and increasing light intensity and air temperature prevailed. Simulation of morning drying conditions in the laboratory produced similar results with *C. carpophilum*. Spore release by *C. carpophilum* was maximum when an RH of 32% was maintained while IR increased from 30 to 40 $\mu E \cdot m^{-2} \cdot s^{-1}$ (Fig. 6B—min 20). Further reductions in RH combined with further increases in IR stimulated subsequent peaks of spore release. When an RH of 22% was maintained in conjunction with IR of 60 $\mu E \cdot m^{-2} \cdot s^{-1}$, spore release gradually decreased (Fig. 5B).

The results of this study support the conclusions of other researchers who have studied spore release by *Cladosporium* sp. The recent investigation of the diurnal periodicity of *C. caryigenum* and *C. fulvum* (2,8) and the previous demonstrations of a diurnal habit for numerous other *Cladosporium* species (4,5,13,14) may be indicative of a diurnal pattern of aerial spore release shared by all members of the genus *Cladosporium*.

LITERATURE CITED

1. Gottwald, T. R. 1982. Spore discharge by the pecan scab pathogen, *Cladosporium caryigenum*. Phytopathology 72:1193-1197.
2. Gottwald, T. R., and Bertrand, P. F. 1982. Patterns of diurnal and seasonal airborne spore concentrations of *Fusicladium effusum* and its impact on a pecan scab epidemic. Phytopathology 72:330-335.
3. Gottwald, T. R., and Tedders, W. R. 1982. Studies on conidia release by the entomogenous fungi *Beauveria bassiana* and *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes) from adult pecan weevil (Coleoptera: Curculionidae) cadavers. Environ. Entomol. 11:1274-1279.
4. Harvey, R. 1967. Air-spore studies at Cardiff. I. *Cladosporium*. Trans. Br. Mycol. Soc. 50:479-495.
5. Hirst, J. M. 1953. Changes in atmospheric spore content: Diurnal periodicity and the effects of weather. Trans. Br. Mycol. Soc. 36:375-393.
6. Keitt, G. W. 1917. Peach scab and its control. U.S. Dep. Agric. Bull. 395. 66 pp.
7. Lawrence, E. G., Jr., and Zehr, E. I. 1982. Environmental effects on the development and dissemination of *Cladosporium carpophilum* on peach. Phytopathology 72:773-776.
8. Leach, C. M. 1977. Influence of relative humidity and red-infrared radiation on violent spore release by *Drechslera turcica* and other fungi. Phytopathology 65:1303-1312.
9. Leach, C. M. 1980. An apparatus for precise control of humidity, temperature, air flow, and light in spore discharge studies. Phytopathology 70:189-191.
10. Leach, C. M. 1980. Influence of humidity and red-infrared radiation on spore discharge by *Drechslera turcica* additional evidence. Phytopathology 70:192-196.
11. Leach, C. M. 1980. Influence of humidity, red-infrared radiation, and vibration on spore discharge by *Pyricularia oryzae*. Phytopathology 70:201-205.
12. Leach, C. M. 1982. Active sporangium discharge of *Peronospora destructor*. Phytopathology 72:881-885.
13. Pady, S. M., Kramer, C. I., and Clary, R. 1969. Periodicity in spore release in *Cladosporium*. Mycologia 61:87-98.
14. Rich, S., and Waggoner, P. E. 1962. Atmospheric concentration of *Cladosporium* spores. Science 137:962-965.