Atmospheric Concentration of Cladosporium Spores

The concentration has a peculiar diurnal cycle, and it may either increase or decrease during rain.

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The aerial burden of fungal spores is of great importance both in biology and in medicine. Besides spreading crop destruction, fungal spores produce allergic responses in some human beings. To understand how the air is filled with spores, one must know something not only of the biology of the fungi but also of the weather factors that induce the flight of mature spores. Thus, the problems involved are both biological and meteorological.

The atmospheric concentration of particular fungus spores may vary with the weather, or in a daily cycle. *Cladosporium* spores, abundant in the summer air, do both.

In 1956 we observed the concentration of *Cladosporium* spores in the air and found a remarkable periodicity. There was a peak in the forenoon, then a midday dip, followed by a second, smaller peak in the afternoon or early evening. The nighttime concentration was low. This periodicity was subsequently reported by Gregory and Stedman (*1*), without an explanation for the pair of daytime maxima (hereafter called the "daytime double maxima").

The Daytime Double Maxima

With an automatic volumetric spore trap (*2*) we measured the concentration of *Cladosporium* spores in the air 46 centimeters above the soil. The trap was set in an 18- by 36-meter field of cucumbers severely infected with *Cladosporium cucumerinum*. The plants were about 50 centimeters tall during the 18 days of sampling between 17 August and 3 September. Rainfall was recorded 200 meters from the field. Wind speed was measured 250 meters from the field and at a height of 15 meters.

The 29 relative maxima and the 28 relative minima of *Cladosporium* spores in the air during the 18 daily cycles are summarized in a frequency diagram (Fig. 1). In the forenoon and afternoon, maxima predominate, and there are occasional maxima in the late evening; at midday and at night, minima predominate.

The most obvious explanations of the daytime double maxima would be (i) that the fungus produces two crops of spores during the daylight hours, or (ii) that spores mature continuously during the day and that weather factors inducing spore flight produce the twin peaks. To find out if either explanation was valid, we turned to the laboratory.

In our laboratory experiments we used sporulating cultures of *Cladosporium cucumerinum* growing on potato dextrose agar.

Are *Cladosporium* spores produced continuously during 24 hours, or is there a diurnal cycle? The concentric rings on colonies growing by a window indicate a diurnal cycle. To find out whether there is such a cycle, we cut squares from the advancing edge of a colony and rubbed off the attached spores. We placed these squares in the dark for various periods before exposing them to the light. We found that *Cladosporium* sporophores require at least 8 continuous hours of dark in order to produce spores. These spores mature within 3 to 4 hours after they begin to form.

In nature, then, *Cladosporium* probably produces only one crop of spores every 24 hours. The spore crop is ready to fly within 4 hours after sunrise, or at about 0900 hours during the summer.

What frees the spores from the sporophores? Is it changes in relative humidity, as is the case in the downy mildews? When we dried colonies with an incandescent lamp, the longer, free sporophores twisted and swayed, but very few spores were released. However, any sudden jarring sent clouds of spores flying. Touching the surface of the colony sufficed to release them.

Does continuous jarring deplete the spore source? We cut squares from sporulating cultures of *C. cucumerinum* and stuck the squares, agar side down, onto glass slides. These slides were inverted 1 centimeter above Vaseline-coated trapping slides. We jarred the sporophores by dropping a 1-gram weight 5 centimeters onto the back of the inverted slides directly over the fungal squares. We found that most of the spores were released by the first five taps. Subsequently, the number of spores jarred loose gradually diminished to just a few at the tenth tap.

Continuous jarring, then, will deplete *Cladosporium*'s single daily crop of spores. Therefore, our laboratory results rule out the simple hypothesis that the daytime double maxima result from two periods of spore liberation.

The weather data, typical for most summer days in our area, generally show increasing turbulence during the morning, reaching maximum turbulence about midday and then gradually calming through the afternoon. Thus, the daytime double maxima are not caused by two periods of turbulence.

Hypothesis for Daytime Double Maxima

Finding no single phenomenon that accounts for the double maxima, we tried a combination. Hewson (*3*) gave us a hint in his analysis of urban air pollution in terms of both emission and turbulence: "Increased activity and . . . heating in the morning taken in conjunction with increasing turbulence associated with solar heating . . . account for the morning maximum; decreasing turbulence in the late afternoon before industrial emission of pollution has been reduced causes the secondary maximum." We followed this hint and considered a combination of emission and turbulence to derive a hypothesis. Conversations with H. K. Weickmann and G. R. Hilst also aided us.

Because *Cladosporium* is ubiquitous,

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we consider that the number n of airborne spores above a plot of land is equal to the number released on the plot, drift on the wind bringing a new supply to replace that carried downwind.

Thus we can reasonably say that the increase in n per unit increase in turbulence T will be proportional to the difference between the number available, m, and the number already airborne, n:

$$dn/dT = C_1(m-n)$$

$$n = m(1 - e^{-C_1T}) \quad (1)$$

where C_1 is a constant of proportionality. (As the need arises, we shall introduce other constants C_i.) The T is merely an indicator of the atmosphere's ability to lift and to dilute a spore cloud. It must increase with such other indicators of turbulence as eddy velocity, exchange coefficient, and the volume of a moving cluster of particles. The ratio T/C_1 is dimensionless.

Once the spores are airborne, the number n could be reduced in two ways. First, as turbulence decays, the spores could settle out, and Eq. 1 assumes that this happens according to the relation that describes their flight. Also, disintegration or scrubbing by rain could remove a proportion C_2t that increases with time t, and we modify the expression for n by multiplying the right-hand term in Eq. 1 by $(1 - C_2t)$ and redefining n. Now

$$n = m(1 - C_2t)(1 - e^{-C_1T}) \quad (2)$$

The concentration of spores in the air, x, is the number n of airborne spores divided by the volume v through which they are dispersed. We need, therefore, to specify v as a function of turbulence T. Because the trap is a finite distance from the soil, v is never less than v_o. But during the day, turbulence expands v. We can reasonably say that v is a linear function of T:

$$v = C_3T + v_o \quad (3)$$

A demonstration that the volume of dilution varies with turbulence is provided by observations taken downwind from a steady source of pollution. The concentration varied inversely with the turbulence (*4*); since the quantity suspended was constant, this indicates that our setting of the volume of dilution, v, proportional to turbulence T is reasonable.

Underlying Eq. 3 is the assumption that the spores remaining in the air as turbulence and volume decrease do not collect near the bottom of that volume —that decreasing turbulence maintains an even distribution of fewer spores (Eq. 2) in a shrinking volume (Eq. 3). This view seems reasonable because the mechanically generated turbulence near the ground persists into the evening hours quite as well as the turbulence aloft (*5*). Scorer (*6*) has picturesquely described the maintenance of turbulence near the surface: "Sometimes puffs of cold air can be observed on calm evenings after a day of well developed convection. These may be very shallow, rustling only the lower leaves of a tall tree. . . . They consist of air descending to replace thermals rising from places which have remained hotter than their surroundings in the cooling evening."

Table 1. Relative concentrations of airborne spores (x/m) for time measured in hours and minutes after sunrise.

Time	x/m
0	0
1 min	0.61
5 min	1.4
1 hr	1.5
2 hr	1.2
4 hr	0.9
6 hr	0.8
8 hr	0.8
10 hr	1.0
11 hr	1.2
11 hr, 55 min	1.0
11 hr, 59 min	0.5
12 hr	0

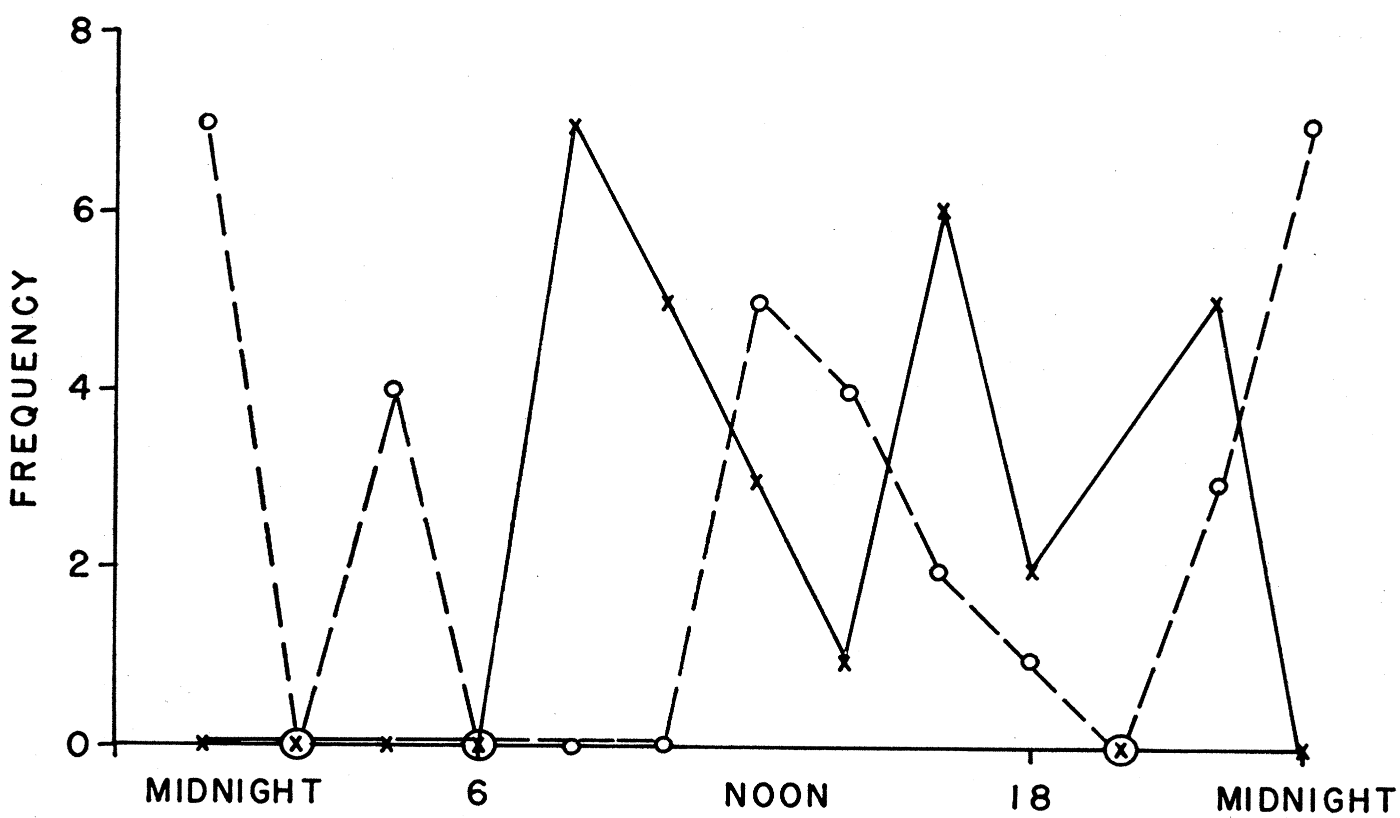

Fig. 1. The diurnal distribution of maxima and minima of *Cladosporium* spores in the air during 18 days in August and September, 1956. The frequency for each 2-hour period is shown above the even-numbered hour concluding that period. (Solid lines and ×'s) maxima; (dashed lines and o's) minima.

Now, if we are to predict the diurnal cycle, we must express turbulence T as a function of time t. Because turbulence generally increases from sunrise to noon and then falls again, a reasonable relation between turbulence T and time t during 12 hours of daylight is

$$T = C_4 \sin(\pi t/12) + T_o \quad (4)$$

where T_o is the turbulence during the dark hours.

This enables us to express n, v, and hence x as a function of time:

$$x = \frac{m(1 - C_2 t)\left\{1 - e^{[-C_1 C_4 \sin(\pi t/12) - C_1 T_o]}\right\}}{C_3 C_4 \sin(\pi t/12) + C_3 T_o + v_o} \quad (5)$$

The consequences of this hypothesis are now evident. If the nighttime turbulence T_o is great and spores are available, the spores are released whenever they are formed, and the dilution remains almost constant throughout the day and night, as in

$$x \approx m(1 - C_2 t)\,[(1 - 0)/C_3 T_o] \quad (6)$$

Hence, the diurnal cycle will depend only upon the maturing of spores, m, and the disintegration. If the rate of disintegration is low, a morning's crop could persist in the air throughout the night, as it evidently did on the nights of 19–20 August (Fig. 2) and 31 August–1 September, when turbulence was great because winds were a relatively high 2.2 to 2.7 meters per second, and the nighttime minimum was in fact eliminated. Of course, if the trap is held high, v_o is large, the volume is never low, and the x sampled will be low until many spores are airborne.

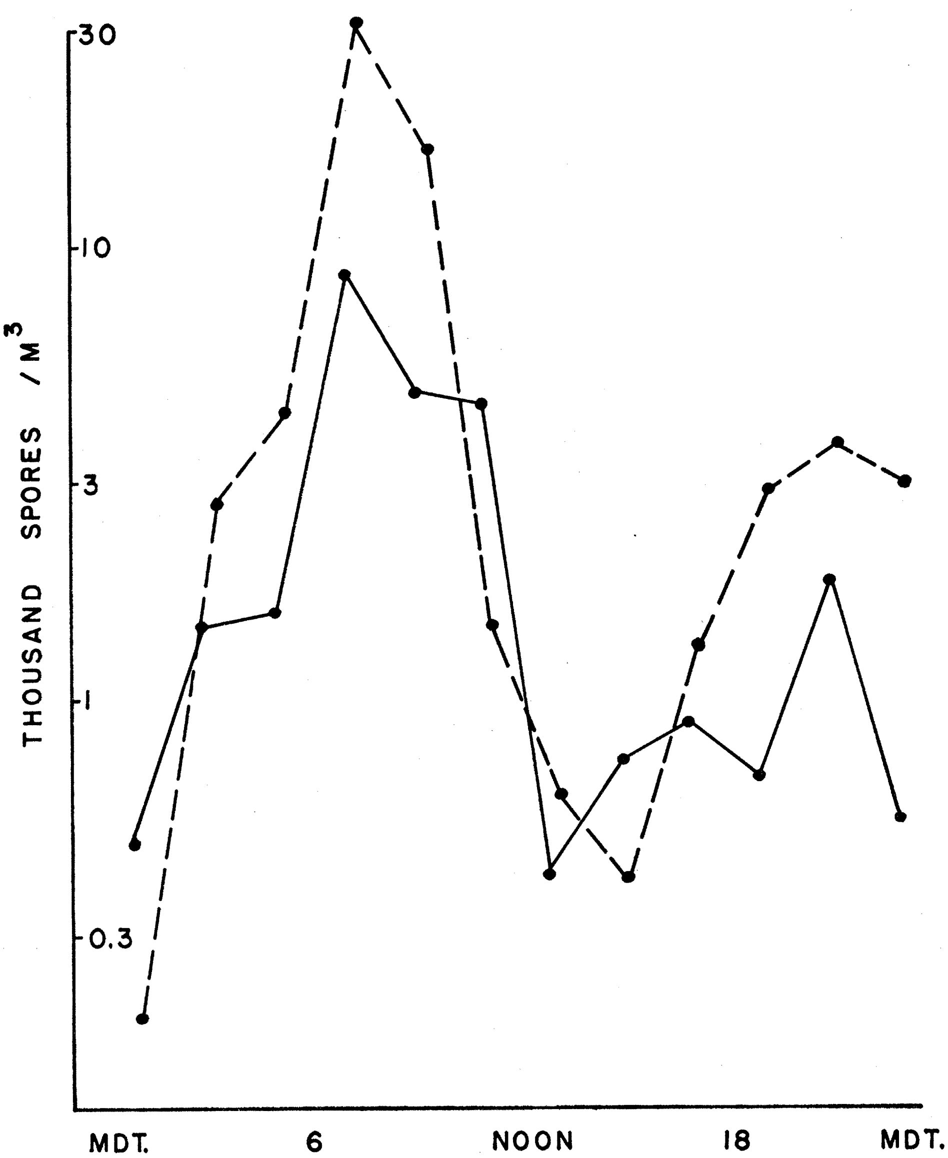

Fig. 2. The course of concentration of airborne *Cladosporium* spores on 2 days in August 1956. (Dashed line) 19 August; (solid line) 23 August.

In a situation more often encountered, T_o is negligible and disintegration C_2 is small. Then

$$x \approx \frac{m(1 - C_2 t)\left\{1 - e^{[-C_1 C_4 \sin(\pi t/12)]}\right\}}{C_3 C_4 \sin(\pi t/12) + v_o} \quad (7)$$

The operation of the hypothesis can be seen if we calculate x/m, the relative concentration of airborne spores, for C_1C_4 equal to 2, C_2 equal to 0.02 per hour, v_o equal to 0.01, and C_3C_4 equal to 1 unit of volume. For time measured in hours and minutes after sunrise, we obtain the values given in Table 1. Thus, the hypothesis leads to a diurnal cycle resembling that of *Cladosporium*: a morning maximum, a relative minimum at noon, and a relative maximum after noon.

If the disintegration C_2t were negligible, the hypothesis would predict an afternoon maximum equal to the morning one. On the other hand, if disintegration or dilution by spore-free air were great, the afternoon maximum would disappear. This may be why *Phytophthora* (*7*) and *Peronospora* (*8*) have a single morning maximum: the destruction of the spores in the dry, sunlit air and the dilution of the air from upwind, where no hosts lie, diminish the supply about and above the trap, preventing the appearance of an afternoon maximum.

Thus, we have a workable explanation for the diurnal cycle of spore concentration, and we turn now to the effect of weather.

Rain and Spore Concentration

How does rain influence the aerial concentration of *Cladosporium* spores? Reports by others vary. Hirst (*7*) said, "There is a suggestion that [light rains] reduced *Cladosporium* concentrations." Ainsworth (*9*), however, observed "an increase during the rain that was unexpected," and Gregory (*10*) agreed that "there was a rapid increase in the *Cladosporium* spore concentration . . . during the showers."

During 5 of our 18 days of observation, rain began at a known hour. A close sequence of observations showed the trends indicated in Table 2.

The first two events, a thunderstorm and a shower, are storms caused by instability and turbulence in the atmosphere. Clearly the concentration of airborne spores is increased sharply by storms of these types.

In contrast to these, the last three events, light rain and drizzle, are associated with stability in the atmosphere. Clearly, the concentration is not increased by rain. In fact, during the light rain that began at 0800 hours on the third day, the usual increase of the morning did not appear; instead, the concentration decreased from a maximum at 0530 hours, and we suspect that the light rain was scrubbing spores from the air.

The explanation of the varying effect of rain seems obvious from the data of Table 2: gusty rainstorms carry more spores aloft, and gentle rains wash them down. This can also be seen in the observations of other workers. Gregory (*10*) called the rains that increased concentrations "showers," and Ainsworth (*9*) mentioned a "sharp thunderstorm." Hirst (*7*) said a decrease in concentration was suggested when "rain was never heavy," and when an increase occurred he mentioned a "thunderstorm."

The heavy wind-driven drops of turbulent storms could propel spores into the air by two mechanisms. One would be the sudden jarring of the infected leaves as they whip in the wind and rain. The second would be the effect described by Davies (*11*), who demonstrated in the laboratory that wind-driven droplets detach 10 to 2000 times more *Cladosporium* spores than are detached by dry air moving at the same speeds.

Table 2. Thousands of spores per cubic meter of air before and during precipitation.

Before		During	
Hour	Spores (*n*)	Hour	Spores (*n*)
	Thunderstorm		
1800	8	2100	85
	5		214
1900	5	2200	8
	25		8
2000	120	2300	29
	53		
	Shower		
	1		16
0500	2	0800	48
	1		180
0600	3	0900	210
	3		150
0700	2		
	Light rain (morning)		
0500	26	0800	5
	27		0
0600	0	0900	2
	1		0
0700	6	1000	0
	1		
	Light rain (evening)		
1900	2	2200	1
	1		0
2000	8	2300	1
	2		1
2100	3	2400	1
	5		
	Drizzle		
	0		1
1900	7	2200	1
	4		0
2000	0	2300	0
	4		0
2100	1		

Conclusions

Daytime double maxima in the concentration of airborne *Cladosporium* spores are common. Apparently the double maxima are not caused by the production of two crops of spores during the day. Nor are they caused by two flight-inducing periods in connection with a continuously produced crop of spores. Neither of these hypotheses is tenable—first, because *Cladosporium* requires 8 hours of darkness to produce spores ready for flight, hence few spores are produced after 0900 hours; second, because flight is induced by jarring, which generally reaches a single maximum during midday turbulence.

We propose the following hypothesis to explain the daytime double maxima.

The mature spores become airborne as the infected leaves are jarred and vibrated by the increasing morning turbulence. The number of spores increases until the spore source is depleted. This occurs in the forenoon.

The number of airborne spores is now constant. Further turbulence carries them ever higher into the air over the fields. This increases the air volume in which the spores are carried. In effect, the concentration of the spores is lowered, accounting for the midday minimum.

During the afternoon or early evening, turbulence lessens. The spores begin to settle, occupying less air volume and giving rise to the second maximum. Gradually the spores settle out of the air, and their number falls to the nighttime minimum.

Rain evidently has two effects on the concentration of airborne *Cladosporium* spores. A light, misting rain that wets the leaves without vibrating them washes the spores down onto the leaf surface, not into the air. At the same time the mist scrubs the airborne spores from the air, reducing their number. Conversely, the heavy, wind-driven drops of thunderstorms and summer showers increase the number of airborne spores. Not only do these turbulent storms jar and whip infected leaves but the wind-driven drops are extremely efficient in detaching any remaining conidia.

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