AVOIDANCE OF *PHYCOMYCES* IN A CONTROLLED ENVIRONMENT

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ABSTRACT The sporangiophore of the fungus *Phycomyces* bends away from nearby objects without ever touching them. It has been thought that these objects act as aerodynamic obstacles that damp random winds, thereby generating asymmetric distributions of a growth-promoting gas emitted by the growth zone. In the interest of testing this hypothesis, we studied avoidance in an environmental chamber in which convection was suppressed by a shallow thermal gradient. We also controlled pressure, temperature, and relative humidity of the air, electrostatic charge, and ambient light. A protocol was established that yielded avoidance rates constant from sporangiophore to sporangiophore to within ±10%. We found that avoidance occurred at normal rates in the complete absence of random winds. The rates were smaller at 100% than at lower values of relative humidity, but not by much. Remarkably, at a distance as great as 0.5 mm, avoidance from a 30-μm diam glass fiber (aligned parallel to the sporangiophore) was about the same as that from a planar glass sheet. However, the rate for the fiber fell more rapidly with distance. The rate for the sheet remained nearly constant out to ~4 mm. We conclude that avoidance depends either on adsorption by the barrier of a growth-inhibiting substance or emission by the barrier of a growth-promoting substance; it cannot occur by passive reflection. Models that can explain these effects are analyzed in the Appendix.

INTRODUCTION

The mycelium of the fungus *Phycomyces* sends up into the air a long thin tube ~0.1 mm in diameter that develops at its tip a spherical sporangium ~0.5 mm in diameter. Growth occurs in a tapered zone extending 2–3 mm below the base of the sporangium. When the sporangiophore is mature (stage IVb, ~2-cm long), it grows steadily at ~3 mm/h, twisting clockwise (as viewed from above) at ~2 rev/h. The sporangiophore changes its direction of growth in response to light, gravity, mechanical deformation, wind, odoriferous chemicals, and nearby objects. We deal here with the latter sensory modality, recognizing at the outset that avoidance also might involve air movement and olfaction.

Avoidance Response

The avoidance response was discovered independently by Wortmann (1881) and Elfving (1881), who observed growth in the dark away from damp pasteboard or plaster, respectively. It was rediscovered by Shropshire (1962). Wortmann followed the growth of sporangiophores emerging from a hole in a glass plate near pieces of wet pasteboard; the sporangiophores bent away from the pasteboard without colliding with it. No response was observed with dry pasteboard, so Wortmann concluded that he was dealing with growth away from a source of water. Elfving found that when a piece of damp plaster was mounted above a culture at an angle from the horizontal, the sporangiophores veered off before reaching the plaster and grew parallel to its surface. When the plaster was mounted horizontally, the sporangiophores either turned at right angles and grew horizontally with some nutation or made a U-turn and grew downwards. A moist zinc plate gave similar results; however, the sporangiophores grew directly into dry glass that had been cleaned with alcohol. Shropshire placed a cylindrical glass lens (0.16-mm diam) parallel to a sporangiophore (0.12-mm diam) at a distance of 0.14 mm. He was interested in interfering with the optical properties of the growth zone, but he found that the sporangiophore grew away from the glass cylinder, even in the dark. This was the first report of avoidance of a dry surface, and the first minute-by-minute description of bending angles.

The state of knowledge in 1969 was summarized by Bergman et al. (1969) in their monumental review: "A sporangiophore placed close to a solid barrier grows away from it. The response begins about 3 min after placing the barrier 2 to 3 mm from the sporangiophore. The rate of response in the steady state varies with the distance, about 1°/min at 2 to 3 mm, about 2°/min at 1 mm. Total angle of bend in both cases is about 50°. If the barrier is present for 3 min and then removed, the response begins at the end of the presentation time and continues for about 5 min. In the tropostat, the response can be kept up indefinitely. How the sporangiophore senses a barrier we do not know. So far,

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only negative evidence is available as to the source of information for the sporangiophore. The following facts appear to be definite. (i) If a sporangiophore is placed between two closely opposed barriers or inside a tube with internal diameter of a few millimeters, it shows a transient growth response. (ii) The avoidance response occurs in complete darkness. (iii) It occurs at 100% humidity. (iv) Seemingly, neither the material nor the color of the barrier has a strong influence on the response: glass, wood, plastic, black tape, or a crystal transparent for infrared radiation of a black body at room temperature are equally effective. (v) The solid barrier can be replaced by a vertical glass rod (diameter, 150 μm), by a copper wire mesh, by a single horizontal copper wire (diameter, 150 μm), by a horizontal human hair (diameter, 75 μm), or by a horizontal silk thread (diameter, 15 μm). In the experiments with horizontal cylindrical objects, the latency is independent of the diameter of the object, but the thinner the object the closer it has to be placed and the more localized is the response. Heating a horizontal copper wire anywhere between 0.1°C and several °C does not modify the effect."

Since then, speculations have centered around the idea that a growth-promoting gas emitted by the growth zone develops a higher concentration on the side of the sporangiophore proximal to the barrier than on the distal side. The concentration gradient of this gas across the growth zone causes the bending. In support of this idea, Bergman et al. (1969) and Ortega and Gamow (1970) found that when a sporangiophore was placed between two parallel barriers or inside a cylindrical tube, its growth rate increased some 20% for ~10 min and then returned to normal; the sporangiophore did not bend. This is what one would expect were the concentration of a growth-promoting gas to increase uniformly. It also has been thought that gradients of the avoidance gas are built up by suppression of random winds. Johnson and Gamow (1971) found that bending did not occur in still air (in a sealed 2.5 × 2.5 × 7.6 cm glass chamber), but that it did occur when the air was stirred (when the chamber was moved back and forth). They studied bending near a barrier in air moving between 0.2 and 1 mm/s (too small a velocity to generate a wind response) and concluded that both moving air and a barrier are required to initiate an avoidance response. Cohen et al. (1975) found that bending did occur in still air (in a sealed lucite box, 6.2 cm on a side), but after a long series of experiments they arrived at a similar conclusion, i.e., that avoidance required random winds.

Wind Response

The wind response was discovered by Cohen et al. (1975), who found that sporangiophores grew into a transverse wind, provided that its velocity was greater than ~1–2 cm/s (too small to act via mechanical deformation). An air current of 15–30 cm/s blowing vertically downward on a sporangiophore completely abolished the avoidance response. There was a negative growth response when such horizontal or vertical winds were switched on, and a positive growth response when they were switched off. However, no change in rate occurred when a sporangiophore was exposed alternately to room air or to room air passed through a chamber containing some 1,000 sporangiophores.

Lafay and Matricon (1982) studied the interrelationships of avoidance and wind response in more detail. They found that while a sporangiophore avoided a 250 μm mesh stainless-steel screen placed 1 mm away at the rate of 2°/min and bent into a 1 cm/s wind at 0.3°/min, it did not bend at all when the wind was blown at the sporangiophore through the screen. They also devised a number of experiments with moving barriers, by which wind gradients could be manipulated. When wind currents were higher on the proximal side of the sporangiophore (between the sporangiophore and the barrier) than on the distal side, the avoidance response did not change sign. Nor did the sporangiophore react to a pure wind gradient, e.g., when placed midway between two moving belts, one moving upward, the other downward. In this case, the sporangiophore grew straight upwards. These authors concluded that the avoidance response and the wind response are distinct sensory modalities.

Aiming Errors

Both avoidance and wind responses are subject to aiming errors. Gamow and Böttger (1982*a*) found that sporangiophores did not grow directly away from a barrier, but rather at an angle (with a clockwise deviation when seen from above). Rotation of the growth zone had been shown by Dennison and Foster (1977) to provide a mechanism by which the sporangiophore avoids complete adaptation during phototropism: a new part of the growth zone continuously rotates into the region of most intense illumination, thus converting an apparently spatial stimulus into a temporal one. Similar arguments apply to the avoidance response. They also apply to the wind response, as shown inadvertently by Gamow and Böttger (1982*b*), who generated the wind with a moving barrier.

Olfactory Response

The olfactory response was discovered by Elfving (1893, reviewed 1916–1917) and rediscovered by Cohen et al. (1979). Elfving reported that sporangiophores bent toward pieces of rusted iron, sealing wax or rosin, or toward platinum that had been exposed (at a distance) to any one of a variety of volatile chemical substances (but not toward platinum that had been degassed by heating). Bending also was observed toward a drop of a volatile liquid spread on a ground-glass surface previously cleaned with potassium dichromate-sulfuric acid (but not toward the cleaned glass alone). Responses were recorded for nitric or hydrochloric acid (but not for acetic or osmic acid), various halogens and halogenated hydrocarbons, carbon disulfide and

hydrogen sulfide, and a wide range of volatile organics. A number of weakly volatile organic solids attracted sporangiophores when held near a growing culture with a bit of wax at the end of a copper wire. Elfving believed that all of these chemicals acted by inhibiting growth on the proximal side of the growing zone, but he did not test for growth inhibition per se. Cohen et al. (1979) studied effects on growth rates of 22 volatile substances. All of these substances (except water) induced negative growth responses. The concentration required for 50% inhibition correlated well with the human olfactory threshold: in short, if we can smell it, *Phycomyces* can smell it. Russo (1977) and Russo et al. (1977) found that ethylene and ethane induced a positive growth response. Since a sporangiophore generates ethylene, they argued that ethylene is the avoidance gas. Unfortunately, the concentrations of ethylene required to induce a growth response are some 10^6 times larger than the concentration of this gas normally found in the vicinity of the growth zone.

Effects of Water Vapor

Interlaced throughout this literature are references to effects of water vapor, long regarded as the avoidance gas by Gamow and his co-workers (e.g., Johnson and Gamow, 1971; Gamow and Böttger, 1982*b*; Pellegrino et al., 1983; Gyure et al., 1984). As noted above, the idea that sporangiophores avoid water goes back to Wortmann (1881), who obtained different results with wet and dry pasteboard. Steyer (1901) repeated Wortmann's experiments using wet filter paper at an ambient relative humidity of 50%, and found a bending response, but only when the sporangiophore was within 5 mm of the paper. Similarly, Walter (1921) failed to find a response in a humidity gradient (30–100% in 30 cm) unless the sporangiophore was close to a wet wall. Materials that actively absorb water, such as NaOH, KOH, or plaster saturated with $CaCl_2$, did not attract sporangiophores (Elfving, 1916–1917). Attempts to generate growth responses to step-changes in relative humidity have consistently failed (Cohen et al., 1975, 1979; Gyure et al., 1984). Gyure et al. (1984) found that sporangiophores grew more steeply (over periods of several hours) into wet winds then dry winds, but the relevance of this to avoidance is not clear.

Experimental Rationale

Given such a complicated state of affairs, it seemed to us wise to simplify the problem by reducing the number of variables. We chose to do this by eliminating winds altogether, by isolating the sporangiophore from exogenous odors, and by working at a fixed pressure, temperature, and relative humidity. Cohen (1976) once wrote, "The observation of avoidance behavior in *Phycomyces* is simple enough for a child to perform. Yet the mediation of this response is so sophisticated as to have eluded explanation for nearly 100 years." In our view, if the measurements were more sophisticated, perhaps the response would prove to be relatively simple. This report describes our first steps along this path.

METHODS

Cultures

Sporangiophores of wild-type *Phycomyces* strain NRRL 1555(−) were grown in shell vials (8.5-mm diam by 30-mm tall) containing 1.1 ml of 4% potato dextose agar (Difco Laboratories Inc., Detroit, MI) with 6 μg/ml thiamine HCl (Sigma Chemical Co., St. Louis, MO). Following Bergman et al. (1969), spores suspended in 2 ml distilled water at a concentration of ~50 viable spores/ml were heat-shocked at 49 ± 1°C for 15 ± 5 min. One drop of this suspension (0.05 ml containing an average of about three spores) was then inoculated into each vial. The vials were incubated inside 10-cm diam by 8-cm tall glass culture jars (No. 3250; Corning Glass Works, Corning, NY) at 97 ± 2% relative humidity at 19 ± 1°C, and under continuous overhead room light (four 40-W fluorescent bulbs located 2 m above the cultures). Stage IVb sporangiophores usually appeared after 3 d, and the sporangiophores were plucked daily so that a fresh crop was ready the next day. In general, only the third through the sixth crop of sporangiophores were used in experiments. In experiments demonstrating reproducible avoidance rates under fixed conditions, only third-crop sporangiophores were used, from cultures aged 120–150 h since inoculation.

Environmental Chamber

The experiments were carried out in the chamber shown in Fig. 1. The main body of the chamber (*m*) was a 10.2-cm diam cylinder machined from aluminum (2024 alloy rod: 4.4% Cu, 1.5% Mg, 0.6% Mn), pierced by three intersecting mutually orthogonal 2.5-cm diam holes. The temperature at the top of this cylinder was regulated by a heating coil (*h*) and the temperature at the bottom by a pair of heating and cooling coils (*h'*, *k*). The sporangiophore (*f*) in its growth vial (*g*) was inserted into the vertical hole from below. The top part of this hole served as a viewing port. It contained a hollow cylindrical plug (*a*) machined from aluminum (6061 alloy tubing: 1.0% Mg, 0.6% Si, 0.25% Cu, 0.2% Cr) fitted with two red cutoff filters (*c*: RG-610 glass discs, 2.2-cm diam by 3-mm thick; Schott Optical Glass Inc., Duryea, PA) and capped with a round glass coverslip (*e*). Plugs of identical design were set into the front and back parts of the horizontal hole running along the viewer's line of sight (not shown). The ends of the second horizontal hole contained solid cylindrical plugs (*d*), machined from aluminum (2024 alloy) and capped with round glass coverslips (*e*), one of which served as the avoidance barrier. A plug of more elaborate design was used in some experiments: this plug (not shown) was pierced by a hole (3.2-mm diam, 8 mm from the plug axis) containing a sliding rod (aluminum welding rod) that carried the barrier at its inner tip. The bottom port contained a micrometer with a nonrotating shaft (*mm*: No. 153-203; Mitutoyo/MTI Corp., Paramus, NJ) that carried a delrin (DuPont Co., Wilmington, DE) support (*j*) for the sporangiophore and allowed its height to be adjusted for growth. This micrometer was mounted on a circular plate with annular extension (*r*) that could be moved in the horizontal plane on a sliding O-ring seal (o_3) so that the sporangiophore could be centered with the chamber remaining airtight. The ports and plugs were machined to a tolerance of $\sim 10^{-3}$ cm, lapped by hand, and assembled with silicone high-vacuum grease (Dow Corning Corp., Midland, MI) to provide an airtight seal and adequate thermal conductivity. They were held in place by split-ring clamps (*b*) and could be positioned at will. A vent (not shown), closed by a stainless steel needle valve inserted from the outside, allowed air to enter or leave the chamber when the plugs were moved. This vent was 0.25 cm in diameter, 3.8-cm long, and drilled in a direction normal to the vertical axis of the chamber, 0.5 cm below the bottom edge of the side ports (3.5 cm above the bottom heater coil). The mycelium and agar in the growth vial (*g*) were covered with a layer of paraffin oil (*i*; Baker Co., Sanford, ME). A

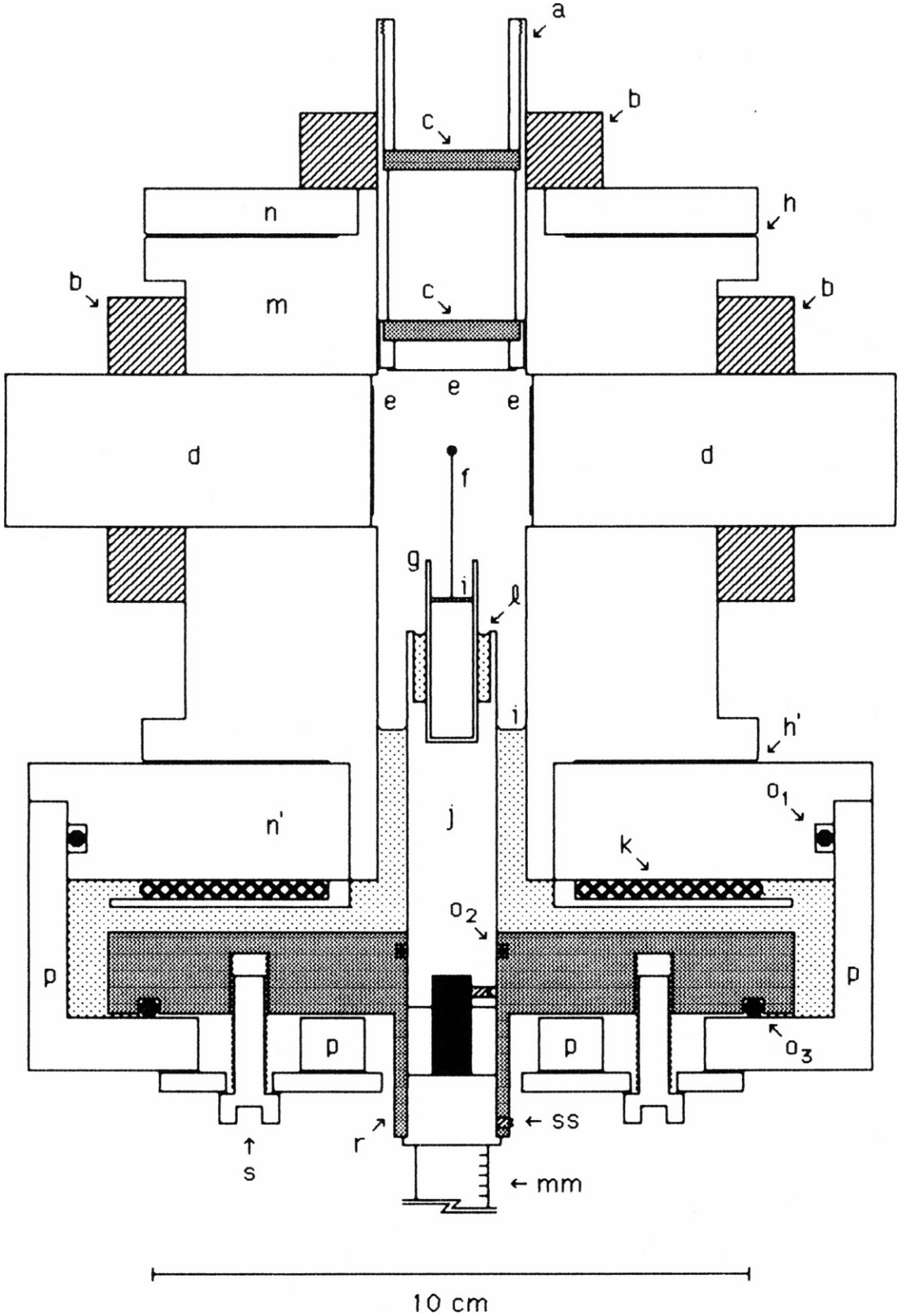

FIGURE 1 Cross-sectional view of the environmental chamber. (*a*) Top plug, (*b*) clamp for plug, (*c*) red filter, (*d*) side plug, (*e*) round glass coverslip, (*f*) sporangiophore, (*g*) glass vial, (*h*, *h'*) top, and bottom heater coils, (*i*) paraffin oil, (*j*) delrin holder for vial, (*k*) water cooling coil, (*l*) solution used to control relative humidity, (*m*) main body, (*mm*) nonrotating micrometer head, (*n*, *n'*) press-fit rings, (o_1) static O-ring seal, (o_2o_3) sliding O-ring seals, (*p*) bottom housing, (*r*) sliding circular plate with annular extension that supports the delrin holder, (*s*) clamp-down bolts for the sliding circular plate (three spaced equally on a 6.8-cm bolt circle; only one is actually visible in cross section, but two are shown for clarity), (*ss*) set screw. Not labeled: a second set screw clamping the delrin holder to the micrometer shaft. Not shown: horizontal sensing holes for the upper, and lower thermistor probes, 2.2-cm deep and located 0.65 cm below the top heater coil and 0.65 cm above the bottom heater coil; horizontal vent hole, 0.5 cm below the bottom edge of the side ports, closed on the outside with a stainless steel screw (opened during movement of plugs); cooling-coil tubing entering and leaving the apparatus through vertical holes, sealed with epoxy, in the bottom press-fit ring; drain line for paraffin oil in bottom housing; three support legs, attached to the underside of the bottom housing.

salt solution used to control the relative humidity (see below) filled an annular well in the delrin holder (ℓ). For most experiments, the bottom part of the apparatus was filled with paraffin oil (*i*) to a level 0.5 cm above the bottom heater coil. Thus, the only materials normally exposed to a sporangiophore during an experiment were aluminum alloy, stainless steel, glass, delrin, silicone grease, paraffin oil, and the solution used to control the relative humidity. The inside volume of the chamber was ~25 cm^3, with the oil added and with the plugs positioned as shown in Fig. 1.

Temperature Control

As noted above, the temperature at the top of the chamber was regulated by heating and at the bottom by heating and cooling. The heating coils were 20-m lengths of No. 32 magnet wire (No. 8082; Belden Electronic Wire and Cable, Richmond, IN; ~0.6 ohm/m) noninductively wound in a flat spiral (54 bifilar turns starting at the midpoint of the wire) extending 1.8–4.4 cm from the axis of the chamber, vacuum impregnated with paraffin. The cooling coil was a bifilar winding of copper tubing (3-mm outer diam) held in place with epoxy. The temperature was sensed by two thermistors (No. GB31J1; Fenwall Laboratories, Berkeley, CA) mounted in holes near the heating coils at positions indicated in the legend to Fig.1. These thermistors each comprised one leg of a bridge circuit used (in conjunction with an operational amplifier and a power transistor) to control the current flowing in the corresponding heater coil (gain 25 A/°C). The cooling coil carried water from a constant temperature bath (No. K-2/RD; Lauda Div., Brinkmann Instruments Co., Westbury, NY; run at 2.8 cm^3/s). The thermistors were calibrated with a thermometer traceable to the National Bureau of Standards. Normally, the temperature was held at 20.05°C at the top of the chamber and at 20.00°C at the bottom, while the bath was run between 19.0° and 19.5°C. With the bath at 19.0°C and the room at 20.0 ± 0.15°C, the current in the top coil was 0.20 ± 0.04 Å, and the current in the bottom coil was 0.56 ± 0.02 Å. The variations in current were caused by small changes in room temperature.

Viewing Arrangement

The sporangiophore was viewed horizontally from the front of the chamber with a low-power microscope (60-mm focal length; Gaertner Scientific Co., Chicago, IL) equipped with a goniometer for measuring the bending angle of the sporangiophore (accurate to about ±0.5°). This microscope was mounted on a micrometer-driven x-y-z stage (accurate to ±10 μm). A 30-W tungsten Koehler illuminator (No. 77914; Nikon Inc., Garden City, NY) run at 5 W provided dim back illumination. This light passed through two infrared blocking filters (No. KG-3, 2-mm thick; Schott Optical Glass Inc.) to prevent heating of the sporangiophore. Red cutoff filters in the viewing plugs (described above) prevented phototropic responses. The sporangiophore was viewed from above with another low-power microscope (80-mm focal length; Gaertner Scientific Co.) equipped with a crosshair and mounted on a micrometer-driven x-y stage (accurate to ±10 μm). When this microscope was used, the intensity of the illuminator was temporarily increased to full power, so that the sporangiophore could be seen by scattered light.

Air Movements

Convective stirring was monitored by injecting a 10-ml suspension of smoke particles into the chamber; in some cases, with a sporangiophore in place avoiding a planar barrier at a distance of 1–2 mm. The particles were produced either by burning a 2.5-cm long magnesium ribbon (3-mm wide by 0.2-mm thick; Sargent-Welch Co., Skokie, IL) inside a 500-ml flask containing 5% O_2 and 95% N_2 at a relative humidity above 90% or by burning ~50 mg of No. 5 filter paper (Whatman Inc., Clifton, NJ; held by a coil of hot nichrome wire) inside a similar flask containing room air. The particles were illuminated with a 1-mW helium-neon laser (No. 133; Spectra-Physics, Inc., Mountain View, CA) either by passing the beam horizontally through an observation plug inserted in the horizontal port opposite the barrier, or vertically down through the top observation plug with the chamber in its standard configuration (Fig. 1). The particles were viewed from the front with the horizontal telescope by scattered light. Measurements were made in the focal plane of the sporangiophore either 1 mm above the sporangium and 1 mm away from the barrier, or at the level of the center of the growth zone 1 mm on either side. In each observation, the vertical velocities of 10–20 different smoke particles were determined by timing their movement along two minor divisions of a reticle inside the eyepiece (a distance in the object plane of 130 μm). Steady horizontal movement of the particles was negligible. The mean sedimentation rate of the particles was estimated from observations made within 0.5 mm of the barrier surface. It varied anywhere from 1 to 10 μm/s. This was subtracted from the mean vertical velocity to give the values reported below. Brownian motion and sedimentation introduced an error into the measurement of wind speed near the sporangiophore of up to ±10 μm/s. The wind speed was checked once every 50–100 experiments.

Relative Humidity

The relative humidity was controlled by placing 0.5 ml of a saturated salt solution in an annular well at the base of the glass vial (Fig. 1, ℓ). At 20°C, the relative humidity at the surface of the saturated solutions used in the experiments was as follows: Na_2SO_4, 93%; K_2HPO_4, 92%; $Na(CH_3COO)$, 76% (Weast, 1975, p. E46). Since water vapor can diffuse 3 cm in ~20 s, the humidity inside the chamber should approach within 1% of its equilibrium value a few minutes after the chamber is closed.

The first observations of avoidance were made without filling the bottom of the chamber with paraffin oil. These included the demonstration of reproducible avoidance rates under fixed conditions, as well as most of the measurements of the humidity dependence. Since the base of the chamber was up to 1.0°C colder than the upper part, the vapor pressure of water there was lower, so that water could have diffused down from the annular well and condensed on the inside surfaces of the base. This flux would reduce the relative humidity at the level of the sporangiophore. However, this could not occur at relative humidities <94%, when the vapor pressure of water in the base (at 19.0°C) would be higher than that near the salt solution (at 20.0°C). This problem was avoided in later experiments by filling the base of the chamber with paraffin oil, as shown in Fig. 1.

Cleaning the Apparatus

The lower part of the apparatus was not usually cleaned, since it was filled with fresh oil at the beginning of each experiment. The bottom port and the vent hole also were not usually cleaned, since they were never greased. The remaining parts of the chamber were cleaned as follows. Kimwipes (13 × 22 cm, No. 34155; Kimberly-Clark Corp., Roswell, GA) were used to wipe off visible silicone grease from the inside surfaces of the top and four horizontal ports, from all surfaces of the solid and hollow plugs, including the inner cutoff filters and their retaining rings, and from the delrin sporangiophore holder. Kimwipes dipped in *n*-heptane (reagent grade; Mallinckrodt Inc., St. Louis, MO) held with a disposable polyvinyl chloride glove (American Scientific Products Div., American Hospital Supply Corp., McGaw Park, IL) were used to remove the remaining traces of grease from the top and four horizontal ports; a fresh Kimwipe was used to wipe them dry. This was repeated once with heptane, twice with RBS-35 alkaline detergent (Pierce Chemical Co., Rockford, IL; filtered through Whatman No. 5 paper) and twice with glass-distilled water. The remaining parts (as above, plus the needle valve) were rinsed several times in heptane and dried with Kimwipes, until the glass filters (Fig. 1 *c*) showed no visible traces of grease. All of these parts were then soaked in a 20% solution of RBS-35 alkaline detergent (filtered as above) in glass-distilled water at 90°–92°C for ~30 s. Any hydroxide layers formed on the aluminum parts were wiped off with a disposable PVC glove (also worn for all subsequent steps), and then the parts were immersed in glass-distilled water at room temperature. They were rinsed

5 to 10 times in glass-distilled water, until a soap bubble no longer appeared inside a retaining ring when it was removed from the rinse solution. All of the parts were then dried uncovered overnight in room air, by placing them on a double layer of Kimwipes (38 × 43 cm, No. 34255), with the surfaces that normally faced the sporangiophore in the apparatus turned upward and not touching the paper.

Cleaning the Barriers

Normally, 2.2-cm diam round glass coverslips (thickness No. 1; VWR Scientific Div., Univar, San Francisco, CA) or 30-μm diam Pyrex glass wool fibers (No. 3950; Corning Glass Works) were used as barriers. They were cleaned overnight before an experiment by soaking at room temperature in 90% fuming nitric acid (Aldrich Chemical Co., Milwaukee, WI). They were removed from the acid one by one with a pair of stainless steel forceps, rinsed twice in glass-distilled water, and stored under fresh glass distilled water in a Pyrex beaker covered with parafilm.

Standard Experimental Protocol

Normally, the apparatus was left assembled, except for the delrin holder and micrometer assembly. The bottom port was left open, and the delrin holder was covered with a Pyrex shell vial. A vial containing a vertical 1.5–3-cm tall sporangiophore was selected and all other sporangiophores in the vial were plucked with forceps. The mycelium was covered with a 1-mm deep layer of paraffin oil, and the vial was placed in the delrin holder and inserted into the experimental chamber from below. The illuminator was turned on and the sporangium was positioned to lie in the plane containing the axes of the horizontal ports, within 2 mm of the axis of the vertical port. If the sporangiophore was not vertical, it was inclined toward the barrier. Static charge on the sporangiophore was neutralized by holding a polonium-210 source (from a No. IC200 Staticmaster brush; Nuclear Products Co., South El Monte, CA) inside the chamber 1 cm away for 15 s. Clean air-dried coverslips were attached to the solid plugs with silicone vacuum grease. They were positioned as shown in Fig. 1. The sporangiophore was allowed to adapt to its new environment for at least 10 min before the barrier was moved into place.

The vertical growth of the sporangiophore was measured by lowering it approximately every 10 min, using the micrometer at the bottom of the chamber (accurate to ± 10 μm), so that the top of the sporangium was level with a horizontal hairline inside the eyepiece of the horizontal microscope. The diameter of the sporangium and the diameter of the sporangiophore's stalk 1.0 mm below the base of the sporangium were measured at the beginning of each experiment using a vertical hairline inside this eyepiece. The point 1.0 mm below the sporangium was located using the calibrated reticle. The distance between the axis of the sporangiophore at this point (the center of the growth zone) and the surface of the barrier was measured in the same way. The vertical telescope was used to measure the horizontal position of the sporangiophore once before bringing up the barrier and once again at the end of the avoidance response, 20–30 min later. Sometimes the horizontal position was checked during the course of the response. These data were used to estimate the sporangiophore's aiming error (see below).

Data Analysis

We wanted to know the bending rate away from the barrier in the plane of the bend, $d\theta/dt$, given the rate observed in the focal plane of the horizontal telescope, $d\alpha/dt$, and the aiming error obtained from the vertical observations, ϕ. The latter two parameters were determined as follows. The angle with respect to the vertical, α, of the top 0.5-mm segment of the growth zone was measured with the goniometer every few minutes and plotted as a function of time. The bending rate, $d\alpha/dt$, was taken to be the slope of the steepest line that could be fit to these data over a 10-min interval after the onset of the response. The aiming error, ϕ, for this 10-min interval was estimated from a plot of the position of the sporangium in the horizontal plane, as viewed from above. Now, horizontal displacements in the plane of the bend are foreshortened on the focal plane of the horizontal telescope by a factor $\cos\phi$, while vertical displacements remain unchanged. Let the horizontal displacement of the top segment of the growth zone in the plane of the bend be x and that in the focal plane be $\rho = x\cos\phi$; let the vertical displacements be z. Then $\theta = \tan^{-1}(x/z) = \tan^{-1}(\rho/z\cos\phi) = \tan^{-1}(\tan\alpha/\cos\phi)$. For angles $<30°$, the angle and its tangent are approximately equal, so that $\theta \simeq \alpha/\cos\phi$ and $d\theta/dt \simeq (d\alpha/dt)/\cos\phi$, the required result.

Next, we wanted to estimate the speed of elongation of the sporangiophore in a direction parallel to the growth zone, v, given the vertical speed, dz/dt, and the bending angle and rate θ and $d\theta/dt$. The vertical speed was determined from the slope of a plot of the vertical displacement as a function of time. The vertical displacement was read from the setting of the micrometer at the bottom of the chamber, as described above. There are two independent contributions to the vertical speed. One is just $v\cos\theta$, the projection of v on the vertical axis. The other is due to the downward bending of the sporangiophore, which we approximate as bending about a hinge a distance $\ell = 2$ mm from the top of the growth zone. This contribution to the vertical speed is $d(\ell\cos\theta)/dt = -\ell\sin\theta(d\theta/dt)$. Thus, $dz/dt = v\cos\theta - \ell\sin\theta(d\theta/dt)$ or $v = (1/\cos\theta)[dz/dt + \ell\sin\theta(d\theta/dt)]$. Since θ was not large, this correction was relatively small.

Finally, the bending rate, $d\theta/dt$, was normalized to a standard growth rate, $v_s = 50$ μm/min, by multiplying it by the factor v_s/v. We refer to this product as the normalized bending rate.

The results of an experiment were discarded if the initial angle of the sporangiophore toward the barrier was outside the range $1° \le \alpha \le 15°$, if the aiming error was outside the range $0° \le \phi \le 35°$ in either direction, or if the growth rate in a direction parallel to the growth zone was outside the range 30 μm/min $\le v \le$ 65 μm/min.

RESULTS

Air Movements

The mean speed of the air 1 mm from the barrier was determined in a series of observations of 10–20 smoke particles (Fig. 2). A sporangiophore was present for the points obtained at −0.015°, 0.045°, and 0.16°C. The only significant movement observed was in the vertical direc-

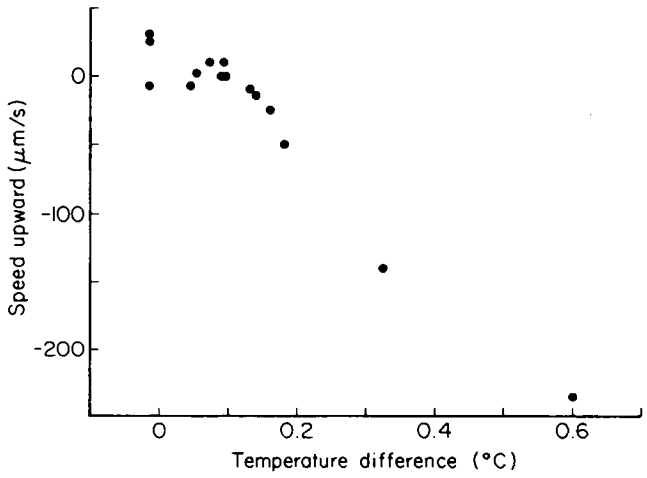

FIGURE 2 Mean upward speed of smoke particles (corrected for sedimentation) as a function of the difference in temperature sensed by the two thermistors (top minus bottom, with the bottom at 20.00°C). The standard deviation for each point was $\sim\pm 10$ μm/s at temperature differences below 0.15°C, and $\sim\pm 30$ μm/s otherwise. The negative temperature difference was generated by cooling the room to 19.0°C, and turning off the top heater.

tion. For temperature differences between 0° and 0.1°C, the mean speeds were less than the experimental error of ~10 μm/s; therefore, a temperature difference of 0.05°C was chosen as the normal operating point. These measurements were made with the horizontal laser beam (see Methods). Similar results were obtained with the vertical beam (data not shown). In particular, measurements made at a temperature difference of 0.05°C with a sporangiophore present always yielded mean speeds that were less than experimental error. Since a large molecule in air with a diffusion coefficient as small as 10^{-2} cm^2/s can diffuse 1 mm (the nominal distance between the sporangiophore and the barrier) in ~0.5 s, while transport over this distance by bulk flow at the rate 10 μm/s requires 100 s, we conclude that the effects of convection are completely negligible.

Conditions for Reproducible Avoidance

An initial series of experiments was carried out to see if we could find conditions under which avoidance rates were reasonably constant from sporangiophore to sporangiophore. We made a single measurement on each of a series of 15 sporangiophores over a period of ~3 wk; the distance from the barrier was 1 mm. The other conditions used were as defined in Methods, unless otherwise noted. The annular well contained distilled water, no oil was used in the bottom part of the apparatus, and the cooling coil was run at 19.0 ± 0.1°C, so the relative humidity near the growth zone was ~97%. Fresh coverslips were attached to the two solid plugs before each measurement. The apparatus was not cleaned between measurements; however, the delrin support and solid plugs were removed and stored in Pyrex culture jars, while the bottom port was left open and the side ports were blocked with Kimwipes. 11 of the 15 sporangiophores satisfied the criteria for acceptable aiming errors, growth rates, and initial bend angles defined in the section on data analysis. For these sporangiophores, there was a steady decline in the normalized bending rate from specimen to specimen of ~0.03°/min. When corrected for this decline, the mean and standard deviation for these data were 2.4 ± 0.1°/min. Thus, avoidance can occur at a sizeable and reproducible rate in the absence of random winds, i.e., in the diffusion limit. Two additional measurements were made with sporangiophores at 0.5 and 2 mm from the barrier, giving values for the normalized bending rate of 2.7 and 2.3°/min, respectively (corrected for the decline), suggesting a shallow distance dependence (see below). Finally, the original bending rate at a distance of 1 mm (2.4°/min) was restored when the apparatus was allowed to stand for 1 wk.

Other observations were of interest: The normalized bending rate was independent of the diameter of the growth zone (range 0.14–0.18 mm). When the illuminator was turned up to full power for a brief sighting through the vertical telescope in the first 8 min after the barrier was brought up, the bending rate was depressed by ~30% (to 1.7°/min); this effect was absent if the illuminator was turned up later, any time after 10 min; neither procedure appeared to affect the growth rate (cf. Harris and Dennison, 1979). There was a relatively large scatter in aiming errors. Correlations between aiming error and the following parameters were looked for but not found: diameter of the growth zone, diameter of the sporangium, length of the sporangium, growth rate, age of mycelium, relative humidity (range 76–98.5%), time in the chamber before the barrier was brought up, sequence in a series of experiments carried out in a given day, and replacement of coverslips on the viewing plugs. There was a small correlation with the initial bend angle. For 48 sporangiophores tested (as above, but at relative humidities ranging from 76 to 98.5%), half started at an initial angle toward the barrier of 0–6° and gave aiming errors ranging from 0–37° (mean and standard deviation 20.4 ± 12.2); the other half started at 7 to 20° and gave aiming errors ranging from 0 to 58° (mean and standard deviation 26.3 ± 22.1). The reasons for this correlation are not known.

The avoidance rate did depend on relative humidity (Fig. 3) but weakly. As noted above, with no oil in the bottom of the apparatus, the values of relative humidities >94% were suspect; therefore, a comparison of bending rates at 93 and 100% relative humidity (water in the annular well and a wet annular glass-fiber filter at the top viewing plug) was made under the conditions used for studies of distance dependence (see below). The point at 100% relative humidity (Fig. 3) was inferred from these measurements.

Inhibition in a Clean Apparatus

The procedure for cleaning the apparatus described in Methods was devised in the hope that it would prevent the slow decrease in avoidance rate noted above. To our

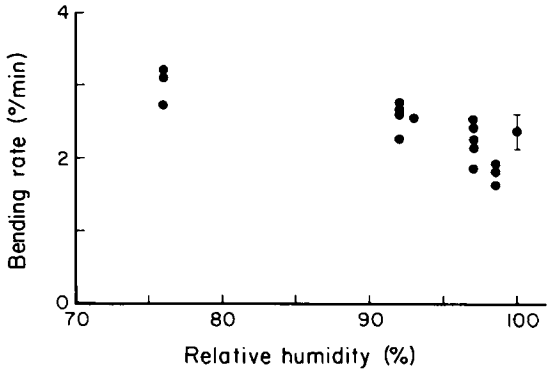

FIGURE 3 Normalized bending rate as a function of relative humidity. The barrier was a glass coverslip (2.2-cm diam) 1 mm away from the center of the growth zone. Each point represents the measurement of a different sporangiophore, except for the point at 100% relative humidity, which was inferred from the ratio of the bending rates at 100 and 93% measured when the bottom part of the apparatus was filled with oil: 0.99 ± 0.08°/min (mean ±SE) at 100% relative humidity (17 measurements on 10 sporangiophores); 1.06 ± 0.07°/min at 93% relative humidity (22 measurements on 20 sporangiophores).

surprise, it markedly increased the latency of the response and limited its duration. These experiments were done at a relative humidity of 93%, with the bottom part of the apparatus filled with paraffin oil. If the chamber and plugs were cleaned just before the experiment, the sporangiophore would bend away from its initial angle of 5 to 10° toward the barrier until it was approximately vertical and then stop; the mean bending rate fell to 0.46°/min and the mean angle of bend after 45 min fell to −0.6° (i.e., toward the barrier; 19 experiments). If the chamber and plugs were not cleaned during the previous few experiments but were allowed to stand in the open air uncovered, the mean bending rate rose to 0.87°/min, and the mean angle of bend after 45 min rose to 14.6° (12 experiments). In some cases, with a freshly cleaned apparatus, no response was observed for at least 30 min. Then, if 50–100 ml of room air was drawn through the chamber (by inserting a 4.3-cm long piece of 1.9-mm o.d. polyethylene tubing into the vent hole and pulling on it with a vacuum at the rate of ~3 ml/s) an avoidance response was initiated of normal latency, speed, and duration. Blowing 50–100 ml of room air or pure air into the chamber (through the same tube at the same rate) gave identical results. When blowing, the air was equilibrated with a saturated solution of Na_2SO_4, so that its relative humidity was 93%; the pure air contained 20 ± 1% O_2, balance N_2, no CO_2, and typically $<10^{-5}$ ppm hydrocarbons (<0.5 ppm guaranteed; UHP air, Big Three Industries, Inc., Houston, TX). A control was run to see whether freshly cleaned aluminum (2024 to 6061 alloy) might poison the system. Aluminum disks (2-cm diam by 0.3-cm thick) were cleaned in the standard manner and attached to the face of the plug opposite the barrier; the rest of the apparatus was not cleaned. The aluminum disks did not inhibit the avoidance response.

Avoidance gradually returned to normal as the apparatus was used over a period of several weeks (not cleaned, without replacing the barrier). However, a difference was noted depending upon whether (*a*) the plugs were removed and, along with the chamber, kept in the open air between experiments, or (*b*) the plugs and the chamber were kept in the open air but covered with a Kimwipe, or (*c*) the plugs were left in the apparatus (as in the standard experimental protocol). In case *a*, the avoidance rate increased with the time that a sporangiophore was in the chamber, from 0 to ~1°/min at 2.5 h, and then leveled off. In cases *b* and *c*, the rate started out at a high level and remained fairly constant, at ~1.0 ± 0.2°/min. Therefore, in the procedure adopted for the remainder of the work, only the delrin holder-micrometer assembly was removed between experiments. Fresh coverslips were used on the solid plugs for each sporangiophore. This gave a somewhat higher avoidance rate, ~1.2°/min at 1 mm (see below). Note that these rates were about half as large as those described in the previous section. The difference probably was due to the smaller volume of the chamber, which was reduced by a factor of about three by the addition of paraffin oil.

We do not understand the inhibition due to cleaning, but it is evident that the inner surface of the chamber either emits or adsorbs some substance, and that the concentration of this substance on the surface of the chamber or in the air inside it affects the response. The rate at which the surface is recontaminated or purged between experiments is sensitive even to the interposition of a Kimwipe.

Distance Dependence

These experiments were carried out over a period of several months. There was more scatter in bending rates than in the earlier experiments (above), but there was no long-term upward or downward trend. Data for avoidance of round or half-round glass coverslips are summarized in Fig. 4. Measurements were made by the standard protocol at distances of 1–7 mm (53 measurements on 45 sporangiophores; *closed circles*), by suspending the coverslip at the end of a thin rod at distances of 0.5 and 1 mm (12 measurements on eight sporangiophores; *open circles*), or by suspending a half-round coverslip at the end of a thin rod at distances of 0.1, 0.5, and 1 mm (40 measurements on 36 sporangiophores), respectively. With the standard protocol, as many as five measurements were made on a single sporangiophore (by withdrawing the barrier and bringing it up again) over periods of more than 6 h. The response did not decrease over this time period (data not shown). The decline in avoidance rate at large distances did not appear to be due to the proximity of the second barrier, which could be pulled back 5 mm without effect. Note that the change in avoidance rate with distance was relatively small out to distances of at least 4 mm. Note also that the

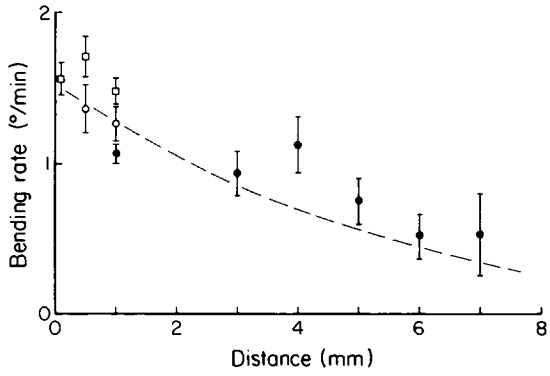

FIGURE 4 Normalized bending rate as a function of the distance between the midpoint of the growth zone and the surface of a glass coverslip (2.2-cm diam). The coverslip either was attached to the face of one of the solid plugs (*closed circles*), to the end of a thin rod passing through a solid plug (*open circles*), or it was cut in half and attached to the end of the thin rod (*open squares*) so that its upper (straight) edge was ~50 μm below the bottom of the sporangium. The bars are standard errors in the mean for 22, 7, 9, 5, 7, and 3 measurements (*left* to *right*, *closed circles*), 5 and 7 measurements (*left* to *right*, *open circles*), or 11, 16, and 13 measurements (*open squares*), respectively. The dashed curve is the prediction for the model involving emission of a growth promoter with decay length R = 0.6 cm, outlined in the Appendix.

avoidance rate did not increase dramatically as the barrier was moved close to the growth zone; compare the bending rates for the half-round coverslips at 0.1, 0.5, and 1.0 mm. The avoidance rates for coverslips suspended on the thin rod (Fig.4, *open symbols*) were consistently higher than for coverslips attached to the plug (*solid symbols*). This difference might also be due to changes in the volume of the chamber (see above), which was reduced by movement of the plug. But this would not explain why avoidance from the half-round coverslips was somewhat higher than that from the round ones (Fig. 4, *open squares* and *circles*, respectively). One other difference should be noted: in moving the thin rod, it was not necessary to open the vent, so with this technique the chamber remained completely isolated.

Data for avoidance of thin glass fibers are summarized in Fig. 5. These measurements (69 on 55 sporangiophores) were made by suspending the barrier at the end of the thin rod. At the beginning of the experiment, the rod was advanced to a point several millimeters above the sporangiophore, with the fiber pointing upwards. At the end of the adaptation period, it was rotated 180° to bring the fiber in juxtaposition to the growth zone. The rotation cycle was repeated as many as six times with a single sporangiophore over periods of more than 8 h. With the possible exception of measurements made at 1 mm, the response did not decrease over this time period (data not shown). Note that at a distance of ~0.5 mm, the avoidance rates for the fibers and the coverslips (cf. Fig. 4) were approximately the same. However, the drop in avoidance rate with distance was much greater for the fibers than for the coverslips. At large distances, an increasing fraction of measurements gave bending rates that were zero or negative (1/7 and 1/3 at 6 and 7 mm in Fig. 4, and 7/15 and 1/3 at 3 and 4 mm in Fig. 5, respectively).

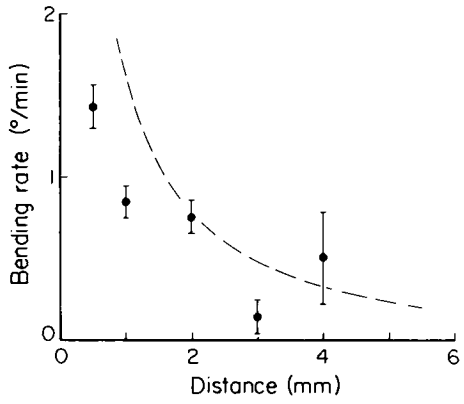

FIGURE 5 Normalized bending rate as a function of the distance between the midpoint of the growth zone and the axis of a parallel glass fiber (30-μm diam by ~2-cm long) attached to the end of a thin rod. The bars are standard deviations in the mean for 11, 20, 20, 15, and 3 measurements (*left* to *right*), respectively. The dashed curve is the prediction for the model involving emission of a growth promoter with decay length R = 0.6 cm, outlined in the Appendix.

Lafay et al. (1975) studied the distance dependence for avoidance of planar iron or brass barriers (in open air). They found a relatively shallow dependence, except at distances less than ~0.5 mm. As noted above, we did not observe a steep rise at short distances (Fig. 4, *open symbols*). Cohen et al. (1975) studied the distance dependence for avoidance of horizontal tungsten or nylon fibers (50-μm diam, in a sealed lucite box, 6.2 cm on a side). They also found a relatively shallow dependence, but data were not collected for distances >1 mm.

DISCUSSION

In summary: (*a*) Normal avoidance occurs in the absence of convection; it does not require random winds. (*b*) The variation in avoidance rate for different sporangiophores tested under identical conditions can be as low as ±5%. (*c*) The response falls off slowly with increasing relative humidity; it does not approach zero at 100% relative humidity. (*d*) The response is sensitive to the size of the experimental chamber, and it is inhibited if the chamber is cleaned. Under certain conditions, the response increases the longer a sporangiophore has been enclosed. Thus, the response depends on the chemical composition of the air inside the chamber, of the surfaces in the vicinity of the sporangiophore, or both. (*e*) The avoidance rate falls off very weakly with distance. It is nearly constant for a planar barrier placed 0.5–4 mm away. It is of the same order of magnitude for a fiber 30 μm in diameter 0.5 mm away. However, the rate for the fiber falls off more rapidly with distance than that for the planar barrier. (*f*) A normal response can be obtained repeatedly if the barrier is brought up to the growth zone several times over the course of several hours.

These results argue strongly for the existence of a diffusible chemical substance that affects the growth rate of the sporangiophore. As argued by earlier workers (see the Introduction), avoidance occurs when changes in the concentration of this substance cause the proximal side of the growth zone (the side facing the barrier) to grow more rapidly than the distal side. We have found that such changes can be effected by diffusion alone. Winds were of no consequence in the experiments reported here. Note that diffusion can work effectively even in the presence of random winds, provided that their speeds are not large. A small molecule in air his a diffusion coefficient, D, of ~0.1 cm^2/s. It can diffuse a distance, d, in a time of order $d^2/2D$. If the air moves at velocity, v, the molecule will be carried this distance in a time d/v. Diffusion will be faster if $d^2/2D < d/v$, or $v < 2D/d$. For d = 2 mm (the length of the growth zone and a typical distance to the barrier) diffusion wins for v < 1 cm/s. The winds in our apparatus, if any, were a thousand times smaller than this. However, winds in the range 15–30 cm/s blowing in a direction parallel to the axis of the sporangiophore should inhibit avoidance, as observed by Cohen et al. (1975).

The diffusible chemical has been regarded as a growth-promoting substance. But note that if it were present in the ambient air and adsorbed by the barrier, it could equally well be a growth-inhibiting substance. A large number of volatile, growth-inhibiting substances are, in fact, known (Elving, 1916–1917; Cohen et al., 1979). Such substances also could mediate the transient increase in growth rate effected by symmetrical barriers (Bergman et al., 1969; Ortega and Gamow, 1970) or growth into a wind (Cohen et al., 1975). The only argument against such a mechanism based on our data is that the same barrier can be used repeatedly in an enclosed environment. One would expect (particularly with a fiber) that available adsorption sites would soon be occupied.

Whether avoidance occurs through adsorption of a growth inhibitor or emission of a growth promoter, the barrier must play an active role. A mechanism involving passive reflection cannot explain why a thin fiber should be nearly as effective as a plane, or why a plane should show such a shallow distance dependence (see below). Remarkable as it might seem, an adsorbent fiber of length $2a$ can remove particles of a diffusible substance from its surroundings at nearly the same rate as a one-sided disk of radius a: for such adsorbers immersed in an infinite medium, the ratio is $\sim\pi/\ln(2a/b)$, where $b \ll a$ is the radius of the fiber (Berg, 1983, pp. 27–29). For $2a = 2.2$ cm and $b = 15$ μm, this ratio is 0.43. In short, a particle wandering at random near the surface of an imaginary disk has a reasonably good chance of bumping into a fiber stretched along the diameter of that disk. If the particle is adsorbed by the fiber and, thus, removed from the environment, the fiber will perturb concentrations a long distance away. If the particle simply bounces off or is adsorbed and re-emitted without chemical transformation, the perturbation will be much smaller. This argument, and the fact that avoidance works well at 100% relative humidity, rules out water vapor as a possible avoidance gas. An alternative hypothesis is that the sporangiophore emits a growth-promoting substance in the form of an inert precursor: after adsorption by the barrier, this material decomposes and is re-emitted in active form. This is the hypothesis that we favor.

In the Appendix, we consider three models in detail: reflection of a growth promoter, emission of a growth promoter, and adsorption of a growth inhibitor. We predict bending rates for each model by finding an approximate solution to the steady-state diffusion equation for a thin cylinder (the growth zone) placed near a parallel plane or wire. From this we estimate the relative difference in concentration or flux of a putative signal molecule across the growth zone. Assuming that the bending rate of the sporangiophore is proportional to this difference, we then decide whether or not a given model is consistent with the results of Figs. 4 and 5. The solutions for the second and third models are less rigorous than the first, because the effects of the avoidance gas are felt over a longer distance, and we have neglected perturbations of the boundary conditions at one surface (except at the growth zone) due to emission or adsorption at another. For intermediate steps in these calculations, see Meyer (1986, Appendix 3). The results are summarized in Table I.

For reflection of a growth promoter, the bending rate expected for the plane is more than 4,000 times larger than that for the wire, and the rates fall off as $1/d^2$ or $1/d^4$, respectively, where d is the distance between the sporangiophore and the barrier (Table I). Both of these predictions contradict the results of Figs. 4 and 5. For emission of a growth promoter, the bending rates expected for the plane and the wire are of the same order of magnitude; the distance dependence for the plane is relatively shallow, while that for the wire falls off as $1/d$ (Table I). This is shown by the dashed curves in Figs. 4 and 5. For adsorption of a growth inhibitor, the two bending rates also are of the same order of magnitude, but they both fall off as $1/d$ (Table I); a shallow distance dependence for the plane requires the ad hoc assumption that the response saturates at a bending rate of $\sim 1.2°$/min.

If avoidance requires adsorption and/or emission of a specific chemical substance, as our results imply, then bending rates ought to depend on the chemical composition and the adsorbing power of the barrier. If the avoidance gas is exogenous, then the response also should depend on the purity of the surrounding air. We hope to test these predictions in a controlled environment. But the ultimate

TABLE I
PREDICTIONS OF THREE AVOIDANCE MODELS OUTLINED IN THE APPENDIX*

Model	Signal	Signal level (and distance dependence) for	
		Plane at 2 mm	Wire at 2 mm
Reflection of growth promoter	$\Delta c/c$	$3.2 \times 10^{-3}\ (1/d^2)$	$7.2 \times 10^{-7}\ (1/d^4)$
Emission of growth promoter	$\Delta F/F$	$1.0 \times 10^{-1}\ [\exp(-d/R)]$	$7.3 \times 10^{-2}\ (1/d)$
Adsorption of growth inhibitor	$\Delta F/F$	$3.1 \times 10^{-1}\ (1/d)$	$4.2 \times 10^{-2}\ (1/d)$

*c is the concentration and F the flux of the signal substance at the surface of the growth zone; d is the distance between the axis of the growth zone and the surface of the plane or the axis of the wire; R is the decay length of the growth promoter.

solution to this mystery requires the isolation and characterization of the avoidance gas. Our results argue that it is worth looking for.

APPENDIX

Reflection of a Growth Promoter

We assume that the growth zone is a right circular cylinder of length L = 0.2 cm and radius a = 0.005 cm that emits a growth-promoting gas X of diffusion coefficient D (in cm^2/s), at a uniform flux F (in molecules/cm^2 per s). The sporangiophore stands in open air that is free from convection. If there is a parallel plane or wire barrier, call its distance from the axis of the growth zone d. We assume that the sporangiophore is vertical and ignore the fact that it bends away from the barrier during the response. We also ignore edge effects due to the sporangium. Further, we assume that if a gradient of X is imposed across the growth zone, the bending rate of the sporangiophore is proportional to the relative difference in concentration of X between opposite sides of the growth zone, measured at its midpoint ($L/2$ from either end). We denote this relative difference by $\Delta c/c$, where Δc is the concentration of X on the side of the growth zone facing the barrier minus its concentration on the opposite side, and c is the average concentration of X around the circumference.

To compute $\Delta c/c$, we first estimate the concentration of X in the horizontal plane, P, passing through the midpoint of the growth zone. We approximate the growth zone by a finite vertical line source of length L located on the axis and emitting X at the same rate; this is a good approximation except at the ends of the growth zone. The line source must emit X along its length at a rate $2\pi aF$ molecules/cm per s. Thus, an infinitesimal segment, dz', of the line source emits X at a rate $2\pi aF dz'$ molecules/s. The concentration at any given point due to a particular segment dz' is $c(r') = aF dz'/2Dr'$, where r' is the distance between the point and the segment. This is the appropriate Green's function solution for the diffusion equation at steady state, $D\nabla^2 c = 0$ (Laplace's equation; cf. Smythe, 1950). Integrating $c(r')$ along our line source, we find that the concentration of X at any point in the horizontal plane, P, at a distance r from the source, is $c(r) = (aF/2D)\ln\{[\xi(r) + 1]/[\xi(r) - 1]\}$, where $\xi(r) = [1 + (2r/L)^2]^{1/2}$. Note that for $r << L$, close to the line source, $c(r)$ reduces to $c(r) \simeq (aF/D)\ln(L/r)$, while for $r >> L$, far from the line source, $c(r) \simeq (aF/D)(L/2r)$. These approximations simplify the calculations that follow. In practice, for L = 0.2 cm, they are good to within ~5% for $r < 0.05$ cm or $r > 0.17$ cm, respectively. It is convenient to use the first approximation when considering the effects of the emission of X on the growth zone itself (at $r = a \simeq 0.005$ cm) and the second approximation when considering perturbations due to a barrier (at a distance $d \simeq 0.2$ cm away).

Next we determine the effect of nearby barriers on the concentration of X at the growth zone. A parallel, plane reflecting barrier located at a distance d from the axis of the growth zone is equivalent to a parallel, image growth zone (line source) located at a distance $2d$. The concentration of X at the growth zone due to this image is $c(r) \simeq (aF/D)(L/2r)$, with $r = 2d$. To find the magnitude of the concentration difference induced across the growth zone by the barrier we take the derivative of this expression with respect to r, evaluate the result at $r = 2d$, and multiply by the width of the growth zone. We find $\Delta c = a^2FL/4d^2D$. The average concentration at the growth zone is $c(a)$ due to the growth zone plus $c(2d)$ due to its image, $c = (aF/D)[\ln(L/a) + L/4d]$.

Note that the image source perturbs the uniform-flux boundary condition at the surface of the growth zone. This perturbation can be offset by the addition of a line dipole along the axis of the sporangiophore. As shown for the wire barrier (below), the strength of this dipole can be adjusted to cancel the flux, F_r, at the position of the growth zone due to reflection of X by the barrier. The outward flux due to this dipole at the surface of the growth zone is $F_r\cos\phi$, where ϕ is the azimuthal angle around the axis of the growth zone, and $\phi = 0$ is toward the barrier. One can show that this dipole produces a concentration difference across the growth zone that is higher on the side facing the barrier by the amount $2aF_r/D$, which is just the concentration difference that would be induced by F_r alone (Meyer, 1986). Thus, the effect of the dipole is to double Δc.

Taking this into account, we find for $d > 0.17$ cm that $\Delta c/c = aL/2d^2[\ln(L/a) + L/4d]$. In particular, if L = 0.2 cm, a = 0.005 cm, and d = 0.2 cm, we get $\Delta c/c = 3.2 \times 10^{-3}$. The distance dependence is $1/d^2$.

A parallel, reflecting wire is equivalent to a line dipole located along the axis of the wire and lying in the plane containing both the axis of the wire and the axis of the growth zone. The dipole's line source is located at the distance ϵ from the axis of the wire on the side facing the growth zone, and its line sink is located the same distance from this axis but on the opposite side. If this source and sink emit and adsorb X at a rate f molecules/cm per s along their lengths, then the dipole moment needed to cancel the flux of X at the surface of the wire (as required if the wire is to reflect X) is $2f\epsilon = \pi\rho_0^2 aLF/d^2$, where ρ_0 is the radius of the wire. The concentration of X due to this dipole at a distance ρ from the axis of the wire (small compared with its length) is $c(\rho) = aFL\rho_0^2/2d^2D\rho$. Proceeding as before, and including the correction for the constant-flux boundary condition at the surface of the growth zone, we find $\Delta c = 2a^2FL\rho_0^2/d^4D$ and $c = (aF/D)[\ln(L/a + L\rho_0^2/2d^3]$. Ignoring the second term in the brackets, which is negligible, we get $\Delta c/c = 2aL\rho_0^2/d^4\ln(L/a)$. Note that this result is smaller than that for the plane barrier, given above, by the factor $4\rho_0^2/d^2$. For L = 0.2 cm, a = 0.005 cm, d = 0.2 cm, and ρ_0 = 0.0015 cm, $\Delta c/c = 7.2 \times 10^{-7}$. This value is more than 4,000 times smaller than that for the plane barrier, and the distance dependence is much steeper, $1/d^4$.

Emission of a Growth Promoter

Here, the growth zone emits an inactive precursor that adsorbs to nearby surfaces, including the surface of the growth zone itself, and then decomposes into a volatile growth promoter that we call X_E. X_E escapes into the surrounding air, where it diffuses with diffusion coefficient, D, and decays with decay time, τ, to form an inert product. The corresponding decay length, R, is $(D\tau)^{1/2}$. If R is small compared with the dimensions of the chamber (e.g., R = 0.5 cm) and the sporangiophore is placed near a barrier (e.g., at d = 0.2 cm), then the concentration of X_E will be greater on the side of the growth zone facing the barrier than on the opposite side, and the sporangiophore will bend away from the barrier. To find the concentration of X_E in the vicinity of the growth zone, or near barriers, we solve a version of the diffusion equation modified to take into account the decay of X_E, namely, $D\nabla^2 c = c\tau$, or $\nabla^2 c = c/R^2$.

For simplicity, we assume that the concentration of X_E is approximately constant near all surfaces and that the response is proportional to the relative difference in flux of X_E across the growth zone, $\Delta F/F$. The concentration will be approximately constant near a surface if escape from the surface is limited by diffusion in the surrounding air and not by the rate of evaporation. If changes in flux are relatively small, the concentration of X_E on the surface of the growth zone will rise and fall inversely with F, but not by much. We assume that the growth zone senses these variations.

At distance x from the center of a square plane barrier of height $h >> x$, $c(x) = c_0\exp(-x/R)$, where c_0 is the concentration at the surface. If this barrier forms one end of a rectangular box of width w, then $c(x) = c_0\{\exp(-x/R) + \exp[(x - w)/R] + 4\exp(-h/2R)\}$. Here, we have added the solutions for all six walls, ignoring mutual perturbations of their uniform-flux boundary conditions.

The concentration $c(r)$ due to the emission of X_E at the surface of an isolated sporangiophore, which we approximate by a cylinder of infinite length and radius $a << R$, is $c(r) = c_0K_0(r/R)/K_0(a/R)$, where r is the distance from the axis of the sporangiophore, and K_0 is the zero-order modified Bessel function of the second kind (Meyer, 1986, p.130). Thus, the total concentration at the surface of the growth zone inside the box is $c = c_0\{K_0(r/R)/K_0(a/R) + \exp(-d/r) + [\exp(d - w)/R] + 4\exp(-h/2R)\}$.

Returning to the expression for $c(x)$, we take the derivative with respect to x and multiply by D to determine the flux at the growth zone (at $x = d$) due to the barrier. The flux difference is twice this value. A correction for the perturbation of the uniform-concentration boundary condition at the surface of the growth zone (similar to that used for the constant-flux boundary condition, above) provides another factor of 2. This gives for the magnitude of the flux difference across the growth zone, $\Delta F = (4c_0D/R)\{\exp(-d/R) - \exp[(d - w)/R]\}$. The magnitude of the flux at the surface of the sporangiophore is $F = (c_0D/R)K_1(a/r)$, where K_1 is the first-order modified Bessel function of the second kind. Thus, $\Delta F/F = 4\{\exp(-d/R) - \exp[(d - w)/R]\}K_0(a/R)/K_1(a/r)$. Since in our experiments $(d - w) = 1.3$ cm, the distance dependence for small values of d is $\exp(-d/R)$. In particular, for $R = 0.6$ cm, $a = 0.005$ cm, and $d = 0.2$ cm, we get $\Delta F/F = 0.10$. This solution is shown in Fig. 4 (*dashed line*).

For a thin wire barrier the solution is $c(\rho) = c_0K_0(\rho/R)/K_0(\rho_0/R)$, where ρ is the distance from the axis of the wire, and ρ_0 is its radius. To get the flux difference across the sporangiophore, we take the derivative of $c(\rho)$ with respect to ρ and multiply by D, evaluate this product at $\rho = d$, and multiply by 4 (see above). This gives $\Delta F = (4c_0D/R)K_1(d/R)/K_0(\rho_0/R)$. Thus, $\Delta F/F = 4K_1(d/R)K_0(a/R)/K_0(\rho_0/R)\,K_1(a/R)$. For $d < R$, the distance dependence is $1/d$. For $R = 0.6$ cm, $a = 0.005$ cm, $d = 0.2$ cm, and $\rho_0 = 0.0015$ cm, we get $\Delta F/F = 0.073$. This solution is shown in Fig. 5 (*dashed line*).

Adsorption of a Growth Inhibitor

A growth inhibiting gas X_1 is present in the ambient air and is adsorbed so efficiently by all surfaces, including the surface of the sporangiophore itself, that its concentration falls to zero there. We assume that the sporangiophore measures the adsorbed flux of the inhibitor at its surface, and that the bending rate of the sporangiophore is proportional to the relative difference in flux of X_1 between opposite sides of the growth zone, $\Delta F/F$, measured at its midpoint. Here, F is the average adsorbed flux of X_1 around the circumference. Note that the sporangiophore could measure these fluxes by measuring the local concentration of inhibitor in the cell wall. This concentration will not rise indefinitely, because the growth zone continually elongates, adding nascent wall above and leaving behind mature wall below. This dilution will offset the influx of inhibitor, leading to a steady-state concentration that depends on the local flux. If an adsorbent barrier is placed next to the growth zone, the flux of X_1 will be smaller on the side of the growth zone facing the barrier than on the opposite side, and the sporangiophore will bend away from the barrier.

First, consider the case of a perfectly adsorbing plane barrier. We assume that the barrier is h by h cm square and forms one end of a rectangular box of width w. The end opposite to the barrier also is adsorbing, but the other four walls of the box are not. We assume further that the inhibitor gas X_1 is produced uniformly throughout the volume of the box at a rate Q molecules/cm^3 per s. We solve a version of the diffusion equation modified to take into account this production, namely, $D\nabla^2c = -Q$ (Poisson's equation), working only in one dimension. We find that the concentration along the axis of the box as a function of the distance from the barrier, x, is $c(x) = Q(wx - x^2)/2D$. We differentiate this with respect to x and multiply by D to determine the flux at the growth zone due to the barrier. The flux difference is twice this value. A correction for the perturbation of the uniform concentration ($c = 0$) boundary condition at the surface of the growth zone (see above) provides another factor of 2. Thus, $\Delta F = 2Q(w - 2d)$. We assume that the average flux of X_1 into the growth zone at a distance d from the barrier is the same as the flux into a growth zone located in open air with background concentration $c(d)$—an exact solution would require solution of Poisson's equation for a thin adsorbing fiber placed next to a parallel, adsorbing plane. We use the approximation $c(d) = (aF/D)\ln(L/a)$ and invert to find the average flux of X_1 into the growth zone. We find $F = c(d)D/a\ln(L/a) = Q(wd - d^2)/2a\ln(L/a)$. This gives $\Delta F/F = (4a/d)\ln(L/a)(w - 2d)/(w - d)$, which falls off as $1/d$ for small d. Note that our experimental chamber is 1.5-cm wide when one barrier is moved to within 0.2 cm of the sporangiophore. For $L = 0.2$ cm, $a = 0.005$ cm, $w = 1.5$ cm and $d = 0.2$ cm, we find $\Delta F/F \simeq 0.31$.

Finally, consider the case of a perfectly adsorbing wire barrier. Now the growth zone is located at the center of the box ($w = h$), where gradients due to adsorption of X_1 by the walls are zero. The concentration of X_1 in this region is $Qh^2/8D$, so the flux into the wire is approximately $Qh^2/8\rho_0\ln(L_w/\rho_0)$, where L_w is the length of the wire, and r_0 is its radius. This reduces the concentration a distance r away ($r << L_w$) by the amount $c(r) = Qh^2\ln(L_w/r)/8D\ln(L_w/\rho_0)$. Proceeding as before, we differentiate with respect to r and multiply by D to determine the flux at the growth zone, multiply by 2 to get the flux difference, and then by another factor of 2 to correct for the $c = 0$ boundary condition. We find $\Delta F = Qh^2/2d_w\ln(L_w/\rho_0)$, where d_w is the distance between the wire and the sporangiophore. The average flux, F, is as given by the formula in the previous paragraph, with $w = h$ and $d = h/2$, so that $F = Qh^2/8a\ln(L/a)$. This gives $\Delta F/F = 4a\ln(L/a)/d_w\ln(L_w/\rho_0)$, which is smaller than the result for the plane barrier (for $d << w$) by the factor $\ln(L_w/\rho_0)$. The distance dependence is the same, $1/d$. In particular, for $L_w = 2.2$ cm and $\rho_0 = 0.0015$ cm, $\ln(L_w/\rho_0) = 7.3$, so that $\Delta F/F = 0.042$.

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