MODELS FOR THE AVOIDANCE RESPONSE IN PHYCOMYCES

Thesis by

Ivan J. Matus

In Partial Fulfillment of the Requirements

for the Degree of

Doctor of Philosophy

California Institute of Technology

Pasadena, California

1988

(Submitted March 4, 1987)

ACKNOWLEDGEMENTS

I have to thank my advisor Dr. Howard Berg and his wife Mary for their help and encouragement. I have to mention Paul Meyer, who introduced me to the work in Phycomyces. A large part of what is presented in this thesis is a continuation of his efforts and ideas.

A number of friends made my student life more bearable. I am very grateful to Ariel Caticha and the rest of the "Brazilian community" that always were very friendly and supportive. I have to mention Professor Ricardo Gomez and Clarita for their hospitality and friendship.

Finally, I recognize the patience and understanding of the rest of my examination committee: Dr. Henry Lester, Dr. Jerome Pine, Dr. Charles Brokaw, and Dr. Jean-Paul Revel. I am grateful to Candace Hochenedel and Joanne White for their help in the typing of the thesis.

ABSTRACT

The sporangiophore of the fungus Phycomyces is able to avoid obstacles placed a few millimeters from its growing zone. The work described in this thesis presents evidence that the avoidance response is mediated by gases. Avoidance occurs in still air in the diffusion limit, even at relative humidities close to 100%. It is shown that the effect of the surfaces of the obstacles cannot only be to reflect these gases: the surfaces must play a more active role. Models in which the surfaces adsorb growth-inhibitors or adsorb an inert precursor that is re-emitted as a growth-promoter that decays are in agreement with our experimental observations.

TABLE OF CONTENTS

List of Figures and Tables

Figures

Tables

CHAPTER 1

General Introduction

During its life cycle the fungus Phycomyces develops what is called the sporangiophore, a long thin cylindrical extension that grows upward into the open air; see Fig. 1.1. At the tip of this structure, a spherical sac is located: the sporangium. There, the organism stores approximately 10^5 spores, which, if spread in the appropriate environment, can germinate to reproduce the fungus. The strategy is clear: Phycomyces should direct its sporangiophore, as far as possible, in directions where there is a good chance that its spores will escape and develop. The fungus prefers to live in damp places, near decomposing matter. The sporangiophore has several sensory responses with which to guide itself. They include: phototropism, which enables the sporangiophore to bend towards illuminated places; geotropism, which allows it to sense gravity and grow upward, reaching heights of more than 10 cm; olfactory responses to a variety of chemicals that modify the direction of growth of the sporangiophore; and wind responses, which make the sporangiophore bend in the direction of incoming winds of speeds greater than 10 cm/sec. Of all the above, the sensitivity that has been best studied and understood is phototropism. On the other hand, the avoidance response, i.e., the ability of the sporangiophore to avoid obstacles placed near its growing zone, has eluded a satisfactory explanation even at the level of basic understanding of the stimulus. The growing zone corresponds to the first two millimeters of stalk below the base of the sporangium. All of the tropisms mentioned above result from bendings that occur in that portion of the cell wall. The work that is described in this thesis can be considered as a refinement of the many previous experimental attempts to study the avoidance response. See Chapter 2 for a summary of the history of the previous work.

As mentioned above, it is known that the sporangiophore is able to sense winds and bend into them. One clear effect of the obstacles can be to lower the velocity of random winds near its surface, leaving the distal side of the sporangiophore more exposed to winds. This could cause the sporangiophore to bend away from the surface. Experiments in which the growth rate of the sporangiophore was measured as a function of wind speed (Cohen et al., 1975) show that measurable changes are observed for wind velocities of more than 10 cm/sec. In these experiments the sporangiophore was subjected to vertical air flows, and the growth rate showed a transient decrease when the wind was turned on and a transient increase when the wind was turned off. In both cases the sporangiophore adapted to the new wind regime, and its growth rate returned to the normal value after approximately 10 min. In other experiments designed to distinguish avoidance responses from wind responses, moving barriers were used (Lafay and Matricon, 1982). In this way an inverted gradient of winds was achieved across the stalk of the sporangiophore, the winds on the proximal side being larger than those of the distal side. In these experiments the sporangiophore showed a noticeable avoidance response, but again away from the surface. This showed that the avoidance response and the wind response are two different sensory modalities and provided strong evidence against models of avoidance in which the sporangiophore senses wind gradients across its growing zone.

The background of most of the rationale of the experimental work carried out until today is the assumption that the basic mechanism involved in avoidance is the ability of the sporangiophore to detect fractional differences in the distribution of gases present in the vicinity of the growing zone. The basic experimental evidence that supports this hypothesis is the noticeable increase in growth rate that is observed when the sporangiophore is subjected to bilateral

stimulation with glass barriers placed symmetrically a few millimeters from either side of the sporangiophore (Johnson and Gamow, 1971). This is interpreted to mean that the barriers increase the concentration of a growth-promoting gas around the stalk of the sporangiophore. This and other similar evidence have given rise to what is called the "Chemical Self-Guidance Hypothesis." In the case of only one barrier acting on the sporangiophore, the concentration of the effector would be higher on the proximal side of the barrier than on the distal side, inducing greater growth on the proximal side, thus making the sporangiophore bend away from the barrier. The principal problem with this model is the difficulty in detecting the presumed gas. It has been reported before that barriers made of surfaces as adsorbent as activated charcoal produce similar responses to barriers made of glass (Cohen et al., 1975). Also, experiments in which one sporangiophore was subjected to a flow of air that previously had crossed a forest of sporangiophores failed to give significant increases in growth rate (Cohen et al., 1975). This observation led people to assume that the presumed growth-promoting gas had to be rapidly readsorbed at the growing zone or have a short life time, and that most of it would never reach the surfaces of obstacles.

According to the leading hypothesis, the sporangiophore would sense small changes in the concentration of the promoter that random air currents produce in the immediate vicinity of the growing zone. This model cannot explain the results from the moving barrier experiments. In addition, the only gases that are known to be produced by the sporangiophore and to elicit positive growth responses are ethylene and ethane, but the concentrations needed to produce a noticeable effect are much larger than those normally present around the growing zone. See Russo et al. (1977) and Chapter 2.

A candidate that has always been present in many discussions is water vapor. It is known that a softening of the cell wall is observed when the stalk of the sporangiophore is submerged in water (Gamow and Böttger, 1982a,b). This led these authors to believe that the basic mechanism in both avoidance and wind responses is a differential softening of the cell wall produced by different concentrations of water vapor around the surface of the growing zone. This difference would be produced by the aerodynamic effects of barriers and winds in increasing the concentration on one side of the growing zone in preference to the other. The driving force in producing the bending of the stalk would be turgor pressure that keeps the sporangiophore erect. A good analogy is a long balloon that is being inflated from one end with a membrane that suffers changes in visco-elasticity at the other end, giving rise to kinks. The only problem with this model is that attempts to measure changes in growth rate when the sporangiophore is exposed to sudden changes in relative humidity have produced mixed or negative results (Cohen et al., 1975; 1979). Also, it has been found that the sporangiophore avoids perfectly well even at 100% relative humidity. This make water a controversial candidate as the effector gas in the avoidance response. It is known (Elfving, 1916-1917; Cohen et al., 1979) that a multitude of volatile substances are able to produce negative growth responses. It is not known if these gases are produced by the sporangiophore, but they can play a role in olfactory responses if they are present in the environment and also interfere with avoidance by being adsorbed to the surfaces of the obstacles, producing a gradient of inhibitor that the sporangiophore can sense.

Another very important observation that has to be taken into account is that two sporangiophores avoid one another. There can be a close interrelation between olfactory responses and avoidance. The question is whether the gases involved are released by the sporangiophore or are present in the environment,

and in particular, if they are adsorbed or emitted at the surfaces of the obstacles. The surfaces can also have the role of increasing the concentration of gases at the proximal side of the growing zone simply by reflecting the putative growth-promoting gas.

Another simple explanation for avoidance that can be put forward is that the barriers interfere with phototropism. In an illuminated environment, the barriers can produce shade on the proximal side of the stalk and cause the sporangiophore to bend towards the more illuminated side. The main argument against this possibility is that avoidance occurs even in complete darkness. Also, the colors of the surfaces seem not to have any significant effect on the magnitude of the response (Cohen et al., 1975). Nevertheless, it has been reported that sudden changes in illumination can modify the strength of the avoidance response (Harris, 1979).

Other more sophisticated explanations involve electric effects. It is known that the sporangium and stalk are generally charged, so the barriers can modify the distributions of charge in these structures. The sporangiophore can sense distortions due to electrical forces and produce a tropic response. However, experiments in which the dielectric properties of the surfaces are changed show no effect on the avoidance response (Cohen et al., 1975).

The complicated interplay between different possible effects required major sophistication in the design of our experiments. It was necessary to separate, in a controlled manner, different variables that, in principle, could play a significant role in producing the avoidance response. The basic improvement with respect to previous experimental work was the use of a chamber that provides an airtight environment and allows one to control the temperature, pressure, relative humidity and winds around the sporangiophore during the experiments. See Chapter 2 for a description of this chamber.

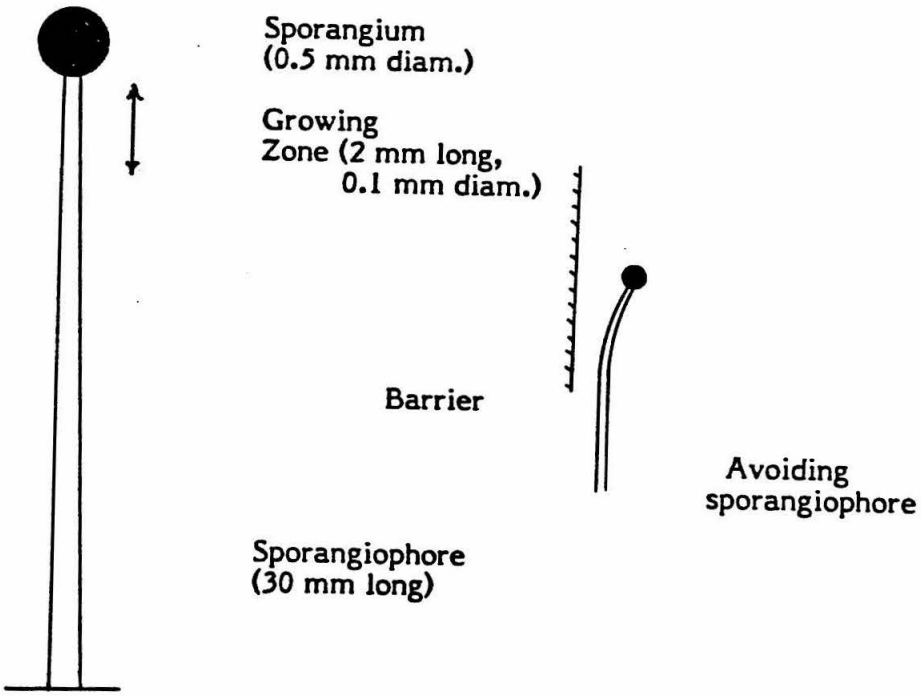
The chamber and associated electronics were designed and built by Howard Berg and Paul Meyer in 1980, and a basic description is found in Chapter 2. See also Paul Meyer's Ph.D. thesis (1986). Paul Meyer had to struggle with the first attempts to obtain reproducible avoidance rates. It became clear that the state of the chamber prior to each experiment was very important, and a large amount of effort was involved in determining the right working conditions. Subtle effects like the state of cleanliness of the internal surfaces of the chamber, or the question of the chamber was left open or closed between experiments, etc. would drastically modify the avoidance rate. Paul finally developed a protocol that enabled him to achieve reproducibility close to 10% between experiments. Using this protocol, he completed most of the avoidance rate vs. relative humidity measurements and established a calibration curve for wind velocities inside the chamber vs. temperature gradient between the top and bottom walls; see Chapter 2. I also determined this curve and obtained identical results. Also, calibration curves were developed for the stabilization of the thermally controlled room where the chamber was located. We found that differences of 0.5°C in the temperature of the room could have significant effects on the temperatures in the walls of the chamber. Paul measured avoidance rates from a flat glass barrier at the low wind regime, and found that an avoidance response occurred in the diffusion limit, i.e., in the absence of measurable winds. The state of the surfaces of the chamber was so critical that I myself was baffled by some results that even today are difficult to interpret, especially results connected with the aging of the surfaces when the chamber is left closed for long periods of time (days) between experiments.

Finally, we decided to concentrate on studying the distance dependence of the avoidance rate from flat glass surfaces and thin glass fibers at the "diffusion limit," as described in Chapter 2. I did most of the latter experimental work.

Once the experimental curves for distance dependence of avoidance rate were obtained, the paper reproduced in Chapter 2 was written and some preliminary models for the avoidance response were proposed. Additional results related to those of Chapter 2 are given in the addendum to that chapter. Then I concentrated on a different line of work designed to test whether or not the strength of avoidance depends on the nature of the surfaces of the barriers. Experiments were designed in which the sporangiophore was subjected to bilateral stimulation with different pairs of surfaces. They are described in Chapter 3. Considering that the avoidance response has to involve differential growth of the cell wall of the sporangiophore, experiments in which variations in the growth rate of the sporangiophore were due to sudden changes in the nature of the surfaces were conducted. The pair of surfaces chosen were glass and activated charcoal. These results are presented in Chapter 4. A crucial test for any model for avoidance is the effect that two sporangiophores have on each other's growth rate. These experiments are described in Chapter 5. Finally, in Chapter 6 a discussion of the new experimental findings is presented and proposals for new experiments are made. In an appendix to this chapter it is shown that the existing data can be fit by a model in which surfaces adsorb a growth-inhibitor to varying degrees.

Fig. 1.1. Sporangiophore of the fungus Phycomyces.

The sporangiophore avoids barriers set a few milimeters away from the growing zone. This section of the stalk corresponds to the first two milimeters below the sporangium. The avoidance response occurs because of the differential growth of the cell wall at the growing zone.


Sporangium
(0.5 mm diam.)
Growing
Zone (2 mm long,
0.1 mm diam.)
Barrier
Avoiding
sporangiophore
Sporangiophore
(30 mm long)

CHAPTER 2

Avoidance of Phycomyces in a Controlled Environment

PAUL W. MEYER, IVAN J. MATUS AND HOWARD C. BERG

From the Division of Biology, California Institute of Technology,
Pasadena, California 91125

Biophys. J., in press.

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-diameter 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 about 4 mm. We conclude that avoidance depends either on adsorption by the barrier of a growth-inhibiting substance or on emission by the barrier of a growth-promoting substance; it cannot occur by passive reflection. Models that can explain these effects are analyzed in an appendix.

INTRODUCTION

The mycelium of the fungus Phycomyces sends up into the air a long thin tube about 0.1 mm in diameter, which develops at its tip a spherical sporangium about 0.5 mm in diameter. Growth occurs in a tapered zone extending 2 to 3 mm below the base of the sporangium. When the sporangiophore is mature (stage IVb, about 2 cm long), it grows steadily at about 3 mm/h, twisting clockwise (as viewed from above) at about 2 revolutions/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 diameter) parallel to a sporangiophore (0.12 mm diameter) 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, 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 about 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 has also 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 x 2.5 x 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 about 1 to 2 cm/s (too small to act via mechanical deformation). An air current of 15 to 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 1000 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 μ 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 (1982a) 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 (1982b), who generated the wind with a moving barrier.

Olfactory Response

The olfactory response was discovered by Elfving (1890, 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 coworkers (e.g., Johnson and Gamow, 1971; Gamow and Böttger, 1982b; 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% to 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 than into dry winds, but the relevance of this to avoidance is not clear.

Experimental Rationale

Given such a complicated state of affairs, it seemed wise to us 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 diameter by 30 mm tall) containing 1.1 ml of 4% potato dextose agar (Difco) with 6 μg/ml thiamine HCl (Sigma). Following, Bergman et al. (1969), spores suspended in 2 ml distilled water at a concentration of about 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 3 spores) was then inoculated into each vial. The vials were incubated inside 10 cm diameter by 8 cm tall glass culture jars (Corning 3250) 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 days, 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 to 150 hours 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-diameter cylinder machined from aluminum (2024 alloy rod: 4.4 % Cu, 1.5 % Mg, 0.6 % Mn), pierced by 3 intersecting mutually orthogonal 2.5 cm-diameter 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: Schott RG-610 glass discs, 2.2 cm diameter by 3 mm thick) 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 in diameter, 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 non-rotating shaft (mm: Mitutoyo 153-203) that carried a delrin (DuPont) 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 about 10^{-3} cm, lapped by hand, and assembled with silicone high-vacuum grease (Dow Corning) 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). A 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 approximately 25 cm^3, with the oil added and with the plugs positioned as shown in Fig. 2.1.

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 #32 magnet wire (Belden 8082, ca. 12 Ω) noninductively wound in a flat spiral (54 bifilar turns starting at the midpoint of the wire) extending 1.8 to 4.4 cm from the axis of the chamber, vacuum-impregnated with paraffin. The cooling coil was a bifilar winding of 3 mm o.d. copper tubing, held in place with epoxy. The temperature was sensed by two thermistors (Fenwal GB31J1) mounted in holes near the heating coils at positions indicated in the legend to Fig. 2.1. 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 (Lauda K-2/RD, 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 A, and the current in the bottom coil was 0.56 ± 0.02 A. 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 (Gaertner, 60 mm focal length) 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 ±10μm). A 30 W tungsten Koehler illuminator (Nikon #77914) run at 5 W provided dim back illumination. This light passed through two infrared blocking filters (Schott KG-3, 2 mm thick) 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 (Gaertner, 80 mm focal length) equipped with a crosshair and mounted on a micrometer-driven x-y stage (accurate to ±10μm). 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 to 2 mm. The particles were produced either by burning a 2.5-cm length of magnesium ribbon (Sargent-Welch, 3 mm wide by 0.2 mm thick) inside a 500 ml flask containing 5% O_2 and 95% N_2 at a relative humidity above 90% or by burning about 50 mg of Whatman #5 filter paper (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 (Spectra Physics #133) 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. 2.1). 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 to 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 to 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 about 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 less than 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. 2.1.

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 (Kimberly-Clark, 13 x 22 cm, #34155) were used to wipe off visible silicone grease from the inside surfaces of the top and 4 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 (Mallinckrodt reagent grade) held with a disposable polyvinyl chloride glove (American Scientific Products) were used to remove the remaining traces of grease from the top and 4 horizontal ports; a fresh Kimwipe was used to wipe them dry. This was repeated once with heptane, twice with RBS-35 alkaline detergent (Pierce, filtered through Whatman #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 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

about 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 x 43 cm, #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 diameter round glass coverslips (VWR, thickness #1) or 30 μm diameter Pyrex glass wool fibers (Corning #3950) were used as barriers. They were cleaned overnight prior to an experiment by soaking at room temperature in 90% fuming nitric acid (Aldrich). 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 to 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 Nuclear Products IC200 Staticmaster brush) 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. 2.1. 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 to 30 min later. Sometimes the horizontal position was checked during the course of the response. These data were used to estimate 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 following 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 less than 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 ℓ = 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 $1° \leq \alpha \leq 15°$, if the aiming error was outside the range $0° \leq \phi \leq 35°$ in either direction, or if the growth rate in a direction parallel to the growth zone was outside the range 30 μm/min $\leq v \leq$ 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 to 20 smoke particles, Fig. 2.2. 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 direction. For temperature differences between 0 and 0.1°C, the mean speeds were less than the experimental error of about ±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 about 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 about 3 weeks; 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 about 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. Eleven 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 about 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 week.

Other observations were of interest: The normalized bending rate was independent of the diameter of the growth zone (range 0.14 to 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 about 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 to 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 to 6° and gave aiming errors ranging from 0 to 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. 2.3, but weakly. As noted above, with no oil in the bottom of the apparatus, the values of relative humidities greater than 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. 2.3) 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 the avoidance rate noted above. To our 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 would 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 to 100 ml of room air were drawn through the chamber (by inserting a 4.3 cm length of 1.9 mm o.d. polyethylene tubing into the vent hole and pulling on it with a vacuum at the rate of about 3 ml/s), an avoidance response was initiated of normal latency,

speed and duration. Blowing 50 to 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 less than 10^{-5} ppm hydrocarbons (less than 0.5 ppm guaranteed; UHP air, Big Three Industries). A control was run to see whether freshly cleaned aluminum (2024 to 6061 alloy) might poison the system. Aluminum disks (2 cm diameter 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 1) the plugs were removed and, along with the chamber, kept in the open air between experiments; or 2) the plugs and the chamber were kept in the open air but covered with a Kimwipe; or 3) the plugs were left in the apparatus (as in the standard experimental protocol). In case 1) the avoidance rate increased with the time that a sporangiophore was in the chamber, from 0 to about 1°/min at 2.5 h, and then leveled off. In cases 2) and 3), the rate started out at a high level and remained fairly constant, at about 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, about 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 3 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. 2.4. Measurements were made by the standard protocol at distances of 1 to 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 8 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 5 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 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. 2.4, 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. 2.4, 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 a thin glass fiber are summarized in Fig. 2.5. 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 mm above the sporangiophore, with the fiber pointing upwards. At the end of the adaptation period, it was rotated 180° to bring the fiber into juxtaposition with the growth zone. The rotation cycle was repeated as many as 6 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 about 0.5 mm, the avoidance rates for the fiber and the coverslips (cf. Fig. 2.4) were approximately the same. However, the drop in avoidance rate with distance was much greater for the fiber 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. 2.4, and 7/15 and 1/3 at 3 and 4 mm in Fig. 2.5, respectively).

DISCUSSION

In summary: 1) Normal avoidance occurs in the absence of convection; it does not require random winds. 2) The variation in avoidance rate for different sporangiophores tested under identical conditions can be as low as ±5%. 3) The response falls off slowly with increasing relative humidity; it does not approach zero at 100% relative humidity. 4) 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. 5) The avoidance rate falls off very weakly with distance. It is nearly constant for a planar barrier placed 0.5 to 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. 6) 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 about 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 to 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 could also 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 about $\pi/\ln(2a/b)$, 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: following 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. 2.4 and 2.5. 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 2.1.

For reflection of a growth-promoter, the bending rate expected for the plane is more than 4000 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 2.1). Both of these predictions contradict the results of Figs. 2.4 and 2.5. 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 2.1). This is shown by the dashed curves in Figs. 2.4 and 2.5. For adsorption of a growth-inhibitor, the two bending rates are also of the same order of magnitude, but they both fall off as 1/d (Table 1); a shallow distance dependence for the plane requires the ad hoc assumption that the response saturates at a bending rate of about 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 should also depend on the purity of the surrounding air. We hope to test these predictions in a controlled environment. But the ultimate 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/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/s. Thus, an infinitesimal segment, dz', of the line source emits X at a rate $2\pi aFdz'$ molecules/s. The concentration at any given point due to a particular segment dz' is $c(r') = aFdz'/2Dr'$, 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 about 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 molecule/cm/sec 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 $2f\varepsilon = \pi\rho_o^2 aLF/d^2$, where ρ_o 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 to its length) is $c(\rho) = aFL\rho_o^2/2d^2D\rho$. Proceeding as before, and including the correction for the constant-flux boundary condition at the surface of the growth zone, we find $\Delta c = 2a^2FL\rho_o^2/d^4D$ and $c = (aF/D)[\ln(L/a + L\rho_o^2/2d^3]$. Ignoring the second term in the brackets, which is negligible, we get $\Delta c/c = 2aL\rho_o^2/d^4\ln(L/a)$. Note that this result is smaller than

that for the plane barrier, given above, by the factor $4\rho_o^2/d^2$. For L = 0.2 cm, a = 0.005 cm, d = 0.2 cm, and ρ_o = 0.0015 cm, $\Delta c/c = 7.2 \times 10^{-7}$. This value is more than 4000 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 to 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, $c(x) = c_o\exp(-x/R)$, where c_o is the concentration at the surface. If this barrier forms one end of a rectangular box of width w, then $c(x) = c_o\{\exp(-x/R) + \exp[(x-w)/R] + 4\exp(-h/2R)\}$. 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 $c(r) = c_oK_o(r/R)/K_o(a/R)$, where r is the distance from the axis of the sporangiophore, and K_o 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 $c = c_o\{K_o(r/R)/K_o(a/R) + \exp(-d/r) + \exp[(d-w)/R] + 4\exp(-h/2R)\}$.

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, $\Delta F = (4c_oD/R)\{\exp(-d/R) - \exp[(d-w)/R]\}$. The magnitude of the flux at the surface of the sporangiophore is $F = (c_oD/R)K_1(a/r)/K_o(a/r)$, where K_1 is the first-order modified Bessel function of the second kind. Thus, $\Delta F/F = 4\{\exp(-d/R) - \exp[(d-w)/R]\}K_o(a/R)/K_1(a/r)$. 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 $c(\rho) = c_o K_o(\rho/R)/K_o(\rho_o/R)$, where ρ is the distance from the axis of the wire, and ρ_o 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 $\Delta F = (4c_o D/R)K_1(d/R)/K_o(\rho_o/R)$. Thus, $\Delta F/F = 4K_1(d/R)K_o(a/R)/K_o(\rho_o/R)K_1(a/R)$. For $d < R$, the distance dependence is $1/d$. For R = 0.6 cm, a = 0.005 cm, d = 0.2 cm, and ρ_o = 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_I" 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_I between opposite sides of the growth zone, $\Delta F/F$, measured at its midpoint. Here, F is the average adsorbed flux of X_I 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 a nascent wall above and leaving behind a 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_I 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 is also adsorbing, but the other four walls of the box are not. We assume further that the inhibitor gas X_I is produced uniformly throughout the volume of the box at a rate Q molecules/cm^3/sec. We solve a version of the diffusion equation modified to take into account this production, namely, $D\nabla^2 c = -Q$ (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_I 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_I 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_I by the walls are zero. The concentration of X_I in this region is

$Qh^2/8D$, so the flux into the wire is approximately $Qh^2/8\rho_o \ln(L_w/\rho_o)$, where L_w is the length of the wire, and ρ_o is its radius. This reduces the concentration a distance r away ($r<<L_w$) by the amount $c(r) = Qh^2\ln(L_w/r)/8D\ln(L_w/\rho_o)$. 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 $\Delta F = Qh^2/2d_w \ln(L_w/\rho_o)$, 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 $\Delta F/F = 4a\ln(L/a)/d_w \ln(L_w/\rho_o)$, which is smaller than the result for the plane barrier (for $d<<w$) by the factor $\ln(L_w/\rho_o)$. The distance dependence is the same, 1/d. In particular, for L_w = 2.2 cm and ρ_o = 0.0015 cm, $\ln(L_w/\rho_o)$ = 7.3, so that $\Delta F/F$ = 0.042.

ACKNOWLEDGMENTS

We thank Markus Meister for his help in finding particular solutions to Laplace's equation. This work was supported by grant DCB-8408858 from the U.S. National Science Foundation. Paul W. Meyer was a National Science Foundation predoctoral fellow and received support from the National Institutes of Health through an NRSA training grant.

Table 2.1. 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 ($1/d^4$)	$\Delta c/c$	3.2×10^{-3} $(1/d^2)$	7.2×10^{-7}
Emission of growth-promoter	$\Delta F/F$	1.0×10^{-1} $[\exp(-d/R)]$	7.3×10^{-2} $(1/d)$
Adsorption of growth-inhibitor	$\Delta F/F$	3.1×10^{-1} $(1/d)$	4.2×10^{-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.

Figure 2.1. 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) non-rotating micrometer head; n,n') press-fit rings; o_1) static O-ring seal; $o_2 o_3$) 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 (3 spaced equally on a 6.8 cm bolt circle; only 1 is actually visible in cross section, but 2 are shown for clarity); ss) set screw. Not labeled: a second set screw clamping the delrin holder to the micrometer shaft. Not shown: 1) horizontal sensing holes for the upper and lower thermistor probes, 2.2 cm deep, located 0.65 cm below the top heater coil and 0.65 cm above the bottom heater coil; 2) 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); 3) cooling-coil tubing entering and leaving the apparatus through vertical holes, sealed with epoxy, in the bottom press-fit ring; 4) drain line for paraffin oil in bottom housing; 5) three support legs, attached to the underside of the bottom housing.

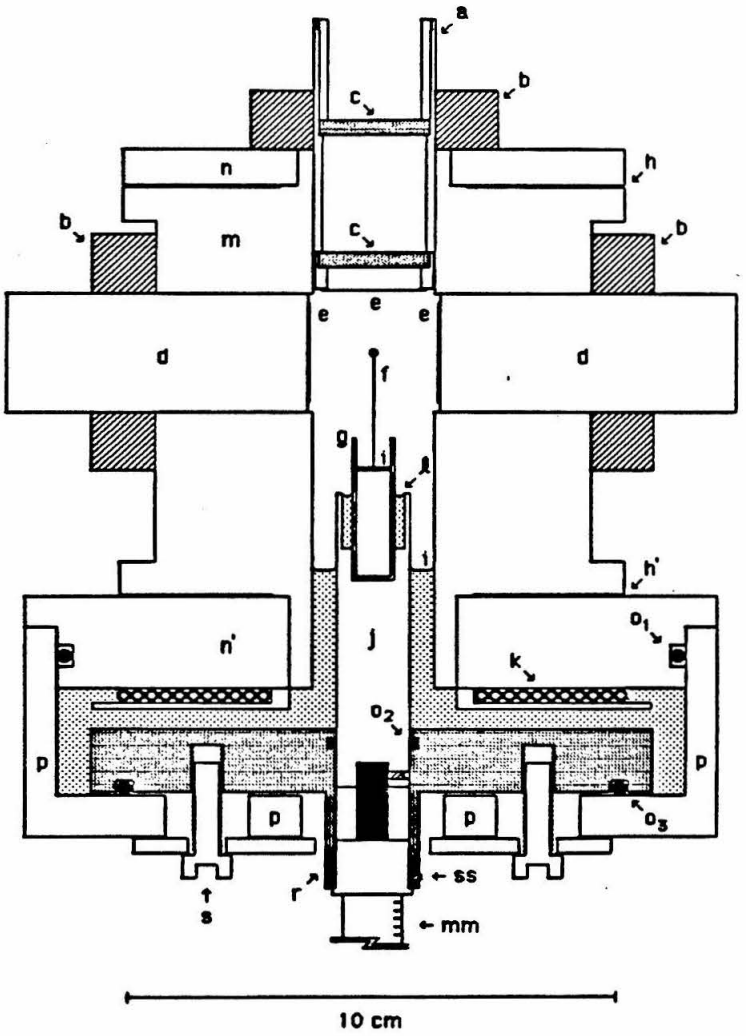

a
b
c
n
h
m
e
d
f
g
i
l
h'
n'
j
k
o_1
o_2
o_3
p
r
s
ss
mm
10 cm

Figure 2.2. 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 about ±10 μm/s at temperature differences below 0.15°C and about ±30 μm/s otherwise. The negative temperature difference was generated by cooling the room to 19.0°C and turning off the top heater.

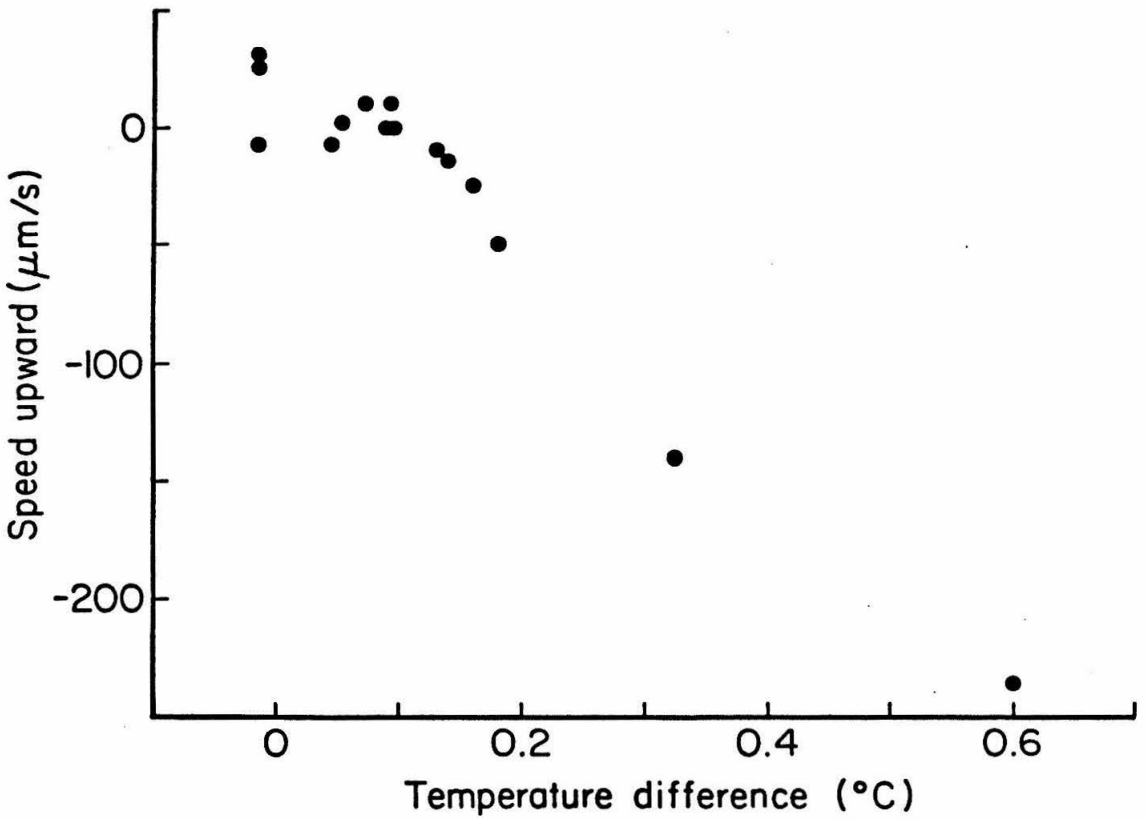

Speed upward (μm/s)
0
-100
-200
0
0.2
0.4
0.6
Temperature difference (°C)

Figure 2.3. Normalized bending rate as a function of relative humidity. The barrier was a glass coverslip (2.2 cm in diameter) 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 $\pm$ 0.08 °/min (mean $\pm$ s.e.) at 100% relative humidity (17 measurements on 10 sporangiophores); 1.06 $\pm$ 0.07 °/min at 93% relative humidity (22 measurements on 20 sporangiophores).

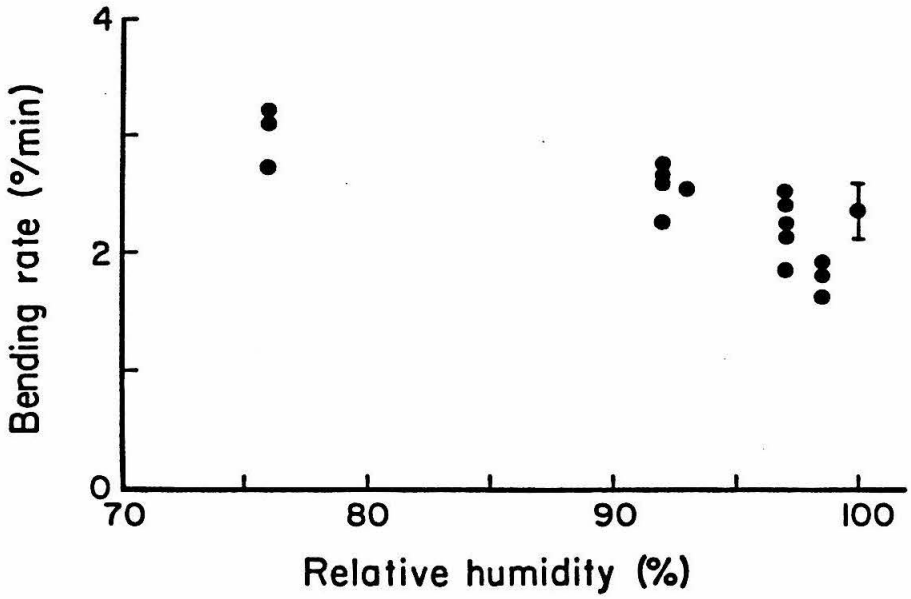

Bending rate (%/min)
4
2
0
70
80
90
100
Relative humidity (%)

Figure 2.4. 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 in diameter). 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 about 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.

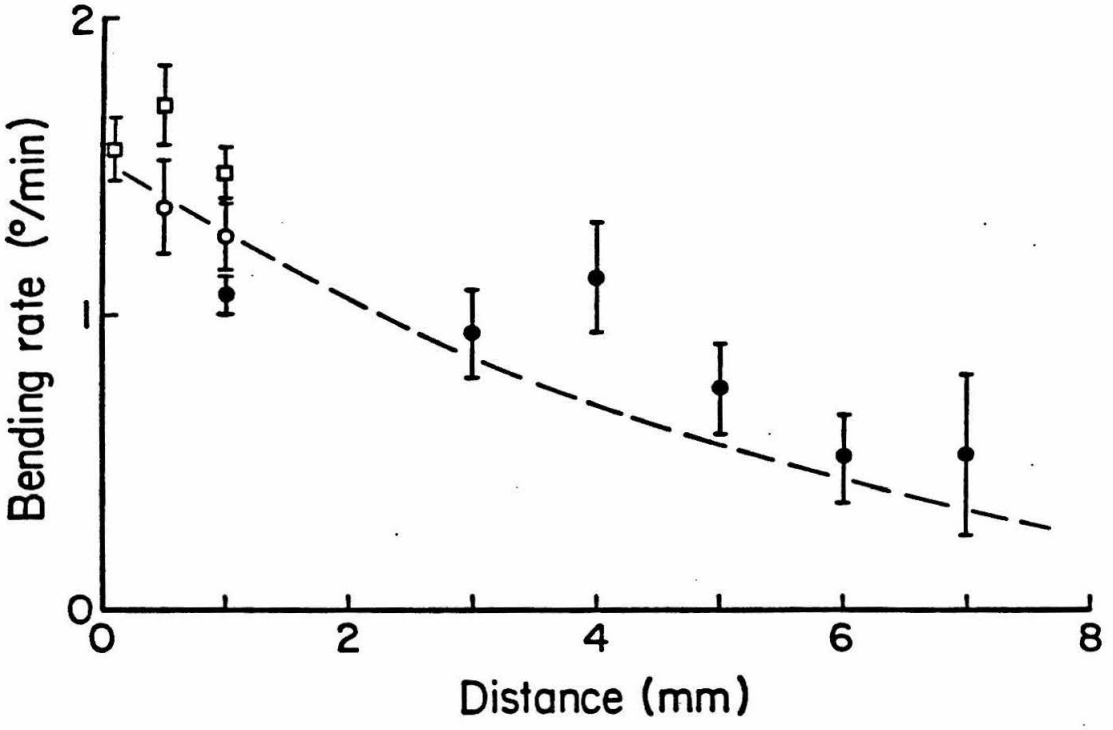
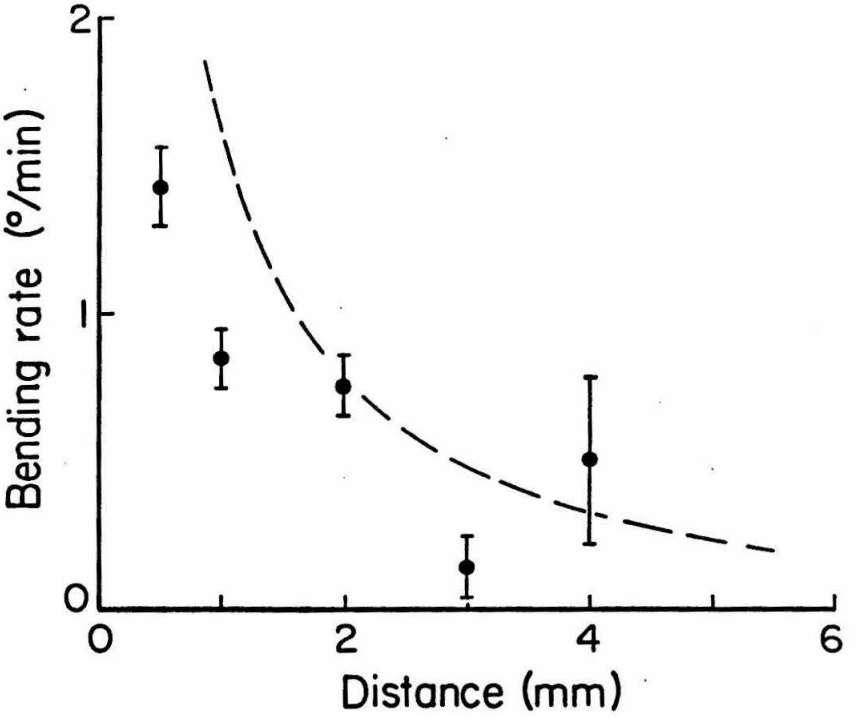

Bending rate (°/min)
2
1
0
0
2
4
6
8
Distance (mm)

Figure 2.5. 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 in diameter by about 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.


Bending rate (°/min)
2
1
0
0
2
4
6
Distance (mm)

ADDENDUM

1) As was mentioned before, one of the main objectives in the design of the experimental chamber was to control the environment around the sporangiophore, so that one could separate variables that might have an influence in avoidance. In particular, by adjusting the electrical currents in the heating coils of the experimental chamber, it was possible to vary the velocity of vertical winds in the vicinity of the growing zone of the sporangiophore. In this way we studied avoidance response at very low wind speeds, thus eliminating the possible influence of convection.

Fig. 2.6 shows results of experiments in which the bending rate of avoidance from a flat glass barrier set 1 mm away from the sporangiophore was measured at different wind velocities. The temperature gradient between the top and bottom walls of the chamber was set at 0.05°C and 0.4°C. According to the calibration presented in Fig. 2, this corresponds to vertical winds with velocities of less than 5 μm/sec or more than 150 μm/sec, respectively. The other conditions in which the experiments were done were the same as those of Fig. 4.

The results show that avoidance is not perturbed by winds of low velocity, as expected if the response is mediated by diffusion of substances of low molecular weight between the barrier and the growing zone (see Chapter 6).

2) A complement of the experiments in which bending rates of avoidance from one glass fiber were measured as a function of the distance between the sporangiophore and the fiber (Fig. 5) consisted in studying the dependence of the bending rates of avoidance from multiple fibers at a fixed distance as a function of the number of fibers. The fibers were set parallel to one another on the end of the sliding hook used in the experiments of Fig. 5. They were evenly distributed with a lateral spread of approximately 3 mm. At the beginning of each experiment, the hook with the fibers was set at the appropriate distance from the sporangiophore but rotated so that the distance from the growing zone to the

fibers was more than 6 mm. After a period of more than 30 min, the hook was rotated and the fibers were facing the growing zone. Then bending rates were measured, following the same protocol described for measurements with only one fiber. The results for the case in which the separation between the sporangiophore and the fibers was 2 mm are presented in Fig. 2.7. In these experiments the relative humidity was set to 93% by adding saturated Na_2SO_4 to the well.

The results show clearly that with a few fibers it is possible to obtain a bending rate that is similar to that obtained with a wide flat glass. Again, this is a strong argument against a model in which the barriers reflect a growth-promoter gas emitted by the sporangiophore. As noted in Table 2.1 (above), for that model a bending rate 10^4 times smaller in comparison with the flat glass should be obtained for a fiber.

On the other hand, the asymptotic shape of the curve of bending rate vs. the number of fibers suggests that the fibers are adsorbing a gas that participates in the avoidance response. In effect, any molecule that reaches the vicinity of the fibers has a large chance of hitting one during its random walk and disappearing from the flow. In other words, the effective cross section for adsorption of diffusing molecules is much larger than the geometrical cross section (cf. Berg, 1983, pp. 27-36). The diffusion current to an adsorbing ellipsoid of revolution with semi-axes $a>b=c$ and $a^2>>b^2$ is $I_e=4\pi DaC_o/\ln(2a/b)$, where C_o is the concentration far away from the ellipsoid. The diffusion current to a disk like adsorber of radius s is $I_d=4DsC_o$. If we assume a=1 cm, b=0.003 cm and s=1 cm, we obtain $I_e=1.9DC_o$ and $I_d=4DC_o$. The values show that a thin fiber adsorbs at approximately the same rate as a wide flat glass, in agreement with our experimental observations. Furthermore, following an analogous derivation to the one presented in the above-mentioned reference for the case of a sphere covered with small adsorbers, we can write for the array of N glass fibers:

$$R_N = R_d [1 + s/\alpha\pi Na \ln(2a/b)],$$

where R_N is the diffusion resistance of the array of N fibers and $R_d = 1/4Ds$ is the diffusion resistance of a disc of radius s, and α is a constant that takes into account the possible difference in adsorptivity of the surface of the glass fibers and the surface of a flat glass cover slip.

To this resistance we associate the diffusion current I given by:

$$\frac{I}{I_o} = \frac{1}{1 + s/\alpha\pi Na \ln(2a/b)} ,$$

where I_o is the diffusion current to a flat glass cover slip. Introducing the values for a,b and s that were assumed before we obtain:

$$\frac{I}{I_o} = \frac{1}{1 + 2/\alpha N} .$$

In Fig. 2.7 the dashed curve is proportional to this function if we take $\alpha=2$.

It is important to notice that when we talk of adsorption, we could include phenomena in which an innert precursor is modified at the surfaces and effectively disappears in the original form, giving rise to a growth-promoter whose emission current from the surfaces would be proportional to the adsorption current of the precursor. The shape of the curves for bending rates as a function of the number of fibers would be similar. This precursor must decay after it is emitted because no one has been able to detect it by simple procedures (Cohen et al., 1975).

Fig. 2.6. Effect of wind velocity on bending rate.

The sporangiophore was avoiding a flat glass surface 1 mm away from its growing zone. The velocities of the vertical winds were set by adjusting the temperature of the top wall of the chamber according to the calibration presented in Fig. 2.2. The points are the averages of experiments with seven different sporangiophores. The error bars are the standard error of the mean. The relative humidity was 93%.

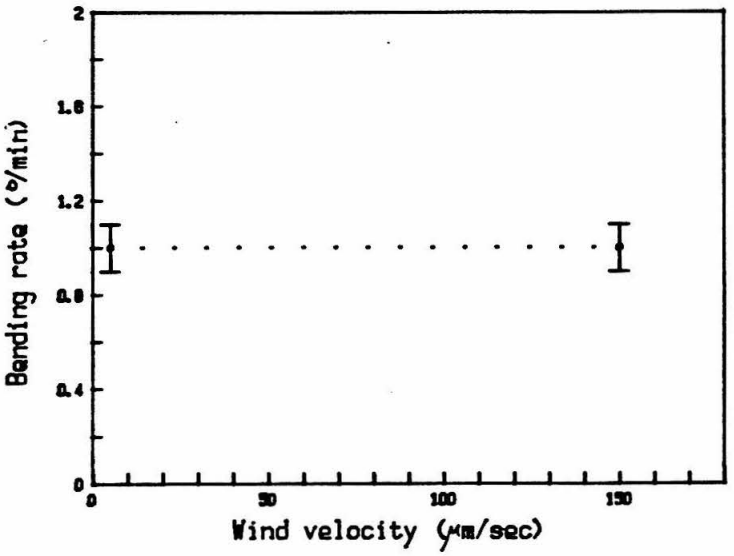
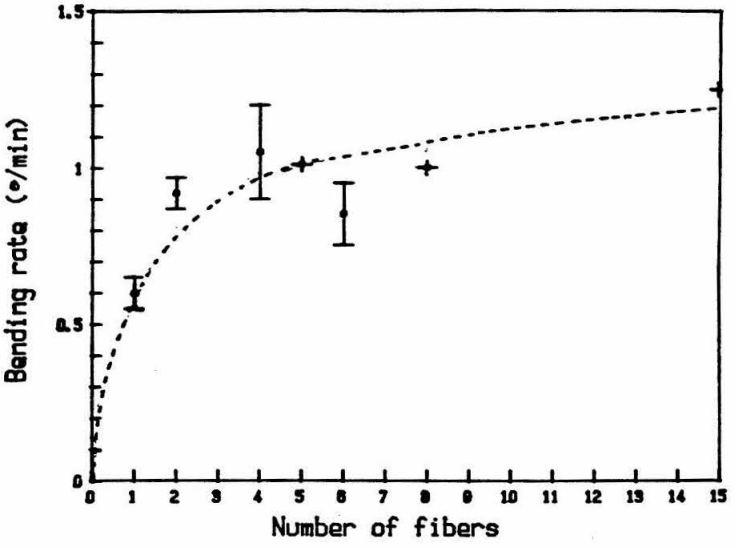

Bending rate (°/min)
2
1.6
1.2
0.8
0.4
0
0
50
100
150
Wind velocity (μm/sec)

Fig. 2.7. Bending rate for avoidance from multiple fibers.

The sporangiophore was set at 2 mm from an array of vertical fibers. In total, 19 different sporangiophores were used. The points represent the mean value of the bending rate for each configuration of fibers. The error bars are the standard errors of the means. The dashed curve is proportional to the function $I/I_0=1/(1+1/N)$, where N is the number of fibers. The relative humidity was 93%.


Bending rate (°/min)
1.5
1
0.5
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Number of fibers

CHAPTER 3

Bilateral Stimulations with Different Surfaces

In this chapter, experiments in which the sporangiophore was exposed to bilateral stimulation with surfaces of different composition are described. Work with activated charcoal is described separately in Chapter 4.

One of the stunning observations that has produced so much controversy about the avoidance response is the apparent independence of the strength of avoidance on the nature of the surfaces. It has been reported before (Cohen, 1975) that surfaces as different as activated charcoal and Teflon produce the same avoidance rate. This made people believe that the only effect of the surfaces was to dampen random winds on the proximal side of the sporangiophore, leaving the distal side more exposed. If the sporangiophore emitted a rapidly re-adsorbed growth-promoter, most of it would never reach distant surfaces, but its local concentration would be affected, because random winds would reduce the concentration on the distal side. This would make the sporangiophore bend away from the surface. The objective of the experiments described in this chapter was to repeat, in the wind-free environment of the chamber, bilateral stimulations of the sporangiophore with different surfaces and to note preferential bendings. Any positive result would argue in favor of a model in which the surfaces play an active role in the avoidance response.

Preparation of the Surfaces

Magnesium and copper slabs (1.2 cm square by a few millimeters thick) were polished, using sand paper (600 grit, 3M wet-or-dry tri-M-ite paper) immediately before, to each experiment. Circular glass cover slips (2.2 cm diam., thickness #1, VWR No. 48380-068), circular glass filters (2.4 cm diam. Glass Fibre Paper GF/A, Whatman), a gold slab (1.2 cm square by less than 1 mm thick), a slab of boron nitride (1.6 cm square by less than 1 mm thick), and Teflon

tape were cleaned in fuming nitric acid (Aldrich) at 60°C and then rinsed many times in glass-distilled water. They were stored under water in a Pyrex beaker covered by Parafilm M (American Can Co.) between experiments.

Description of the Experiments

The sporangiophore was placed in the chamber following the general protocols described in Chapter 2. The chosen surfaces were fixed to the lateral plugs with silicone vacuum grease and moved to the desired distance from the growing zone of the sporangiophore. Liquids were poured on glass filters previously attached to glass cover slips with silicone vacumm grease, and the entire set was fixed to the lateral plugs in the usual manner. In most experiments, in order to detect a preferential bending towards one of the surfaces, it was necessary to wait for more than 1 h. As a control, the experiment was repeated with the surfaces exchanged. If there were a real surface-related effect on the bending of the sporangiophore, it should bend in in the new configuration in the opposite direction but towards the same surface as before. The direction of bending and the bending rate were recorded in each trial.

Results

The results are summarized in Table 3.1. The headings of the columns of the table have the following meanings: Surface 1 and surface 2 correspond to the surfaces that are on barriers 1 and 2 of the chamber, respectively (on the left and right sides of the apparatus shown in Fig. 2.1 of Chapter 2). $\underline{d}$ is the distance from either surface to the sporangiophore in mm; the sporangiophore was equidistant from either surface. $\underline{dir}$ is the direction of bending of the sporangiophore. $\rightarrow$ signifies bending from surface 1 towards surface 2 and $\leftarrow$ the

opposite; 0 signifies no bending. BR is the bending rate in degrees per minute (± the standard error in the mean when multiple measurements were made). RH is the relative humidity inside the chamber. Obs identifies the relevant observation that accompanies the table.

The results indicate that there is an influence of the nature of the surfaces on the strength of avoidance. Some possible correlations with properties of the surfaces can be put forward. It seems clear that the sporangiophore avoids Mg less well than glass or other less-reducing surfaces. This might indicate that if there is a chemical transformation at the surfaces, it might involve an oxidation that could be impeded at the more-reducing surfaces. Another possible correlation is with the acidity of the surfaces. It appears that the sporangiophore avoids acidic surfaces less well than basic ones. This is another indication that some chemical transformation might be occurring at the surfaces. Finally, the sporangiophore seems to avoid surfaces with larger surface areas, such as glass filters, better than flat glass cover slips. This also suggests that some chemical transformation might occur at the surfaces: the ones that present more area or active sites would be more effective in eliciting avoidance. In Chapter 4 we present more evidence that the nature of the surfaces has an effect on the strength of avoidance.

TABLE 3.1.

Bilateral Stimulation with Two Different Surfaces[a]

Surface 1	dir	Surface 2	d (mm)	BR (°/min)	RH %	Obs.
Mg	←	gcs	5	0.19 ±0.09	100	1
Mg	←	gcs	12	0.12 ±0.01	93	2
Cu	→	Al	12	0.12 ±0.05	93	3
Cu	→	Mg	5	0.30 ±0.15	93	4
Boron nitride	0	gcs	2	0.01 ±0.10	93	5
gf	→	gcs	1	0.54 ±0.17	93	6
dry gf	→	H_2O+gf	2	0.46 ±0.20	93-100	7
dry gf	0	Na_2SO_4+gf	2	0.11 ±0.40	93	8
Na_2SO_4+gf	→	H_2SO_4+gf	2	0.11 ±0.10	93	9
dry gf	0	H_2SO_4+gf	2	0	93	10
H_2SO_4+gf	→	H_2O+gf	4	0.48	93-100	11
NaOH+gf	→	dry gf	2	0.47 ±0.09	100	12
NaOH+gf	→	H_2SO_4+gf	2	0.52 ±0.29	100	13
NaOH+gf	→	H_2O+gf	4	0.81 ±0.30	100	14

[a] gcs = glass cover slip; gf = glass fiber filter; the concentrations were Na_2SO_4 2.8 M, H_2SO_4 15%, and NaOH 0.1 M.

Observations Relevant to Table 3.1

1) 4 experiments (exps), 1 sporangiophore (sph). In all of the exps the sph bent in the same direction.

2) 11 exps, 5 sph. In all of the exps the sph bent in the same direction.

3) 10 exps, 6 sph. In 3 exps the sph failed to bend. In 1 it bent in the direction opposite to the shown. In the remaining 6, it bent in the direction shown.

4) 3 exps, 2 sph. In all of the exps the sph bent in the same direction.

5) 6 exps, 3 sph. In 4 exps the sph failed to bend. In 2 it bent in the opposite direction.

6) 5 exps, 4 sph. In all of the exps the sph bent in the same direction.

7) 7 exps, 4 sph. In 3 exps the sph failed to bend. In the others it bent in the same direction.

8) 4 exps, 2 sph. In 1 exp the sph failed to bend. In 2 exps the sph bent in one direction. In the other it bent in the opposite direction.

9) 6 exps, 2 sph. In 5 exps the sph did not bend. In 1 it bent in the direction shown.

10) 1 exp, 1 sph.

11) 1 exp, 1 sph.

12) 2 exps, 1 sph. In both exps the sph bent in the same direction.

13) 2 exps, 1 sph. In both exps the sph bent in the same direction.

14) 3 exps, 2 sph. In all of the exps the sph bent in the same direction.

CHAPTER 4

Experiments with Activated Charcoal

In this chapter we present the results of experiments in which activated charcoal was used on one or the other barrier.

As mentioned in the Introduction and in greater detail in Chapter 3, previous authors reported no significant difference in the avoidance rate of sporangiophores from a wide range of surfaces, including activated charcoal (Cohen et al., 1975). Our objective was to repeat these experiments in the controlled environment provided by the chamber.

Two kinds of experiments were designed. In one group, the sporangiophore was subjected to bilateral stimulation: one of the barriers had particles of activated charcoal fixed on it, and the other did not. We noted the direction of bending of the sporangiophore and measured the bending rate. These experiments are similar to the experiments described in Chapter 3. Another group of experiments was devoted to measurements of the growth rate of the sporangiophore under sudden changes in the disposition of the surfaces.

It is known from the work on phototropism (Foster and Lipson, 1973) that the sporangiophore adapts its growth rate to a wide range of light intensities; i.e., the sporangiophore tries to keep its growth rate constant under any illumination. If this were true for the avoidance stimulus, it would make it difficult, in simple avoidance experiments, to know if the gases involved were promoting or inhibiting growth. This is because the sporangiophore would try to keep its average growth rate constant, and would only respond to fractional differences in gas concentration across its growing zone. Bending would occur either because there was more growth-promoter or because there was less growth-inhibitor on one side of the growing zone than on the other side, but it would not be possible to tell which. This was the motivation for studying the effect of sudden changes in the composition of the barriers on the growth rate of

the sporangiophore. We guessed that changes from activated charcoal to glass and vice-versa could give noticeable growth responses. This proved to be the case.

A complementary approach was to study, for bilateral stimulation, the effect of the wall separation on the growth rate of the sporangiophore. As was mentioned in the Introduction, transient increases in growth rate were observed by others when the sporangiophore was set between two close barriers (Johnson and Gamow, 1971). These observations led these authors to postulate a model in which the sporangiophore would emit a growth-promoter that would be reflected at the surfaces, and therefore would explain the observed transient increases in growth rate. Nevertheless, they could not identify the gas and were forced to postulate that the gas was rapidly re-adsorbed at the surface of the growing zone before reaching the barriers. This was the origin of the "wind-sensing" model in which the sporangiophore could sense small random winds around its surface that could modify the distribution of promoter. They explained the effect of the close barriers by assuming that they were dampening the random winds and therefore increasing the local concentration of promoter gas. The objective of our experiments was twofold: on one side, we wanted to know if, in the "wind-free" environment of the chamber, there is a transient increase in the growth rate of the sporangiophore when two barriers are set close to it, and we wanted to know if this effect depends on the composition of the surfaces. Again, any positive result would argue in favor of a model in which the surfaces play an active role.

Preparation of the Surfaces

Each surface that contained activated charcoal was prepared in the following way. Grains of activated charcoal (gas chromatograph grade, screen size 80/100, base material SK-4, Coast Engineering Lab.) were poured on a dry, acid-cleaned glass filter (2.4 cm diam., Glass Fibre Paper GF/A, Whatman). A second filter was set on top of the grains. Kimwipes were used to completely cover both glass filters. Then, with a clean and dry smooth surface, pressure was exerted on the filters. Finally, the filters were separated with clean forceps. With this simple procedure, a fairly high density of charcoal grains was achieved on both glass filters. For some experiments, it was necessary to cut both filters in half before separation, in order to obtain surfaces half-covered with activated charcoal. In some experiments, rectangular strips of dry paper filter (8 by 20 mm, Whatman #5) were used instead of glass filters. The filters were fixed on top of acid-cleaned glass cover slips with silicone high-vacuum grease, and the entire set was then put on the barriers in the usual manner.

Description of the Experiments and Results

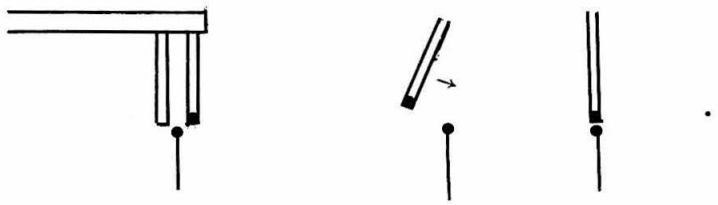
1) Direction of bending in asymmetric stimulation with charcoal.

Two kinds of experiments were realized:

a) One of the barriers was completely free of activated charcoal particles and the other had one-half of its surface covered with activated charcoal particles and the other half free. At the beginning of the experiment, the sporangiophore faced only clean filters. Then, by rotating the barrier that had one half covered with activated charcoal by 180°, the sporangiophore was subjected to a sudden asymmetric stimulation, as shown below:

The direction of bending and the bending rate were recorded. These results are presented in Table 4.1.

b) A variation of the previous experiment consisted in the use of two glass capillary tubes cut to a length of 1 cm (0.8 - 1.1 mm diam., Kimax 51). One had a small charcoal grain in one of its apertures (charcoal coconut activated, 8 to 12 mesh, Matheson Coleman and Bell); the other did not. Both capillaries were fixed with silicone grease to the sliding hook used in the experiments with glass fibers (Chapter 2), as shown below:



The axes of the capillaries were 1.7 mm apart. After the chamber was closed, the capillaries were set above the sporangium but with their apertures far away from the growing zone ($d > 6$ mm). After an adaptation time of approximately 30 min, the sliding hook was rotated to set the apertures of the capillaries an equal distance from the sporangium at a distance of approximately 1 mm above its top. The results are presented in Table 4.2 .

Comments

a) It is clear for both kinds of experiments that activated charcoal produces more avoidance than glass.

b) The experiments with capillary tubes show that the influence of the barriers can be felt even in situations where the barriers are not in front of the growing zone. This is very useful for the sporangiophore when it needs to avoid an obstacle ahead of it that could touch the sporangium.

2) Variation of growth rate of the sporangiophore under sudden changes in the composition of the surfaces (activated charcoal and glass).

In these experiments the sporangiophore was set equidistant to two barriers. Each one had half of its surface covered with activated charcoal grains and the other half clean. The distance between the barriers was kept constant. The disposition of the barriers was such that similar compositions were opposite one another. The sporangiophore was moved vertically with the graduated micrometer so that in less than 1 min the composition of the opposing surfaces was changed from activated charcoal plus glass to clean glass, or vice versa. The sporangiophore remained in each configuration for approximately 13 min. Growth rates were measured as a function of time by recording the readings of the graduated micrometer every 2 min, taking their difference, dividing by 2, and plotting the results at the point corresponding to the time at the middle of the interval.

The results of these experiments are presented in Fig. 4.1 for 93% relative humidity with the charcoal grains on the lower halves of the surfaces. Figure 4.2 shows the results for 100% relative humidity with the charcoal grains on the

lower halves of the surfaces, and Fig. 4.3 shows the results at this humidity with the charcoal grains on the upper halves of the surfaces. In Fig. 4.4 we present a combination of the data of Fig. 4.2 and Fig. 4.3.

We can estimate the magnitude of the change in growth rate by defining the growth response, GR, as:

$$GR = \frac{\text{g.rate (after)} - \text{g.rate (before)}}{\text{g.rate (before)}} ,$$

where g.rate (after) is the maximum or minimum growth rate for two successive measurements during the first five minutes after the surface was changed, and g.rate (before) is the growth rate for two successive measurements immediately prior to the change. The calculated values for GR and their standard errors for the different experiments are given below:

Fig. 4.1. Effects of changes in surface composition on growth rate (charcoal-glass). 93% relative humidity.

a) Change from charcoal to glass:

g.rate (before) = 40 $\pm$ 4 μm/min (average of points 13 and 15 of the second part of the cycle).

g.rate (after) = 36 $\pm$ 4 μm/min (average of points 3 and 5 of the first part of the cycle).

$$GR \text{ (charcoal} \rightarrow \text{glass)} = -0.10 \pm 0.15$$

b) Change from glass to charcoal:

g.rate (before) = 38 $\pm$ 3 μm/min (average of points 11 and 13 of the first part of the cycle).

g.rate (after) = 56 $\pm$ 3 μm/min (average of points 3 and 5 of the second part of the cycle).

GR (glass → charcoal) = 0.47 ± 0.12

Fig. 4.4. Effects of changes in surface composition on growth rate (charcoal-glass). 100% relative humidity.

a) Change from glass to charcoal:

g.rate (before) = 53 ± 2 μm/min (average of points 15 and 13 of first part of the cycle).

g.rate (after) = 61 ± 2 μm/min (average of points 1 and 3 of the second part of the cycle).

GR (glass → charcoal) = 0.15 ± 0.06

b) Change from charcoal to glass:

g.rate (before) = 44 ± 2 μm/min (average of points 11 and 13 of the second part of the cycle).

g.rate (after) = 43 + 2 μm/min (average of points 1 and 3 of the first part of the cycle).

GR (charcoal → glass) = -0.02 + 0.06

Comments

a) These experiments suggest that there is a growth response of the sporangiophore following a sudden change in the composition of the surfaces from activated charcoal to glass and vice versa. The growth rate increases when the change is from glass to charcoal and decreases or remains the same for the inverse change.

b) The effect occurs at 100% relative humidity but is less vigorous.

c) The growth response lasts approximately 10 min.

In Chapter 6 we use these observations to discuss the possible models that could explain the avoidance response.

3) Variation in growth rate with separation between the barriers.

In this series of experiments the sporangiophore was set equidistant to two barriers. The composition of the surface of either barrier was the same. Growth rates were measured as a function of time when the separation between the barriers was varied. Surfaces made of glass cover slips and glass filters covered with activated charcoal were employed. The results are presented in Fig. 4.5 for activated charcoal particles on filters and in Fig 4.6 for clean glass.

With the definition for the growth response given before we calculated the following values for GR:

Fig. 4.5. Effects of changes in wall separation on growth rate (8mm - 2mm). Filters with activated charcoal particles.

a) Change from d = 2 mm to d = 8 mm:

g.rate (before) = 33 $\pm$ 2 µm/min (average of points 15 and 17 of the first part of the cycle).

g.rate (after) = 26 $\pm$ 2 µm/min (point 7 of second cycle).

$$GR\ (d_{small} \rightarrow d_{large}) = -0.21 \pm 0.09$$

b) Change from d = 8 mm to d = 2 mm:

g.rate (before) = 40 $\pm$ 2 µm/min (average of points 13 and 15 of the second part of the cycle).

g.rate (after) = 49 $\pm$ 2 µm/min (point 5 of first part of the cyle).

$$GR\ (d_{large} \rightarrow d_{small}) = 0.25 \pm 0.08$$

Fig. 4.6. Effects of changes in wall separation on growth rate (8mm - 2mm). Glass cover slips .

a) Change from d = 2 mm to d = 8 mm:

g.rate (before) = 51 $\pm$ 2 μm/min (average of points 11 and 13 of the first part of the cycle).

g.rate (after) = 44 $\pm$ 4 μm/min (point 7 of the second part of the cycle).

$$GR\ (d_{small} \rightarrow d_{large}) = -0.14 \pm 0.10$$

b) Change from d = 8 mm to d = 2 mm:

g.rate (before) = 48 $\pm$ 4 μm/min (average of points 11 and 13 of second part of the cycle).

g.rate (after) = 56 $\pm$ 2 μm/min (point 5 of the first part of the cycle).

$$GR\ (d_{large} \rightarrow d_{small}) = 0.17 \pm 0.11$$

Comments

a) There is a growth response when the separation of the surfaces changes. The growth rate shows a transient increase when the separation is diminished and a transient decrease when the separation is enlarged. The response is larger when the surfaces are covered with particles of activated charcoal than when they are not.

b) The growth response lasts approximately 10 min.

See Chapter 6 for the implications of these findings.

TABLE 4.1

Bilateral Stimulation with Filters Covered with Activated Charcoal Particles and with Clean Filters

The column headings are defined in the text preceding Table 3.1[a].

No	Surface 1	dir	Surface 2	d (mm)	BR (°/min)
1	ch+gf	→	gf	1	1.32
2	ch+gf	→	gf	1.3	1.28
3	ch+pf	→	pf	1	1.7
4	ch+pf	→	pf	1	2.7

[a] gf=clean glass filter, ch+gf=glass filter covered with particles of activated charcoal, pf=clean paper filter, ch+pf=paper filter covered with particles of activated charcoal. Experiments 1 and 2 were done with one sporangiophore and experiments 3 and 4 with a different one. The relative humidity was set at 93% by adding saturated Na_2SO_4 to the well.

TABLE 4.2

Bilateral Stimulation with Capillaries with Activated Charcoal Particles and with Clean Capillaries

The column headings are defined in the text preceding Table 3.1[a].

No	Capillary 1	dir	Capillary 2	d (mm)	BR (°/min)
1	ch+c	→	c	0.85	0.24
2	c	←	ch+c	0.85	0.63

[a] c=clean capillary, ch+c=capillary with charcoal particle in aperture. The relative humidity was set at 93% by adding saturated Na_2SO_4 to the well.

Fig. 4.1. Effects of changes in surface composition on growth rate (charcoal grains - glass).

The left half of the figure shows the growth rate after the surface was changed from activated charcoal to glass. The right half of the figure shows the growth rate after the surface was changed back from glass to activated charcoal. The changes were made cyclically, beginning at the times shown by the arrows. The points are the mean values for 4 cycles taken on 1 sporangiophore. The error bars are the standard errors of the means. The time taken to change the surfaces was less than 1 min. The separation between the surfaces was 4 mm, and the sporangiophore was midway in between. The relative humidity was set to 93% by adding saturated Na_2SO_4 to the well. The dashed line is the average of the points.

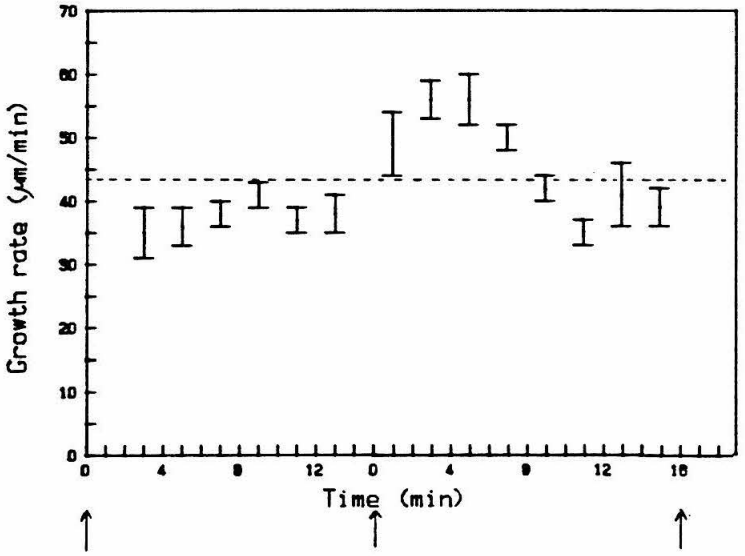

Growth rate (μm/min)
70
60
50
40
30
20
10
0
0
4
8
12
0
4
8
12
16
Time (min)

Fig. 4.2. Effect of changes in surface composition on growth rate (glass–charcoal grains).

The left half of the figure shows the growth rate after the surface was changed from glass to activated charcoal. The right half of the figure shows the growth rate after the surface was changed back from activated charcoal to glass. The changes were made cyclically, beginning at the times shown by the arrows. The points are the mean values of 6 cycles taken on 2 different sporangiophores. The error bars are the standard errors of the means. The time taken to change the surfaces was less than 1 min. The separation between the surfaces was 4 mm, and the sporangiophore was midway in between. The relative humidity was set to 100% by adding H_2O to the well. The dashed line is the average of the points.

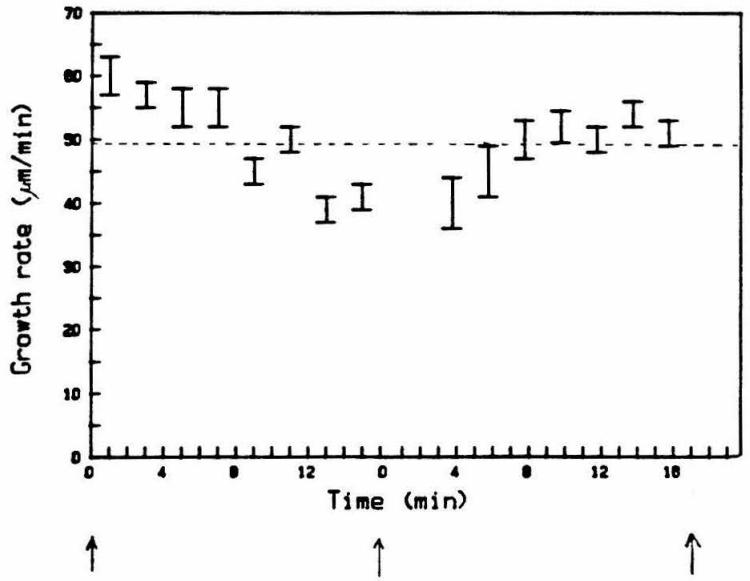

Growth rate (μm/min)
70
60
50
40
30
20
10
0
0
4
8
12
0
4
8
12
16
Time (min)

Fig. 4.3. Effect of changes in surface composition on growth rate (charcoal grains-glass).

The left half of the figure shows the growth rate after the surface was changed from activated charcoal to glass. The right half of the figure shows the growth rate after the surface was changed back from glass to activated charcoal. The changes were made cyclically, beginning at the times shown by the arrows. The points are the mean values of 2 cycles taken on 1 sporangiophore. The error bars are the standard errors of the means. The separation between surfaces was 4 mm, and the sporangiophore was midway in between. The relative humidity was set to 100% by adding H_2O to the well. The dashed line is the average of the points.

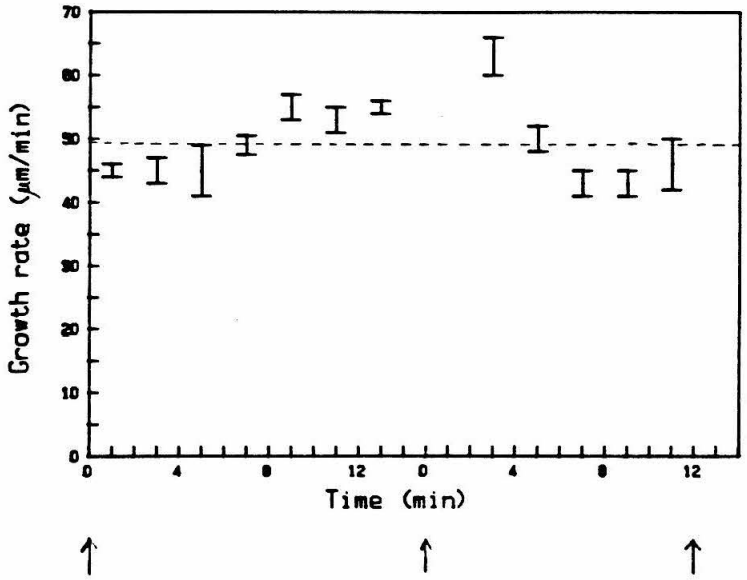

Growth rate (μm/min)
70
60
50
40
30
20
10
0
0
4
8
12
0
4
8
12
Time (min)

Fig. 4.4 Effects of changes in surface composition on growth rate (charcoal grains - glass).

The left half of the figure shows the growth rate after the surface was changed from activated charcoal to glass. The right half of the figure shows the growth rate after the surface was changed back from glass to activated charcoal. The changes were made cyclically, beginning at the times shown by the arrows. The data are a combination of the data of Fig. 4.2 and Fig. 4.3. The dashed line is the average of the points.

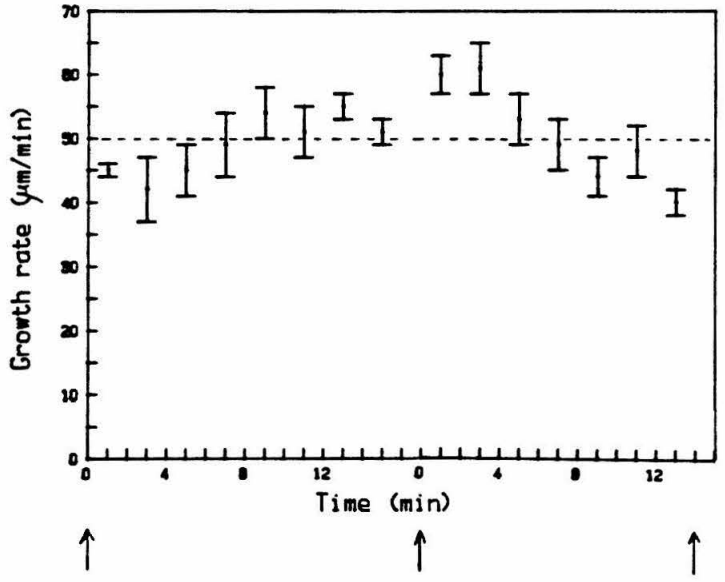

Growth rate (μm/min)
70
60
50
40
30
20
10
0
0
4
8
12
0
4
8
12
Time (min)

Fig. 4.5. Effect of changes in wall separation on growth rate (8 mm-2 mm). The surfaces were glass filters covered with activated charcoal particles.

The left half of the figure shows the growth response when the separation was decreased from d = 8 mm to d = 2 mm. The right half of the figure shows growth response when the separation was increased from d = 2 mm to d = 8 mm. The time taken to make the changes was less than 1 min. The changes were made cyclically, beginning at the times shown by the arrows. The points are the average of 5 cycles taken on one sporangiophore. The error bars are the standard errors of the means. The relative humidity was set to 93% by adding saturated Na_2SO_4 to the well. The dashed line is the average of the points.

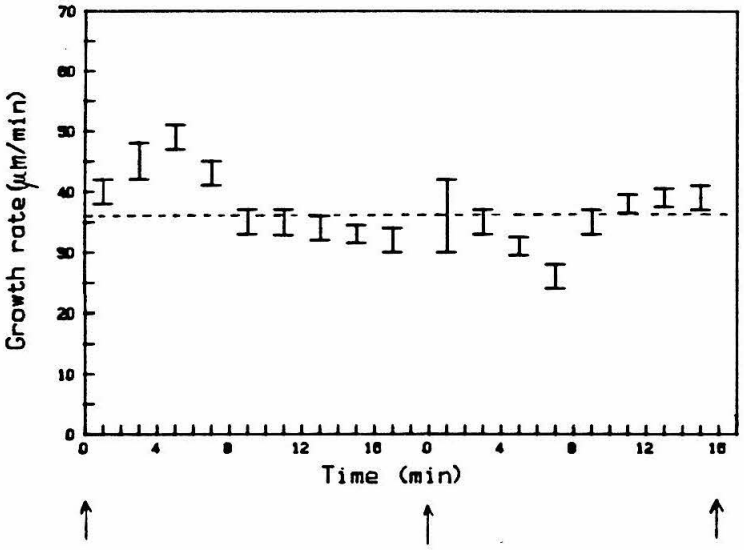
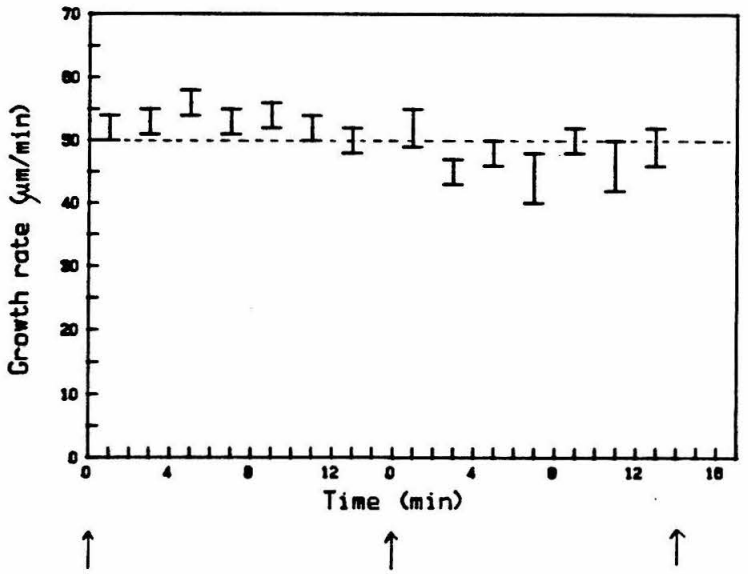

Growth rate (μM/min)
70
60
50
40
30
20
10
0
0
4
8
12
16
0
4
8
12
16
Time (min)

Fig. 4.6. Effect of changes in wall separation on growth rate (8 mm - 2 mm). The surfaces were glass cover slips.

The left half of the figure shows the growth response when the separation decreased from d = 8 mm to d = 2 mm. The right half of the figure shows the growth response when the separation was increased from d = 2 mm to d = 8 mm. The time taken to make the changes was less than 1 min. The changes were made cyclically, beginning at the times shown by the arrows. The points are the average of 4 cycles taken on one sporangiophore. The error bars are the standard errors of the means. The relative humidity was set to 93% by adding saturated Na_2SO_4 to the well. The dashed line is the average of the points.


Growth rate (μm/min)
70
60
50
40
30
20
10
0
0
4
8
12
0
4
8
12
16
Time (min)

CHAPTER 5

Two Interacting Sporangiophores

In this chapter, experiments in which two sporangiophores were made to interact are presented.

"Flaring" of a forest of sporangiophores is well known: when many sporangiophores are growing close together, they diverge radially as if trying to move as far as possible from one another. This is interpreted as mutual avoidance. This avoidance also occurs when only two are interacting. It is interesting to study the effect of one sporangiophore on another because most of the original models for avoidance from inert barriers assumed that the only source for the gases involved in the phenomenon was the sporangiophore itself. According to these models, the sporangiophore could emit a growth-promoter that is reflected at the barriers, causing a gradient of concentration that the sporangiophore could sense, or the sporangiophore could emit a growth-inhibitor that is adsorbed at the barriers, also producing a gradient that the sporangiophore could sense. In either case the sporangiophore would bend away from the surfaces. One thing is certain: if two sporangiophores were suddenly set close to one another (with a separation of about 1 mm between the growing zones) and if they emitted a growth-promoter, then their growth rates should show a transient increase. On the other hand, if they emit a growth-inhibitor, their growth rates should show a transient decrease. If they don't emit any gas but are adsorbing a growth-inhibitor coming from the environment, it is also possible that they will show a transient increase in growth rate. This is because there will be two closely opposed adsorbent surfaces that will reduce the concentration of inhibitor around the growing zones.

These experiments were designed to test these alternatives. In addition to testing two sporangiophores, we devised experiments to study changes in the growth rate of a single sporangiophore when a glass fiber was set suddenly near its growing zone, as in Chapter 2. The idea was to compare the effect of an

"active surface" (live sporangiophore) with the effect of an inert surface, like glass. In the interest of completeness, we tested the effect of the inert stalk of a second sporangiophore on the growth rate of the one under study.

Description of the experiments and results

Mounting of the second sporangiophore. As is described in Chapter 2, the chamber was originally designed to hold only one sporangiophore. It was necessary to adapt the sliding hook used in the experiments with glass fibers to support one additional sporangiophore.

We took advantage of the fact (Bergman et al., 1969) that when an immature sporangiophore (stage 3, yellow head) is carefully plucked from its base and quickly put in contact with water, it keeps its turgor pressure, and with finite probability, continues to develop to maturity (stage 4b, black head), although it grows slowly, not reaching normal heights.

Small "flower pots" were built by cutting 5 mm off the tip of a Pasteur pipette (2 mm diam.) and closing one end with silicone grease. Water was put inside through the other end and the base of the plucked sporangiophore was inserted. Silicone grease was used to fix the stalk to the flower pot and to fix the flower pot to the sliding hook.

At the beginning of the experiment, the plucked sporangiophore was kept more than 6 mm away from the normal sporangiophore, the sporangiophore whose growing rate was under study. It was necessary to wait until the plucked sporangiophore matured before the measurements were begun.

The measurements consisted in recording the growth rate of the normal sporangiophore as a function of time, and noting variations after the interaction with the plucked sporangiophore had started. The growth rate was calculated, using readings of the graduated micrometer taken every 2 min.

The case in which the growing zone of the normal sporangiophore was in front of the inert stalk of the plucked sporangiophore is shown in Fig. 5.1, and the case in which the growing zone of the normal sporangiophore was in front of the growing zone of the second sporangiophore is shown in Fig. 5.2. The ordinates of these figures show the normalized growth rate Ngr, defined as

$$Ngr(t) = \langle gr(t) \rangle_n + \Delta \quad \text{for all } t,$$

$$\Delta = 50 - \langle \langle gr(t) \rangle_n \rangle_t \quad \text{for } t < 0,$$

where $t = 0$ is the time of the beginning of the interaction, $\langle\ \rangle_n$ is the average over the individual measurements for each time t and $\langle\ \rangle_t$ is the average over time. The growth rates are given in μm/min. The value of 50 μm/min has been chosen because it is the average growth rate for a normal population of sporangiophores.

As noted before, we also studied the changes in growth rate when one sporangiophore interacted with a glass fiber. These results are presented in Fig. 5.3. The ordinate is defined as above.

In the same way as in Chapter 4 we can define a growth response GR as the fractional change in growth rate produced when we stimulate the sporangiophore with some barrier.

We obtained the following values:

Response to opposition of growing zone and stalk, Fig. 5.1.

g.rate (before) = 49 ± 2 μm/min (average of points -4, -2, and 0).

g.rate (after) = 53 ± 2 μm/min (average of points 6, 8, and 10).

$$GR = 0.08 \pm 0.06$$

Response to opposition of two growing zones, Fig. 5.2.

g.rate (before) = 51 $\pm$ 3 μm/min (average of points 0 and -2).

g.rate (after) = 63 $\pm$ 4 μm/min (average of points 8 and 6).

$$GR = 0.23 \pm 0.09$$

Response to opposition of growing zone and glass fiber, Fig. 5.3.

g.rate (before) = 50 $\pm$ 4 μm/min (average of points -2 and 0).

g.rate (after) = 52 $\pm$ 3 μm/min (average of points 6 and 8).

$$GR = 0.04 \pm 0.1$$

Comments

a) The results show that when the two growing zones are set facing one another (at a separation of 1 mm) there is a clear transient increase in growth rate that lasts for 12 min.

b) This increase is not observed either when one growing zone faces the inert stalk of a second sporangiophore at a point about 3 mm down from the bottom of its sporangium at a separation of 1 mm or when one growing zone faces a glass fiber. If there is a transient increase in these cases, it is much smaller than observed in a). For the relevance of these results see Chapter 6.

Fig. 5.1. Response to opposition of growing zone and stalk.

At t=0 (arrow), a point 3 mm below, the sporangium of the stalk of the plucked sporangiophore was moved within 1 mm of the growing zone of the normal sporangiophore. The two stalks were parallel to one another. The points are mean values of 4 measurements (n=4) made on one sporangiophore. The error bars are the standard errors of the means. The relative humidity was set at 93% by adding saturated Na_2SO_4 to the well. The dashed line is set at 50 μm/min.

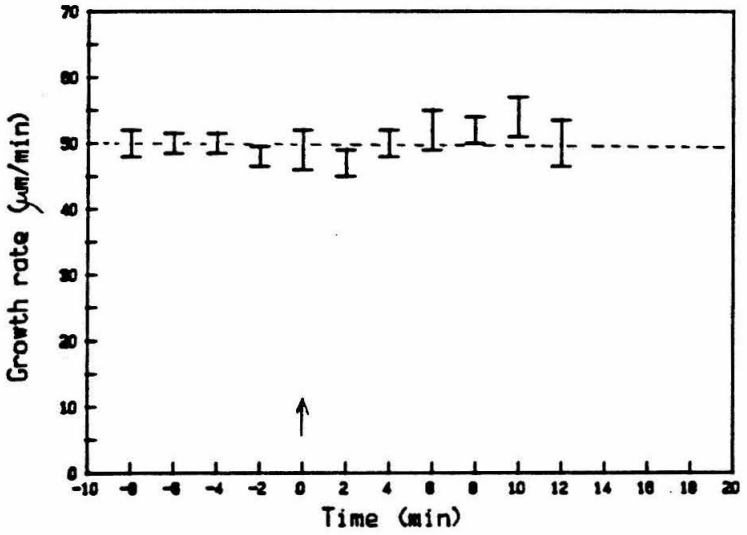

Growth rate (μm/min)
70
60
50
40
30
20
10
0
-10
-8
-6
-4
-2
0
2
4
6
8
10
12
14
16
18
20
Time (min)

Fig. 5.2. Response to opposition of two growing zones.

At t=0 (arrow), the growing zone of the plucked sporangiophore was moved to within 1 mm of the growing zone of the normal sporangiophore. The two sporangiophores were parallel to one another. The points are the mean values of 6 measurements (n=6) on 3 different sporangiophores. The error bars are the standard error of the means. The relative humidity was set at 93% by adding saturated Na_2SO_4 to the well. The dashed line is set at 50 μm/min.

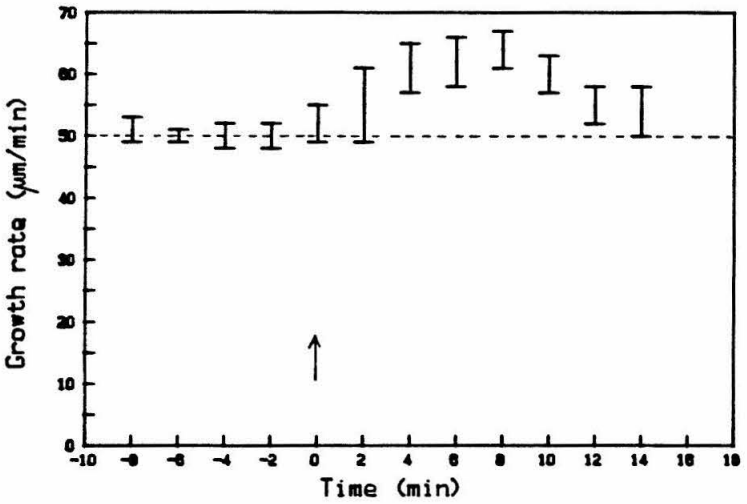
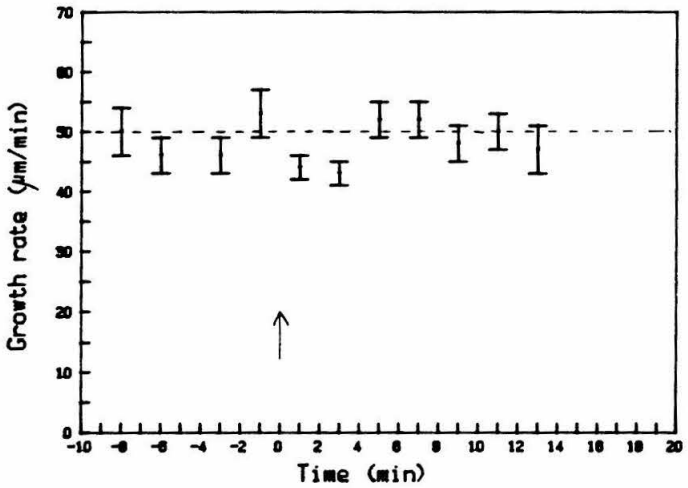

Growth rate (μm/min)
70
60
50
40
30
20
10
0
-10
-8
-6
-4
-2
0
2
4
6
8
10
12
14
16
18
Time (min)

Fig. 5.3. Response to opposition of growing zone and glass fiber.

At t=0 (arrow), a glass fiber was placed 1 mm from the growing zone, as in the experiments of Chapter 2. The points are the mean values of 3 measurements (n=3) on one sporangiophore. The error bars are the standard errors of the mean. The relative humidity was set at 93% by adding saturated Na_2SO_4 to the well. The dashed line is set at 50 μm/min.


Growth rate (μm/min)
70
60
50
40
30
20
10
0
-10
-8
-6
-4
-2
0
2
4
6
8
10
12
14
16
18
20
Time (min)

CHAPTER 6

Discussion

For clarity we list the most important experimental findings:

1) The avoidance response occurs in the diffusion limit, even at wind velocities of less than 10 μm/sec. The bending rate is not affected when vertical winds of velocities of order 150 μm/sec are blown past the growing zone, i.e., when air is made to circulate slowly within the chamber (cf. Chapter 2).

2) There is avoidance even at relative humidities close to 100% (cf. Chapter 2).

3) Avoidance from a few thin fibers is comparable to avoidance from a wide flat barrier of the same height and width as the fiber's length (cf. Chapter 2).

4) The avoidance response depends on the nature of the surfaces (cf. Chapters 3 and 4). In bilateral stimulations, avoidance from activated charcoal is larger than avoidance from glass.

5) In the wind-free environment of our chamber, there are growth responses when the separation between two symmetrical barriers is varied. The sporangiophore shows a transient increase in growth rate when the separation is reduced and a transient decrease in growth rate when the separation is enlarged. These growth responses are larger when the barriers contain particles of activated charcoal than when they are made of clean glass (cf. Chapter 4, Figs. 4.5, 4.6).

6) There also are growth responses to sudden changes in the composition of two symmetrical barriers. The sporangiophore shows a transient increase in growth rate when the change in surface is from glass to activated charcoal and a small transient decrease in growth rate when the change is in the opposite direction (cf. Chapter 4, Figs. 4.1-4.4).

7) There is a transient increase in growth rate when the growing zone of one sporangiophore suddenly faces the growing zone of another (cf. Chapter 5, Fig. 5.2).

8) In our closed chamber, the dependence with distance of the bending rate for avoidance from wide planes is very shallow. The dependence with distance for avoidance from thin fibers is more steep (cf. Chapter 2, Figs. 4 and 5).

These findings support the following general conclusions:

The occurrence of avoidance in the diffusion limit suggests that if avoidance is a chemosensory response, it must be mediated by a volatile substance.

Avoidance in the diffusion limit provides a strong argument against models in which random winds generate differences in concentration of gases around the growing zone (Cohen et al., 1975). These models had previously been challenged on the grounds that the sporangiophore avoids moving barriers that generate inverted wind gradients (Lafay, 1982).

Avoidance at 100% relative humidity, together with the argument in the following paragraph, rules out water as the chemosensory substance.

The effectiveness of thin fibers or set of thin fibers implies that the chemosensory substance is actively adsorbed by or emitted from the surfaces of the barriers. It is not just passively reflected, or adsorbed and re-emitted in an unaltered form. See the discussion in Chapter 2 and in the Addendum to Chapter 2.

The dependence of avoidance and growth rates on the composition of the barriers strengthens the latter conclusion. Evidently; the rates of removal or activation of the chemosensory substance depend on the physical and/or chemical properties of the surfaces of the barriers.

If the surfaces of the barriers adsorb and inactivate or remove the chemosensory substance, then this substance must be a growth-inhibitor. If they activate and thus emit the chemosensory substance, then this substance must be a growth-promoter.

The sporangiophore senses perturbations to the distribution of the chemosensory substance around its growing zone because of the adsorption or emission and bends away from the barriers.

We turn now to the evaluation of specific models:

In the models discussed in Chapter 2, we used as a signal for avoidance either $\Delta c/c$ or $\Delta F/F_{av}$, where Δc is the difference in concentration of the gas at either side of the growing zone, c is the average concentration of the gas at the growing zone, ΔF is the difference in flux of gas adsorbed or emitted at either side of the growing zone, and F_{av} is the average flux that is adsorbed or emitted. We can give the following comments that justify this choice.

It is customary in describing tropic responses in *Phycomyces* for the bending rate of the sporangiophore to be expressed as:

$$d\alpha/dt = \varepsilon\langle v\rangle/r, \tag{6.1}$$

where $d\alpha/dt$ is the bending rate in degrees per minute, $\langle v\rangle$ is the average growth rate in μm/min, and r is the radius of the growing zone in μm. The remaining information is embedded in the coefficient ε, which contains all the unknown biology of the behavior that makes one side of the growing zone grow faster than the other. For phototropism it has been possible to write ε in terms of the intensities of the light sources and internal parameters of the sporangiophore (Bergman et al., 1969). Expression (15-2) of the latter reference explicitly shows that ε is a ratio of the effects of different light beams incident on the growing

zone added *vectorially* to an average of the effect of the same beams. For the case of two opposed light sources of the same spectral quality, this expression reduces to:

$$\varepsilon = K(I_1 - I_2)/(I_1 + I_2), \qquad (6.2)$$

where I_1 and I_2 are the beam intensities incident on opposite sides of the sporangiophore, and K is a constant. Castle (1965) found that this expression agreed with his experiments over a wide range of intensities.

Expressions (6.1) and (6.2) suggest that the bending rate is linearly proportional to the ratio of the difference of intensities that are incident on opposite sides of the sporangiophore to the average intensity that the growing zone is receiving. There has not been any other study of the dependence of ε on stimulation that has been as detailed as that on phototropism. This is explained because of the obvious experimental advantages that the work with beams of light present over that of any other stimulus. No such detailed work has been done on olfaction. Elfving (1917) did observe that the sporangiophore was able to bend towards odors, but he did not study this effect in detail (Meyer, 1986). Cohen et al. (1979) and Russo (1977) studied only growth responses.

At this stage in our understanding of avoidance we can postulate only simple expressions for e that agree with what is known for other sensibilities, in particular, for phototropism. Therefore, we postulate for avoidance and olfaction that ε should be:

$$\varepsilon = K\Delta c/c\ , \qquad (6.3)$$

or, alternatively:

$$\varepsilon = K\Delta F/F_{av} , \qquad (6.4)$$

where K is a constant.

In part B of the appendix to this chapter we present a very simple model for the avoidance response that illustrates how expressions like (6.3) or (6.4) can arise. We use the fact that the magnitudes of the growth responses for increases in the concentration of growth-inhibitors and growth-promoters are linear with the logarithm of the concentration (Cohen et al., 1979; Russo, 1977), and we assume reversibility to justify positive growth responses in response to reductions in the concentrations of growth-inhibitors. This model describes the avoidance response as a manifestation of the growth response. The main difficulty with this procedure is that growth responses, while transient, last about 10 min. They reach a maximum after 5 min from the start of the stimulation and then gradually decrease, returning the growth rate to its initial value. On the other hand, the avoidance response lasts for more than 20 min, with the bending rate remaining approximately constant during the entire period. We could argue, in the same way as has been done for phototropism (Dennison and Foster, 1977), that as the stalk of the sporangiophore rotates (12°/min), the cell wall of the growing zone is continuosly exposed to a new external stimulus. Once the initial perturbation is established, it could remain about the same for an extended period of time, until the growing zone finally moves several mm away from the barrier. It is also known for the case of phototropism that it is possible to mantain an increased growth rate for periods larger than the adaptation time (5 min) if the intensity of light is continuosly stepped up at intervals of time less than the adaptation time (Bergman et al., 1969).

We consider two kinds of models. A) adsorption of growth-inhibitors at the surfaces, and B) emission of growth-promoters at the surfaces.

A) Adsorption of growth-inhibitors at the surfaces

If the gas is a growth-inhibitor, it cannot be produced *solely* by the sporangiophore. This is because, in experiments in which two sporangiophores are made to interact, a transient increase in growth rate is observed, not a transient decrease (cf. Chapter 5, Fig. 5.2). In addition to this, it is known that two growing zones avoid each other with a bending rate not dissimilar to the avoidance of one from a fiber (P. Meyer, private communication). The alternative is that the growth-inhibitor is produced in the environment, for example, as the decay product of an inert precursor emitted by the sporangiophore.

The following comments can be given about a model in which the surfaces adsorb a growth-inhibitor.

A possible argument against this model is that in a closed environment like the experimental chamber, one would expect the avoidance response to disappear with time. This is because the surfaces inside the chamber would become less adsorbent as they became more saturated with growth-inhibitor. This has not been observed, even in the case of avoidance from thin glass fibers, in experiments that lasted many hours. One possible explanation for this lack of saturation at the surfaces of the chamber that might save the model would be that, as the stalk of the sporangiophore elongates, new cell wall constantly is created that continues to adsorb growth-inhibitor. Therefore, the concentration inside the chamber would never rise to saturating levels for the surfaces. Alternatively, surfaces coated with inhibitor might still adsorb more inhibitor, or the adsorbed inhibitor might decompose to some inert product.

Additional information that favors models in which the avoidance gas is a growth-inhibitor is that there is a multitude of gases that are known to be inhibitors of growth for the sporangiophore. Cohen et al. (1979) has found that at least 22 different gases are able to produce notable negative growth responses: the growth rate shows a transient decrease to increases in the concentration of these inhibitors. We assume that had he decreased the concentration of inhibitors, he would have observed transient positive growth responses. There are many clues that suggest that this would happen.

The sporangiophore of Phycomyces shows a reversible behavior for many varied sensibilities, including avoidance. In phototropism there are positive growth responses to positive steps in light intensity and also negative growth responses to negative steps in intensity (Foster and Lipson, 1973). This happens over a range of intensities of more than 9 orders of magnitude. Reversibility is also observed for stretch responses (Dennison and Roch, 1967) and for gravity (Dennison, 1961). Two kinds of behaviors that might be directly related to the avoidance response and that show reversibility are the response to vertical winds and the imprisonment or house response (Cohen et al., 1975; Lafay, 1980). Examples of reversibility related to avoidance are our experiments in which we varied the composition or the separation of the barriers (observations 5 and 6).

In the appendix to this chapter we present a simple model for the avoidance response in which the surfaces are imperfect adsorbers of growth-inhibitors. We give expressions for the avoidance signal and also for growth responses and rates of bending for experiments with symmetrical barriers. This model is a generalization of the model of adsorption of an inhibitor described in the appendix to Chapter 2. The nice aspect of this model is that it predicts dependences of $d\alpha/dt$ with distance from a plane and from a fiber that fits our experimental results remarkably well. It can also fit the experimental curves

obtained by Lafay et al. (1975). His experiments were done under very different experimental conditions. In part C of the appendix to this chapter we give a more general derivation that leads to the same predictions.

In addition to this, this model can explain qualitatively the results of the experiments of Chapter 4, in which the separation or the nature of the surfaces was varied (cf. part A of the appendix, expression A.34). With the definition of growth response GR given in Chapter 4 this model predicts:

$$GR(\text{glass} \rightarrow \text{charcoal}) > 0 \tag{6.5}$$

$$GR(\text{charcoal} \rightarrow \text{glass}) < 0 \, . \tag{6.6}$$

This is observed in our experiments (cf. Chapter 4, Figs. 4.1-4.4). The absolute value of GR(charcoal → glass) in our experiments is smaller than GR(glass → charcoal), but nevertheless it appears to be negative.

The model also predicts that:

$$GR(d_{large} \rightarrow d_{small}) > 0 \tag{6.7}$$

$$GR(d_{small} \rightarrow d_{large}) < 0 \, . \tag{6.8}$$

This also is observed in our experiments (cf. Chapter 4, Figs. 4.5, 4.6). Moreover, the model predicts that (6.7) and (6.8) should be larger when the surfaces are better adsorbers, as it is observed in our experiments, where GR for charcoal was found to be larger than GR for glass.

B) Emission of growth-promoters at the surfaces

As was mentioned in Chapter 2, when we talk of adsorption we include any phenomena in which molecular species disappear at the surfaces of the barriers. They could reappear with a different identity, as growth-promoters, for example. In this case, once reactions between components have reached a steady state, the current of emission of the final species would be proportional to the current of adsorption of the original species. The same principle applies for a problem in which catalysis is involved and two or more molecules interact at the surface to produce a new active one that can diffuse out. This molecule could be a growth-promoter and be sensed by the sporangiophore, eliciting avoidance. The only difference is that in this case the current of adsorption is as many times larger as the number of molecules needed to produce one molecule of the active product. This would happen equally at the surface of a plane or a thin fiber. Once the product molecule is formed and diffuses away from the surface, we have essentially an identical problem regardless of how the the active molecule originated.

A necessary characteristic of this model is that the gas should decay to an inert form. This is true in part because in previous bio-assay experiments (Cohen et al., 1975) it has not been possible to detect the gas. In this experiment, air was blown past a forest of sporangiophores and the gas was directed through glass tubing towards a test sporangiophore placed more than 10 cm downstream. The growth rate was measured and no changes were observed. If a significant fraction of precursor had been transformed at the tubing surfaces into promoter and this gas had a short life time, it would be possible, for the flow velocities that were employed, for most of the promoter to have decayed before reaching the sporangiophore. For a typical diffusion coefficient D=0.1 cm^2/sec and a decay length ρ=0.6 cm, the decay time is $\tau=\rho^2/D$ = 3.6 sec.

Assuming that in the experiments a flow velocity of 1 cm/sec was employed (to prevent wind responses), then most of the promoter might have decayed within 4 cm of the end of the glass tubing.

On the other hand, a model with these characteristics predicts accurately the kind of dependence with distance for the avoidance rate that is experimentally observed. See Chapter 2. In those experiments a decay length of 0.6 cm gave the best fit to the experimental results.

To make the results of our experiments with activated charcoal consistent with a model of emission of a promoter at the surfaces, it is necesary that activated charcoal be a better emitter of promoter than glass. This requires that activated charcoal, in addition to having more adsorbent power than glass, also have more catalytic capacity than glass for the gases involved in avoidance. Presumably, since activated charcoal has more active sites for adsorption than glass, it would bind more precursor molecules. These molecules could then be more easily transformed into the active promoter by a mechanism that we do not know. If so, the promoter would have to be more loosely bound. By this mechanism, more active promoter would be produced by activated charcoal than by glass.

Let P be the concentration of growth-promoter at the growing zone. For the experiments with activated charcoal in which the distance was kept constant, let P_1 be the concentration of growth-promoter when the sporangiophore is in front of activated charcoal, and P_2 the concentration of growth-promoter when the sporangiophore is in front of glass. We expect to have $P_1 > P_2$.

Assuming reversibility, this model also predicts that (6.5) and (6.6) are valid. That is, after the sporangiophore has been adapted to P_1 and suddenly senses P_2, we expect a negative growth response. Reciprocally, after the

sporangiophore has been adapted to P_2 and suddenly senses P_1, we expect a positive growth response. As noted earlier, this has been observed in our experiments.

For experiments in which the composition of the surfaces was fixed but the distance between them varied, we define P_2 as the concentration of growth-promoter when the separation is large and P_1 the concentration of growth-promoter when the separation is small. Given the finite decay time, we expect the concentration of promoter to decrease exponentially with distance from a flat surface. Therefore, we expect $P_1 > P_2$.

Assuming reversibility, we expect that after the sporangiophore has been adapted to P_1 and suddenly senses P_2, a positive growth response should occur; i.e., we expect (6.7) to be valid. Reciprocally, we expect that after the sporangiophore has been adapted to P_2 and suddenly senses P_1, a negative growth response should occur; i.e., we expect (6.8) to be valid. As was said before, both predictions are observed in our experiments.

In summary, on the basis of the experimental information at hand, we cannot make a clear distinction between models in which a growth-inhibitor is adsorbed at the surfaces or in which a growth-promoter is generated following the adsorption and transformation of an inert precursor. We have found that both kinds of models fit the same basic experimental observations. However, models involving the adsorption of a growth-inhibitor have the merit of simplicity and can explain in a natural way why strong adsorbers, such as activated charcoal, are such effective avoidance barriers. Therefore, we have developed this kind of model further in the appendix.

Finally, some new experiments should be considered:

a) In an effort to confirm or reject the model of adsorption of a growth-inhibitor at the surfaces, it is necessary to repeat the experiments of the sort that Russo (1977) made with ethylene and ethane but with growth-inhibitors believed to be produced by the sporangiophore. It is necessary to prove that when the background concentration of inhibitors increases inside a closed box, the magnitude of the avoidance response decreases. This is expected to occur because the average flux that the sporangiophore would sense would be comparatively larger, and the perturbation that the surfaces might produce on the concentration of inhibitor would be comparatively smaller.

b) An analogous experiment would be to compare avoidance responses in a small closed chamber when one of the walls not used as a barrier is covered either with glass or with activated charcoal. We predict that the avoidance response will be larger when the wall is covered with charcoal than when it is covered with glass. This is because charcoal would adsorb any excess background concentration of inhibitor. These experiments can also be used to disprove models of emission of growth-promoters at the surfaces if the distance dependence of the bending rate shows a drastic change of slope. Recall that in those models, the slope is related to the decay length of the promoter (cf. Chapter 2), which should not change.

c) It is necessary to test to see if the sporangiophore shows reversibility in growth responses with respect to increases and decreases in the concentration of inhibitors and promoters. This would support any explanation of the imprisonment experiments and the experiments in which we suddenly varied the nature of the surfaces in terms of increases and decreases in the concentration of effector gases (cf. Chapter 4, Figs. 4.1-4.6).

d) The final objective in the study of the avoidance response has to be the identification of the gases that participate in the phenomenon. If the gas is a growth-inhibitor that is adsorbed at the surfaces there is a good chance that traces could be found in activated charcoal and glass. On the other hand, if the gas is a growth-promoter that is the result of the transformation of a precursor at the surfaces, there is the difficulty that the growth-promoter decays in a short period of time. For a typical diffusion coefficient $D=0.1\ cm^2/sec$ and a decay length $\rho=0.6$ cm, the decay time is $\tau = \rho^2/D = 3.6$ sec.

e) Before planning sophisticated procedures to deal with such problems, it is possible to use our existing techniques to obtain more information about the chemosensory substance and its precursor.

We can estimate the diffusion coefficient D for the precursor and also for the active product with the use of transverse winds and a porous screen. Two porous barriers on opposite sides of the chamber would allow for the flow of transverse winds and could also be used to elicit avoidance. For example, if the sporangiophore were set close to one of the barriers, the wind flow could prevent the promoter from reaching the sporangiophore if the wind were blowing into the barrier, or it could prevent the precursor from reaching the barrier in the first place if the wind were blowing out of the barrier. In either case, no avoidance from the barrier should be observed. Similar arguments apply to the case for adsorption of inhibitor, for if molecules of inhibitor were not able to diffuse back from the barrier to the sporangiophore, the sporangiophore could not know that the barrier was there.

For a transverse wind of velocity v and a sporangiophore set at a distance d from a porous barrier, the precursor will fail to reach the barrier if the time required by diffusion for the molecules to travel from the sporangiophore to the barrier, $d^2/2D$, is larger than the time required for the molecules to be carried

by the wind, d/v. In this case, drift wins when $d/v > d^2/2D$, or $D < vd/2$. Similarly, the promoter will not reach the sporangiophore if the velocity of the incoming wind is such that it takes more time to travel the distance d by diffusion. We arrive at the same expression for the diffusion coefficient, $D < vd/2$.

APPENDIX

A) Adsorption of a growth-inhibitor. Extension to the case in which the surfaces are not perfect adsorbers. The concentration of the inhibitor is not zero near the surface (cf. Berg, 1983, p. 31; DeLisi and Wiegel, 1981).

In the appendix to Chapter 2 (see also Meyer, 1986) we presented the derivation of expressions for the rate of avoidance from perfectly adsorbing planes and thin fibers, assumed proportional to the relative difference in flux of a growth-inhibitor across the growing zone, $\Delta F/F$. We found that the dependence of $\Delta F/F$ on the separation $\underline{d}$ between the sporangiophore and the plane or fiber, for short distances, went as $1/d$ in either case. F is the average flux of inhibitor X_I adsorbed at the growing zone of the sporangiophore, and ΔF is the difference of adsorbed flux between the proximal side and the distal side of the growing zone. We develop in this appendix the expressions for $\Delta F/F$ for the case in which the barriers are not perfect adsorbers.

As before, we assume that Q molecules/cm^3/sec of inhibitor with diffusion coefficient D are created uniformly in the chamber. For the steady state we have to solve Poisson's equation:

$$-D\nabla^2 c = Q . \qquad (A.1)$$

We model the chamber as a box of sizes w_y and w_z in the y and z directions and H in the x direction, and impose the boundary conditions:

$$c(x = -h/2) = c1 \qquad (A.2)$$

$$c(x = h/2) = c2 .$$

The other surfaces are considered perfect reflectors (no dependence of c with y and z in this approximation). In some experiments we vary h, so in general, $h < H$.

Conditions (A.2) indicate that the surfaces S1 and S2 placed at $x=-h/2$ and $x=h/2$ are not perfect adsorbers. If they were perfect adsorbers the boundary conditions would be that the concentration is zero at the surface. The

sporangiophore will be set inside the box and its growing zone will be considered a perfect adsorber.

In this approximation, (A.1) reduces to the 1-dimensional problem:

$$-D\left(\frac{d^2c}{d^2x}\right) = Q, \tag{A.3}$$

with the boundary conditions given by (A.2).

Integrating (A.3) twice and using (A.2) to determine the two constants of integration, we obtain:

$$c(x) = \frac{Qh^2}{8D}\left\{1 - \left(\frac{2x}{h}\right)^2\right\} + \left\{\frac{c2-c1}{h}\right\} x + \left\{\frac{c1+c2}{2}\right\}. \tag{A.4}$$

The flux F(x) associated with this distribution of concentration can be obtained from c(x) by F=-D(dc/dx):

$$F(x) = Qx - D\left\{\frac{c2-c1}{h}\right\}. \tag{A.5}$$

It is apparent that even in the case when the sporangiophore is equidistant from S1 and S2 (set at x=0), there will be a net flux of inhibitor across its growing zone, provided that $c1 \neq c2$. This could explain the reason why, in experiments with bilateral stimulation with different surfaces, the sporangiophore bends toward one surface.

Let $\underline{d}$ be the separation between the sporangiophore set at position x and the barrier S2 at x=h/2. We have x=h/2-d. Sustituting this value for x in (A.4) and (A.5), we obtain :

$$c_b = \frac{Q}{8D} 4d\left\{h-d\right\} + \left\{\frac{c2-c1}{h}\right\} \frac{h}{2} - d + \left\{\frac{c2+c1}{2}\right\} \tag{A.6}$$

$$F_b = \frac{Qh}{2} - Qd - D\left\{\frac{c2-c1}{h}\right\}, \qquad (A.7)$$

where we have defined $c_b = c(x)$ and $F_b = F(x)$ evaluated at $x = h/2 - d$.

The average flux of inhibitor adsorbed by the growing zone can be approximated as the flux that would be adsorbed if the growing zone were in open air with a background concentration c_b. The idea is that the solution for the problem of an adsorbing fiber (the sporangiophore) in a background concentration of value c far away from it is symmetrical to the problem of an emitting fiber with concentration c at its surface and zero concentration far away (Meyer, 1986). Using $c_b = (aF/D)\ln(L/a)$, which is an approximate solution for the latter problem near the surface of the growing zone (of length L and radius a), we invert to get the average flux F_{av} into the growing zone:

$$F_{av} = D\frac{c_b}{a\ln(L/a)} \; . \qquad (A.8)$$

1) Avoidance response.

The stimulus for avoidance can be postulated (for more details see part B of this appendix) as:

$$S = \Delta F/F_{av}, \qquad (A.9)$$

where ΔF is the difference between the fluxes adsorbed at the proximal and distal sides of the growing zone.

As was shown in by Meyer (1986), ΔF is approximately:

$$F = 4F_b, \qquad (A.10)$$

where the sporangiophore is modeled as a cylinder placed in the flux of an inhibitor. One factor of 2 comes from the fact that the perturbation produced by the barriers to the fluxes at both sides of the growing zone point in the same

direction, and a second factor of 2 comes from the imposition of the boundary condition c=0 at the surface of the growing zone. Then, we have:

$$S = 4F_b/F_{av} \,. \tag{A.11}$$

a) Symmetrical stimulation.

We can apply these results to the case in which the sporangiophore is at x=0 (d=h/2) and the surfaces are at positions x=-h/2 and x=h/2. This is the case of symmetrical bilateral stimulation. We obtain :

$$S = \frac{4a}{h} \frac{(c1-c2)\ln(L/a)}{\frac{Qh^2}{8D} + \frac{c1+c2}{2}} \,. \tag{A.12}$$

If we write c1 = c2 + Δc and c2 = co, we obtain :

$$S = \frac{4a\ \Delta c\ \ln(L/a)}{h\left[\frac{Qh^2}{8D} + co + \frac{\Delta c}{2}\right]} \,. \tag{A.13}$$

If we assume further that $\Delta c \ll c_o$, we can write :

$$S = \frac{4a\ \Delta c/co\ \ln(L/a)}{h\left\{\frac{Qh^2}{8Dco} + 1\right\}} \,. \tag{A.14}$$

Clearly, if $\Delta c > 0$ (S2 is a better adsorber of inhibitor than S1), the sporangiophore will bend away from surface S2 in preference to surface S1. We recall that in this model the side of the cell wall that is receiving less inhibitor is growing faster.

b) Avoidance from a flat barrier.

For the case in which the sporangiophore is at a distance d from barrier S2 (x=(h/2)-d), we obtain from (A.6), (A.7), (A.8), (A.9) and (A.10):

$$S = \frac{\frac{4a}{D}\left\{\frac{Qh}{2} - Qd\right\} - D\left\{\frac{c2-c1}{h}\right\}}{Qd\left\{\frac{h-d}{2D}\right\} + \frac{c2-c1}{h}\left\{\frac{h}{2} - d\right\} + \frac{c1+c2}{2}} \ln(L/a) \,. \tag{A.15}$$

For the special case in which both barriers are equally adsorbent (c1 = c2 = co) we obtain :

$$S = \frac{\frac{Qh}{2} - Qd}{Qd\left\{\frac{h-d}{2D}\right\} + co} \frac{4a}{D} \ln(L/a) \quad . \tag{A.16}$$

In our experiments we move one barrier to a distance d from the sporangiophore. The other is kept at a distance H/2. So, h = H/2+d. Introducing h in (A.16) and for the case d << H, we obtain:

$$S = \frac{4a \frac{QH}{4Dco} \ln\left(\frac{L}{a}\right)}{1 + \frac{QH}{4Dco} d} . \tag{A.17}$$

Defining d_o = 4Dco/QH, we can write:

$$S = \frac{4a\ln(L/a)}{d + d_o} \quad . \tag{A.18}$$

The smaller d_o (the better the adsorber or the larger the box), the steeper the distance dependence and the larger the response.

For d_o << d:

$$S = \frac{4a\ln(L/a)}{d} \quad , \tag{A.19}$$

which becomes independent of co. This could explain situations where quite different surfaces give apparently similar avoidance response (Cohen et al., 1975).

For $d_o >> d$:

$$s = \frac{4a\ln(L/a)}{d_o} , \qquad (A.20)$$

which would give a flat and smaller avoidance response.

See part B of this appendix for the fitting of (A.18) to our results of Chapter 2 and to the results of Lafay et al. (1975).

c) Avoidance from a fiber.

We turn now to the problem of a thin fiber set at a distance d from one sporangiophore placed at x=0. We assume that the fiber is not a perfect adsorber and the concentration at its surface is co, the same concentration present at S1 and S2.

The average flux entering the fiber is given approximately by (A.8) with c_b given by (A.6) evaluated at d=h/2 and h=H. The fiber is set close to the center of the chamber and S1 and S2 are at x=-H/2 and x=H/2. L and a are now L_w and r, the length and radius of the fiber.

We obtain:

$$F_{av} = \frac{QH^2}{8r\ \ln(L_w/r)} . \qquad (A.21)$$

The perturbation to the concentration at a distance x from the fiber (for $x << L_w$), which is induced by the adsorption of the fiber is approximately given by:

$$c(x) = \frac{rF}{D}\ av\ \ln(L_w/x), \qquad (A.22)$$

which is the analogue of (A.8), with r the radius of the fiber, L_w the length of the fiber. This perturbation has to be subtracted from the background concentration to get the total concentration at the position of the sporangiophore.

Introducing (A.21), we get:

$$c(x) = \frac{QH^2 \ln(L_w/x)}{8D \ln(L_w/r)} \quad . \tag{A.23}$$

As the sporangiophore is equidistant from S1 and S2, there is no net flux of inhibitor produced by these surfaces [set x=0, and c1=c2 in (A.5)], so the only flux that the sporangiophore is sensing is given by $F_b = -Ddc(x)/dx$ with c given by (A.23).

Calculating F_b, we obtain:

$$F_b = \frac{Qh^2}{8x \ln(L_w/r)} \quad . \tag{A.24}$$

The average flux entering the sporangiophore is evaluated as in (A.8) with c_b given by (A.6) evaluated at d=h/2 less the correction induced by the adsorbing fiber at x=d given by (A.23). Now we have to keep co because for this model, the sporangiophore is a perfect adsorber (c=0 at its surfaces). We obtain:

$$F_{av} = \frac{\frac{D}{a}\left\{\frac{QH^2}{8D} \frac{\ln(d/r)}{\ln(L_w/r)} + co\right\}}{\ln(L/a)} \quad . \tag{A.25}$$

Using (A.11) to evaluate S, we get after reducing the algebra:

$$S = \frac{4a \ln(L/a)}{d\left\{\ln(d/r) + \frac{2d_o}{H} \ln(L_w/r)\right\}} \quad , \tag{A.26}$$

where we have used the definition for d_o given before, $4DC_o/QH$. See part B of this appendix for fitting of this curve to our experimental results of Chapter 2. If $d_o << H$ we obtain:

$$S = \frac{4a\ln(L/a)}{d\ln(d/r)} \quad . \tag{A.27}$$

We can take the ratio $R = S_p/S_f$ of S for the flat plane (S_p) and S for the fiber (S_f) and obtain:

$$R = \frac{d\left\{\ln(d/r) + \frac{2d_o}{H}\ln(L_w/r)\right\}}{d_o + d} \quad . \tag{A.28}$$

For small d_o (good adsorbers or large chambers) (A.28) reduces to:

$$R = \ln(d/r) \quad . \tag{A.29}$$

For d = 1 mm and r = 1.5×10^{-2} mm (A.29) gives 4.6. For d_o = 4 mm, d = 1 mm, H = 22 mm, r = 1.5×10^{-2} mm, L_w = 20 mm (A.28) gives R = 1.41.

d) Avoidance from a second sporangiophore.

We can calculate the avoidance response of one sporangiophore from another when the distance between their growing zones is d. We have to change in (A.26) L_w and r for L and a, respectively, and make $d_o = 0$, because co = 0 at the surface of either growing zone. We obtain:

$$S = \frac{4a}{d}\,\frac{\ln(L/a)}{\ln(d/a)} \quad . \tag{A.30}$$

We note that the discrepancy between the avoidance from a good adsorbing fiber (d_o small) and the avoidance from a second sporangiophore is logarithmic in the radius of the fiber; compare (A.26).

2) Growth response.

We now obtain values for the magnitude of the growth response, assuming that the avoidance response and the growth response are governed by the same model of adsorption of growth-inhibitor at the surfaces.

The basic observation is that in experiments in which the sporangiophore is between two surfaces, when the distance between the surfaces is reduced, there is a transient increase in growth rate of the sporangiophore. Reciprocally, when the distance between the barriers is enlarged, there is a transient decrease in growth rate. In both cases the sporangiophore returns to the normal growth rate after approximately 10 min. An additional feature of the response is that the sizes of the increases and decreases are approximately equal. See Chapter 4 and Lafay (1980).

Assuming that what determines the increases and decreases in growth rate are the changes in the total flux of inhibitor adsorbed by the sporangiophore, we can define a growth response (see Discussion and part B for more details) as:

$$GR = -m \ln(F/Fo), \qquad (A.31)$$

where GR has the same meaning in terms of growth rates as was defined in Chapter 4. The ln() in (A.31) is the relation found by Cohen et al. (1979) for negative growth responses with increases in the concentration of inhibitors. Since in our model the fluxes that the sporangiophore adsorbs are proportional to the background concentration of the inhibitor (see A.8), we postulate that a relation like (A.31) is valid. Here Fo is interpreted as the average flux of the inhibitor before the stimulus (the sporangiophore is assumed to be adapted to that flux), F is the new value of the flux, and m is a parameter that might depend on Fo. It is interesting to note that when $F = Fo + \Delta F$ and $\Delta F \ll Fo$, (A.31) reduces to:

$$GR = m\Delta F/Fo, \qquad (A.32)$$

where we have used $\ln(1+x) = x$ when $x << 1$. This will be related to the avoidance response where we used as signal $\Delta F/F$, with ΔF the difference of fluxes between both sides of the sporangiophore, and F was the average flux of inhibitor. See part B.

a) Growth response from planes.

Using (A.6) and (A.8), we can write for the average flux that the sporangiophore is sensing:

$$F_{av} = D \frac{\frac{Qh^2}{8D} + co}{a\ln(L/a)} . \qquad (A.33)$$

Therefore, (A.31) can be written, for a change from ho,co to h1,c1, as:

$$GR = -m \ln \left[\frac{\frac{Qh1^2}{8D} + c1}{\frac{Qho^2}{8D} + co} \right] . \qquad (A.34)$$

For the case of good adsorbers (c1=co=0), GR reduces to:

$$GR = -2m \ln(h1/ho) . \qquad (A.35)$$

Expressions (A.34) and (A.35) agree with the observations of Chapter 4. For the case in which we vary the separation h between similar surfaces, we could expect that $c1 \simeq co$. In our chamber the changes in h do not appreciably modify the total volume of the chamber; therefore, the effect on co and c1 of a small change in volume is not very important. The same argument applies to Q (in the case in which the inhibitor is the decay product of a precursor, $Q = C_p/\tau$, with C_p the concentration of precursor and τ the decay time of the precursor). The principal effect is in modifiying the total adsorption of inhibitor and therefore reducing the concentration. If h1 > ho (the separation between the surfaces is enlarged), the numerator inside the ln() is larger than the

denominator. So, we get a negative growth response, as it is observed experimentally. For the case in which h1 < ho (there is a decrease in the separation between the surfaces), the inverse is true, and we have a positive growth response, as is also observed in our experiments. An additional comment is that if the surfaces contain grains of activated charcoal, we expect to have better adsorption than in the case of surfaces made of glass. In other words, co and c1 for activated charcoal are smaller than in the case of glass. This is reflected in (A.34) by making the effect of the change in separation h more significant (cf. Chapter 4, Fig. 4.5 and Fig. 4.6). The extreme limit is the case of a perfect adsorber (A.35). These expressions predict increased growth responses when the surfaces are better adsorbers. This has been observed in our experiments with activated charcoal and glass. Expression (A.34) also predicts that if, initially, the sporangiophore faces a poor adsorber and then is made to face a good one (co > c1), with the separation h constant (Q is also assumed constant), there will be a positive growth response. This has also been observed in our experiments when there was a change in surface from glass to activated charcoal. Reciprocally, if the change is the opposite, (A.34) predicts a negative growth response. In our experiments we obtained a response that is smaller than for the first case; nevertheless, it appears to be negative (cf. Chapter 4, Figs. 4.1, 4.2, 4.3, 4.4).

b) Growth response from a fiber or from a second sporangiophore.

We can evaluate the growth response that is obtained when we set a fiber near a sporangiophore. In this model this response is produced by the changes in the average flux of inhibitor F_{av} that reaches the sporangiophore because of the presence of the fiber.

The flux Fo into the sporangiophore before the fiber is set close to it is evaluated. Using (A.8) with c_b given by (A.6) evaluated at d=h/2 (h=H) and $c_1=c_2=co$, we obtain:

$$Fo = D \frac{\frac{QH^2}{8D} + co}{a\ln(L/a)} \quad . \tag{A.36}$$

The flux F into the sporangiophore after the fiber is set close to it is given by (A.21). The growth response given by (A.31) is then:

$$GR = -m \ln \left\{ 1 - \frac{H/2}{H/2 + d_o} \frac{\ln(L_w/d)}{\ln(L_w/r)} \right\}, \tag{A.37}$$

where we have used the definitions for d_o given in (A.18). For d_o very small (the fiber is good adsorber):

$$GR = -m \ln \left\{ \frac{\ln(d/r)}{\ln(L_w/r)} \right\}. \tag{A.38}$$

The growth response from a second sporangiophore is calculated in the same way and one obtains:

$$GR = -m \ln \left\{ \frac{\ln(d/a)}{\ln(L/a)} \right\}. \tag{A.39}$$

Recall that d is the separation of the fiber and sporangiophore (or the two sporangiophores), r is the radius of the fiber, a is the radius of the sporangiophore, and L_w and L are the lengths of the fiber and sporangiophore, respectively.

Comparing (A.38) and (A.39), we could have cases in which fibers could give larger growth responses than sporangiophores if they were good adsorbers and long ($L_w >> L$). In our experiments we observe that a sporangiophore gives more growth response than a glass fiber (cf. Chapter 5). This agrees with the idea that the sporangiophore is a better adsorber than the fiber.

B) Fitting of the experimental results to the model described in part A of the appendix.

We postulate that the sporangiophore shows a positive growth response (a transient increase in growth rate) when the concentration of inhibitor in the environment decreases from the level at which the sporangiophore is adapted. This is complementary to the behavior observed by Cohen et al. (1979). He found that the sporangiophore shows a negative growth response (a transient decrease in growth rate) to increases in concentration of a variety of growth-inhibitors. Cohen found that the negative growth responses depended on the concentration of inhibitor c as: $GR = m \log(c/co)$, where co is a constant that might be connected to the concentration at which the sporangiophore is adapted and m is a constant of proportionality. He found that for some inhibitors the value of m presented a discontinuity at certain high values of c, but it was constant over the range of concentrations. In that paper he suggested that adaptation might be involved, but he did not study the phenomenon in detail. The thresholds for the growth responses were in the range of 100 $pmol.l^{-1}$. We also postulate that the positive growth response GR to a decrease in inhibitor concentration should depend on whether:

$$GR = -m \ln(c/co), \qquad (B.1)$$

where m is a proportionality constant, not necessarily equal to the ones obtained for negative growth responses, co is the concentration before the change, and c

is the concentration after the change. Russo (1977) has found a similar dependence for growth in response to changes in the concentration of ethylene, which is a growth-promoter.

The way in which we make a connection with the model of adsorption of growth-inhibitors discussed in Chapter 2 and in part A of this appendix and with the results of Cohen et al. (1979) is by interpreting (B.1) as a relation between fluxes of growth-inhibitor adsorbed by the sporangiophore, instead of as a relation between background concentrations. We can do this because in our model, the flux of inhibitor adsorbed by the sporangiophore, F, is proportional to the background concentration of inhibitor (see A.8). The proportionality constant in (A.8) depends on the diffusion coefficient of the inhibitor, D, and the dimensions of the growing zone where the adsorption takes place (its radius a and length L). The constant cancels out in the ratio in GR. So, we write:

$$GR = -m \ln(F/F_o), \qquad (B.2)$$

where F_o and F are the fluxes of the inhibitor, before and after the stimulation.

The avoidance response can be considered the result of differential growth in the cell walls of the sporangiophore. If we assume a simple model that for each element of cell wall a relation like (B.2) holds, then we can make the following analysis. Let GR1 and GR2 be the growth responses at opposite sides of the growing zone when a barrier is set close to the sporangiophore. An avoidance response is created when GR1 is different from GR2. Let F_o be the flux of the inhibitor adsorbed on sides 1 and 2 before avoidance and F1 and F2 be the fluxes adsorbed on sides 1 and 2 of the growing zone during avoidance. Let $F1 = F2 + \Delta F$; i.e., $\Delta F = F1-F2$. Then $GR1 = -m \ln(F1/F_o)$ and $GR2 = -m \ln(F2/F_o)$, from which we obtain:

$$GR1-GR2 = -m \ln(F1/F2) . \qquad (B.3)$$

Using the definitions for F1 and ΔF, setting F2 $\simeq F_{av}$, and assuming that $\Delta F << F_{av}$, we can use $\ln(1 + x) = x$, valid for $x << 1$, and obtain:

$$GR1-GR2 = -m\,\Delta F/F_{av} . \qquad (B.4)$$

Let dL1/dt and dL2/dt be the growth rates of the cell wall at opposite sides of the growing zone attained because of the growth responses. We can write dL1/dt = v(1 + GR1) and dL2/dt = v(1 + GR2). Here, v is the average growth rate of the sporangiophore. Assume that dL1/dt > dL2/dt. Then we can write:

$$dL1/dt - dL2/dt = 2a\, d\alpha/dt , \qquad (B.5)$$

where a is the radius of the growing zone and $d\alpha/dt$ is the bending rate (α is the angle of bend of the growing zone). Introducing the definitions for dL1/dt, dL2/dt and using (B.3), we have:

$$d\alpha/dt = m(v/2a)\Delta F/F_{av} , \qquad (B.6)$$

where we have dropped the minus sign.

We recall that $\Delta F/F_{av}$ corresponds to S, the stimulus for avoidance defined in part A. For the case of avoidance from a flat plane we use (A.18), defining $k = 1/d_o$, and obtain:

$$\frac{d\alpha}{dt} = \frac{A}{(1 + kd)} , \qquad (B.7)$$

(Avoidance from a plane)

and for the case of the fiber we use (A.26) and obtain:

$$\frac{d\alpha}{dt} = \frac{A}{d\left[k\ln(d/r) + \frac{2}{H}\ln(L_w/r)\right]} , \qquad (B.8)$$

(Avoidance from a fiber)

where we have defined:

$$A = m\, k\, 2v\, \ln(L/a)\, 180/\pi. \qquad (B.9)$$

We note that A depends on the potency of the growth-inhibitor through m and on the adsorptive properties of the surfaces and environment through $k = 1/d_o$. The other parameters are constants of the sporangiophore.

We now proceed to fit the functions (B.6) and (B.7) to the experimental data presented in Chapter 2. We will also fit (B.6) to the data published by Lafay et al. (1975).

In Fig. B1 we reproduce our experimental results for the case of avoidance from flat glass coverslips. The dashed line is the weighted mean-square fit of the function (B.6). The best values for the parameters are: A = 1.60°/min, and $k = 0.25\ mm^{-1}$ ($X^2 = 4.90$, n = 6).

In Fig. B2 we reproduce our experimental results for avoidance from a fiber. The dashed line is the function (B.7), with r the radius of the fiber (1.5×10^{-2} mm), L_w the length of the fiber (20 mm), and H the width of the chamber in the direction of avoidance (22 mm). A = 1.6°/min and $k = 0.25\ mm^{-1}$, the values determined for the case of avoidance from a flat glass (Fig. B1).

Lafay et al. (1975) performed their experiments in open air. For distances between 0.3 and 3 mm they used as a barrier a brass disk, 20 mm in diameter, set parallel to the axis of the sporangiophore. For shorter distances they used the end section of a rod made of iron, 2 mm in diameter. They fitted their results with the function:

$$d\alpha/dt = 2.2\ 10^2\ d^{-0.6}\ v\ a^{-1}, \qquad (B.10)$$

valid for 100 μm < d < 3000 μm. Here, v is the growth rate of the sporangiophore in μm/min, a is the radius of the sporangiophore in μm, and $d\alpha/dt$ is the bending rate in °/min.

In Fig. B3 we reproduce Lafay et al.'s data normalized to sporangiophores with a growth rate of 50 μm/min. Originally, they plotted the variable ε that is related to the bending rate by: $d\alpha/dt = \varepsilon v a^{-1}$, where v is the absolute growth rate, and a is the radius of the sporangiophore. To compare with our results we calculated their $d\alpha/dt$ from ε, using v = 50 μm/min and their mean value for a = 48 μm. The dashed line is the weighted mean-square fit of the function (B.7). The best values for the parameters are A = 14.6°/min and k = 2.9 mm^{-1} (X^2 = 0.731, n = 6).

We can make the following observation: If we take L = 2 mm , a = 0.5×10^{-1} mm, and v = 50×10^{-3} mm/min and substitute into (B.9) the values for A and k obtained in our experiments (from the fit in Fig. B1) and in Lafay's experiments (from the fit in Fig. B3), we obtain m = 0.3 in our case and m = 0.23 in Lafay's case. These values are very close to one another. On the other hand, the order of magnitude of these values is similar to the ones obtained by Cohen et al. (1979) in his experiments with growth-inhibitors (cf. B.1).

Looking at the results in a different way, if we assume that m is the same in our experiments and in Lafay's, (B.9) predicts that the ratio of the amplitudes of the responses (for similar sporangiophores) has to be equal to the ratio of the values of the parameters k. Comparing Figs. B1, B2, and B3, we find for the ratio of amplitudes 8.9 and for the ratio of the value of the parameters k 11.6, values that agree to within 30%. We must recall that k = QH/4Dco (k = $1/d_o$, d_o = 4Dco/QH), (cf. A.18). If the inhibitor were created uniformly in open air at the same rate that it is created inside the chamber (Q is the same), we could simply argue that H is larger in open air to explain the reason that the value of k obtained in Lafay's experiments is 11.6 times larger than the value obtained in our experiments. For a more general discussion, refer to part C of this appendix.

C) Generalization of the model of adsorption of an inhibitor at the surfaces.

We consider the general case in which the concentration of inhibitor near an adsorbing flat surface depends on the distance to the surface x as:

$$c(x) = co + k1(x/X) + k2\,(x/X)^2 + \ldots\,, \qquad (C.1)$$

where X is a typical distance over which the concentration changes. For small x/X we expect that the first two terms dominate.

The flux of inhibitor near the surface is given by $F_b = -Ddc/dx$:

$$F_b = -Dk1/X - 2Dk2\,(x/X^2) + \ldots \qquad (C.2)$$

The avoidance stimulus is given by (A.11): $S = 4F_b/F_{av}$, where F_{av} is given by (A.8); thus, $F_{av} = Dc(x)/aln(L/a)$. So, we have:

$$S = \frac{-(k1/X + 2k2\ x/X^2)}{[co + k1x/X + k2\ (x/X)^2]}\ 4aln(L/a)\ . \qquad (C.3)$$

When $x/X \ll 1$, we can neglet the terms containing k2 in (C.3) and obtain:

$$S + \frac{-1}{(do + x)}\ 4aln(L/a). \qquad (C.4)$$

Here we have defined $do = Xco/k1$. This expression has the same form as the one obtained in part A of the appendix (cf. A.18), which we used to fit the data of Figs. B1, B2, and B3. The experiments of Figs B1, B2 and Fig. B3 were made under very different experimental conditions. In deriving (C.4) we have not made any requirement as to how the inhibitor is created. The only condition is that the distribution of concentration for the inhibitor is not very dependent on the presence of the sporangiophore. The inhibitor might arise from external

sources far away from the sporangiophore, or it might be produced by the sporangiophore itself (in the form of an inert precursor that gradually decays with time).

In a model in which the inhibitor is the decay product of an inert precursor emitted at the surface of the growing zone, the concentration of the inhibitor is not uniform in open space. It can be shown (Meyer, 1986, with the growing zone modeled as a sphere of radius a) that the concentration of inhibitor c(x) reaches a maximum at a distance of the order of $R_{max} = (2aR_{dp})^{1/2}$ from the growing zone, where R_{dp} is the decay length of the precursor. R_{max} has to be larger than 1 cm, because "flaring" of sporangiophores is observed at distances of at least 1 cm. In open space we could take R_{max} as an estimate of X, and $c(R_{max})$ as a estimate of k1 in (C.1). An important observation is that in open space, for the case of good or bad adsorbers, there will be a gradient of inhibitor near the barrier, because in either case the maximum in the concentration of inhibitor will be located at a distance of the order of R_{max} away from the barrier. For the case of bad adsorbers a gradient of concentration might exist because most of the precursor is reflected at the barrier and has time to diffuse out before decaying into the inhibitor (see below). This might explain why very different surfaces can produce comparable avoidance responses.

In the formulation of the model, co represents the concentration of inhibitor that is not adsorbed at the surfaces. We expect that for similar surfaces, co in open space should be smaller than co inside a closed chamber. This is because in open space most of the inhibitor that is not adsorbed can diffuse out from the vicinity of the barrier. In a closed chamber all the inhibitor is confined, and the molecules of the inhibitor that are not adsorbed remain in the volume and contribute to increase the background concentration. This could make co larger inside a closed chamber. On the other hand, the precursor is also

confined inside the chamber. This can increase the concentration of the inhibitor that is created per unit time (Q can be larger when the chamber is smaller). In general, for each chamber and for surfaces of different quality we expect to have different steady-state values for co and Q and therefore different d_o.

In general, for similar surfaces, we can expect that the value of co outside the chamber will be smaller than the value inside. We also expect, for cases where the inhibitor is the decay product of an inert precursor emitted by the sporangiophore, that the value of Q will be smaller outside the chamber than inside, but we expect Q/co to be about the same. As H will be larger outside, the net effect is to reduce d_o.

We can visualize the meaning of k by considering the problem of a disc of radius s in a semi-infinite medium. The disc is in a background of gas (with diffusion coefficient D) and the concentration far away from the disc is c1. Assume that the disc adsorbs, but the steady-state concentration of gas near its surface is co. The total current of adsorption to that disc is given by: $I = 4Ds(c1-co)$ (Berg, 1983). We can calculate the distribution of concentration near the disc by solving the reverse problem: a disc with concentration co at its surface that is emitting gas with the same emission current into open space (concentration = 0, far away). The average flux at the surface is given by $I/\pi s^2$; i.e. $F = 4D(c1-co)/\pi s$. The first-order term of the concentration distribution, near the center of the disc, is approximately obtained by integrating $F = -Ddc/dx$ with respect to x and requiring that the concentration be co at the surface. We get: $c(x) = co + 4(c1-co)/\pi\ (x/s)$, which is valid for $x << s$. From here we recognize d_o as: $d_o = \pi\ s\ co/4(c1-co)$. For small d_o (C.4) becomes (A.19):

$S = 4a\ln(L/a)/x$. Without showing the intermediate steps we can write the avoidance stimulus for a sporangiophore at a distance x from a fiber of length L_w and radius r:

$$S = \frac{2a\ln(L/a)}{x\left[\ln(x/r) + \frac{2d_o}{X}\ln(L_w/r)\right]}, \qquad (C.6)$$

where we have defined $d_o = Xco/2(c1-co)$, and have assumed that the distribution of inhibitor near the fiber is given by:

$$c(x) = c1 - (c1-co)\ln(L_w/x)/\ln(L_w/r)\,. \qquad (C.7)$$

Here, c1 is the background concentration (at the center of the chamber for example) and co is the concentration at the surface of the fiber. The second term in (C.7) is a correction to the background concentration due to the presence of the adsorbing fiber. It is clear that (C.6) is a generalization of (A.26).

Fig. B1. Avoidance from a plane barrier.

Observations made inside our experimental chamber.

The normalized bending rates for avoidance from flat glass barriers are plotted as a function of the distance between the sporangiophore and the barrier. See Chapter 2, Fig. 2.4. The dashed line corresponds to (B.7) with A = 1.6°/min and k = 0.25 mm^{-1}.

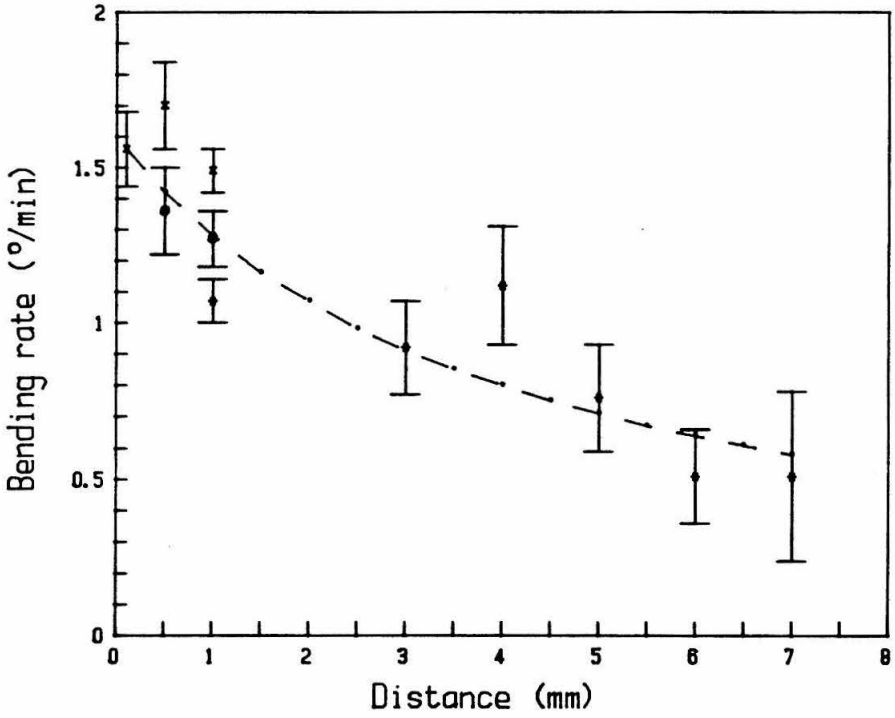

Bending rate (°/min)
Distance (mm)
0
0.5
1
1.5
2
0
1
2
3
4
5
6
7
8

Fig. B2. Avoidance from a thin fiber.

Observations made inside our experimental chamber.

The normalized bending rates for avoidance from thin glass fibers are plotted as a function of the distance between the sporangiophore and the fiber. See Chapter 2, Fig. 2.5. The dashed line corresponds to (B.8) with A = 1.6°/min and k = 0.25 mm^{-1}, the values determined in the fit shown in Fig. B1.

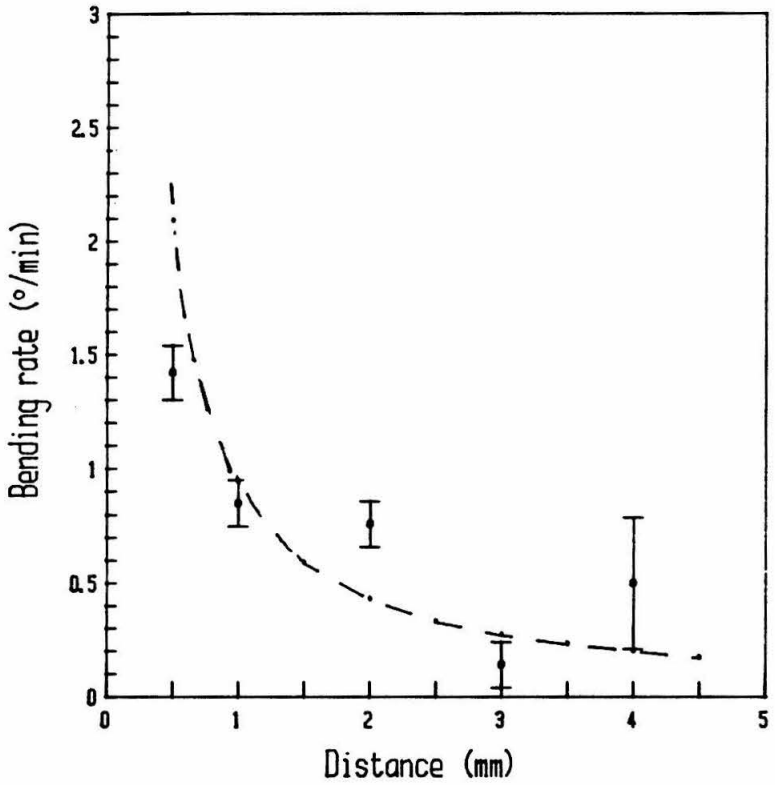
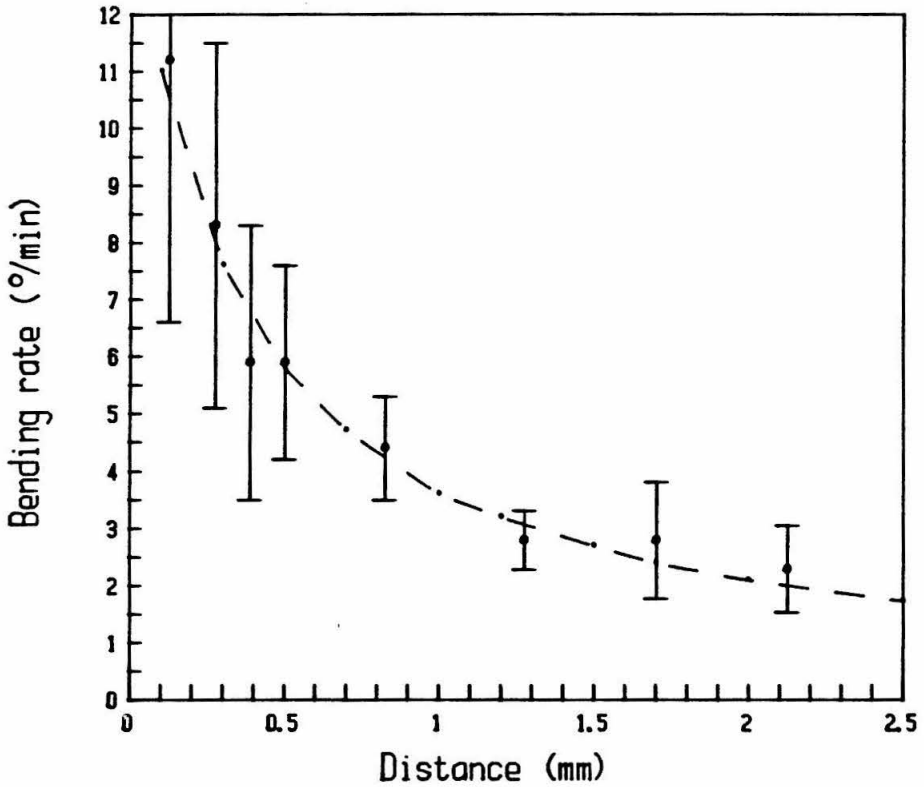

Bending rate (°/min)
Distance (mm)
0
0.5
1
1.5
2
2.5
3
0
1
2
3
4
5

Fig. B3. Avoidance response from flat barriers. Results reported by Lafay et al. (1975) normalized to a growth rate of 50 μ/min.

The bending rate for avoidance from the end of an iron rod (2 mm diam.) or from a brass disk (20 mm diam.) are plotted as a function of the distance between the sporangiophore and the barrier. The rod was used for distances less than 0.3 mm. The experiments were made in open air. The dashed curve corresponds to (B.7) with A = 14.6°/min and k = 2.9 mm^{-1}.


Bending rate (°/min)
Distance (mm)
0
1
2
3
4
5
6
7
8
9
10
11
12
0
0.5
1
1.5
2
2.5

REFERENCES

Berg, H. C. 1983. Random Walks in Biology. Princeton University Press, Princeton, New Jersey.

Bergman, K., P.V. Burke, E. Cerdá-Olmedo, C.N. David, M. Delbrück, K.W. Foster, E.W. Goodell, M. Heisenberg, G. Meissner, M. Zalokar, D.S. Dennison and W. Shropshire, Jr. 1969. Phycomyces. Bacteriological Reviews 33: 99-157.

Castle, E. S. 1965. Differential growth and phototropic bending in Phycomyces. Journal of General Physiology 48: 409-423.

Cohen, R.J. 1976. The avoidance behavior of Phycomyces blakesleeanus: Is mediation by natural convection significant? Journal of Theoretical Biology 58: 207-217.

Cohen, R.J., M.G. Fried and M.M. Atkinson. 1979. Olfactory responses of Phycomyces blakesleeanus. Biochemical Biophysical Research Communications 86: 877-884.

Cohen, R.J., Y.N. Jan, J. Matricon and M. Delbrück. 1975. Avoidance response, house response, and wind responses of the sporangiophore of Phycomyces. Journal of General Physiology 66: 67-95.

DeLisi, C. and F. Wiegel. 1987. Effect of nonspecific forces and finite receptor number on rate constants of ligand-cell bound-receptor interactions. Proc. Natl. Acad. Sci. USA 78: 5569-5572.

Dennison, D. S. 1961. Tropic responses of Phycomyces sporangiophores to gravitational and centrifugal stimuli. Journal of General Physiology 45: 23-38.

Dennison, D.S. and R.P. Bozof. 1973. Phototropism and local adaptation in Phycomyces sporangiophores. Journal of General Physiology 62: 157-168.

Dennison, D.S. and K.W. Foster. 1977. Intracellular notation and the phototropic response of Phycomyces. Biophys. J. 18: 103-123.

Dennison, D. S. and C.C. Roth. 1967. Phycomyces sporangiophores: fungal stretch receptors. Science 156: 1386-1388.

Elfving, F. 1881. En obeaktad känslighet hos Phycomyces. Botaniska Notiser No. 4: 105-107.

Elfving, F. 1890. Uber physiologische Fernwirkung einiger Körper. Commentationies variae in memoriam actorum CCL annorum. Edidit Universitas Helsingforsiensis.

Elfving, F. 1893. Zur Kenntnis der pflanzlichen Irritabilität. Öfversigt af Finska Vetenskaps-Societetens Förhandlingar 36: 77-85.

Elfving, F. 1916-1917. Phycomyces und die sogennante physiologische Fernwirkung. Öfversigt af Finska Vetenskaps-Societeten Förhandlingar 59: 1-56.

Foster, K. W. and E.D. Lipson. 1973. The light growth response of Phycomyces. Journal of General Physiology 62: 590-617.

Gamow, R.I. and B. Böttger. 1982a. Phycomyces: Discovery of the aiming error in the avoidance response. Plant Physiology 69: 742-744.

Gamow, R.I. and B. Böttger. 1982b. Avoidance and rheotropic responses in Phycomyces. Journal of General Physiology 79: 835-848.

Gyure, D.C., B. Böttger and R.I. Gamow. 1984. Phycomyces: Detailed analysis of the anemogeotropic response. Journal of General Physiology 84: 727-738.

Harris, S.S. and D.S. Dennison. 1979. Phycomyces: Interference between the light growth response and the avoidance response. Science 206: 357-358.

Johnson, D.L. and R.I. Gamow. 1971. The avoidance response in Phycomyces. Journal of General Physiology 57: 41-49.

Lafay, J.-F. 1980. Résponses sensorielles d'un système végétal. Thèse, Université de Paris XI.

Lafay, J.-F. and J. Matricon. 1982. Avoidance and wind responses of Phycomyces blakesleeanus: Two distinct sensitivities. Experimental Mycology 6: 244-252.

Lafay, J.-F., J. Matricon and C. Bodéré. 1975. The avoidance response of Phycomyces: Distance dependence of the response. Physiologie Végétale 13: 259-263.

Meyer, P.W. 1986. The avoidance response of Phycomyces in a controlled environment. Ph.D. Thesis, California Institute of Technology, Pasadena, California.

Ortega, J.K.E. and R.I. Gamow. 1970. Phycomyces: Habituation of the light growth response. Science 168: 1374-1375.

Pellegrino, J.J., R.L Sani and R.I. Gamow. 1983. Mass transfer from Phycomyces in the avoidance and anemotropic responses. Journal of Theoretical Biology 105: 77-90.

Russo, V.E.A. 1977. Ethylene-induced growth in Phycomyces mutants abnormal for autochemotropism. Journal of Bacteriology 130: 548-551.

Russo, V.E.A., B. Halloran and E. Gallori. 1977. Ethylene is involved in the autochemotropism of Phycomyces. Planta 134: 61-67.

Shropshire, W., Jr. 1962. The lens effect and phototropism of Phycomyces. Journal of General Physiology 45: 949-958.

Smythe, W.R. 1950. Static and Dynamic Electricity, 2nd edition. McGraw-Hill Book Company, New York.

Steyer, K. 1901. Reizkrümmungen bei Phycomyces nitens. Inaugural-Dissertation, Leipzig.

Walter, H. 1921. Wachstumsschwankungen und hydrotropische Krümmungen bei Phycomyces nitens. Zeitschrift für Botanik 13: 673-718.

Weast, R.C., ed. 1975. Handbook of Chemistry and Physics. CRC Press, Cleveland, Ohio. 56th edition.

Wortmann, J. 1881. Ein Beitrag zur Biologie der Mucorineen. Botanische Zeitung 39: 368-374, 383-387.