EXPERIMENTAL MYCOLOGY 19, 153–159 (1995)

Evidence That Spitzenkörper Behavior Determines the Shape of a Fungal Hypha: A Test of the Hyphoid Model

SALOMON BARTNICKI-GARCIA,*[1] DAVID D. BARTNICKI,† GERHARD GIERZ,‡
ROSAMARÍA LÓPEZ-FRANCO,§ AND CHARLES E. BRACKER¶

Department of Plant Pathology, †Department of Computer Science, and ‡Department of Mathematics, University of California, Riverside, California 92521; §Centro de Biotecnología, Instituto Tecnológico y de Estudios Superiores, 64849 Monterrey, N.L. México; and ¶Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana 47907-1057

Accepted for publication April 12, 1995

BARTNICKI-GARCIA, S., BARTNICKI, D. D., GIERZ, G., LÓPEZ-FRANCO, R., AND BRACKER, C. E. 1995. Evidence that Spitzenkörper behavior determines the shape of a fungal hypha: A test of the hyphoid model. *Experimental Mycology* **19,** 153–159. Hyphae of the fungus *Rhizoctonia solani* have a characteristic Spitzenkörper in their growing tips and a cell shape described by the mathematical hyphoid equation. A mild disturbance of hyphae growing in a slide culture chamber on a microscope stage caused the Spitzenkörper to move away from its usual position next to the apical pole and wander briefly inside the apical dome. Hyphal elongation rate declined abruptly, and the apex became rounded and increased in diameter. As the Spitzenkörper migrated back to its polar position, rapid cell elongation resumed, and the contour of the growing hyphal tip returned to the typical hyphoid shape. The brief dislocation of the Spitzenkörper left a permanent bulge in the hyphal profile. This morphogenetic sequence was mimicked by computer simulation, based on the hyphoid equation which relates the generation of hyphal shape to the linear displacement of a vesicle supply center (VSC). The VSC was programmed to retrace the observed movements of the Spitzenkörper during the above sequence. The resulting similarity of shape between real and computer-simulated cells reinforces the mathematical prediction that the Spitzenkörper acts as a VSC and that its continuous linear advancement generates a typical hyphal tube with the characteristic hyphoid shape. Accordingly, the hyphoid model and its VSC concept provide a plausible hypothesis to explain the cellular basis of polarized growth of fungal hyphae.

INDEX DESCRIPTORS: apical growth; computer simulation; hyphal morphogenesis; hyphoid equation; mathematical model; *Rhizoctonia solani;* Spitzenkörper; vesicle supply center; video microscopy.

The Spitzenkörper is an organized assemblage of vesicles and other small cellular components found in elongating hyphal tips of higher fungi (McClure *et al.,* 1968; Girbardt, 1957; Girbardt, 1969; Grove and Bracker, 1970; Howard, 1981; Roberson and Fuller, 1988). Although this complex was discovered long ago (Brunswik, 1924), its role in hyphal growth has remained unclear. Girbardt (1957, 1969) provided the first strong correlative evidence linking the Spitzenkörper with the growth of hyphae, but the absence of a Spitzenkörper in hyphal tips of lower fungi (McClure *et al.,* 1968; Grove and Bracker, 1970; Grove *et al.,* 1970; Heath *et al.,* 1971) has been a compelling reason to question whether the Spitzenkörper is essential for apical growth of fungal hyphae.

The hyphoid model of fungal morphogenesis (Bartnicki-Garcia *et al.,* 1989; Bartnicki-Garcia, 1990) predicts that the Spitzenkörper functions as a final distribution center for wall-destined vesicles and has provided renewed impetus for assessing the role of the Spitzenkörper in hyphal growth. This mathematical model, based on vesicle dynamics, assumes that wall-building vesicles arise from a hypothetical vesicle supply center (VSC)[2] and migrate outward in all directions. In this model, a hypha is generated by the linear displacement of the VSC, a process de-

[1] To whom correspondence should be addressed. Fax: (909) 787-5113. E-mail: bart@ucrac1.ucr.edu.

[2] Abbreviations used: VSC, vesicle supply center.

0147-5975/95 $6.00

scribed by the hyphoid equation [$y = x \cot (x\ V/N)$], which defines the shape and size of a fungal cell by two physiological parameters; N, the quantity of wall-destined vesicles released from the VSC per unit time; V, the rate of linear displacement of the VSC. A computer simulation based on this model produces two-dimensional images which closely resemble the profiles of living hyphae (Bartnicki-Garcia *et al.*, 1989; Bartnicki-Garcia, 1990). In the hyphoid model, the VSC is centrally located in the apical dome, at a fixed short distance from the apical pole; a distance (d) defined by the ratio N/V. The position of the VSC corresponds to that of the Spitzenkörper in real hyphae and supports this idea that the Spitzenkörper functions as a VSC in the manner described by the model (Bartnicki-Garcia *et al.*, 1989; Bartnicki-Garcia, 1990). The observations presented here provide the first experimental test and support for this concept.

MATERIALS AND METHODS

Hyphae of *Rhizoctonia solani* were grown in a specially designed slide culture chamber with a hinged cover slip (López-Franco, 1992). The specimens were examined by video-enhanced phase-contrast microscopy with a 100× oil immersion objective lens. The images, obtained with a Hamamatsu C2400-01 video camera mounted on an Olympus Vanox microscope, were electronically enhanced and enlarged with an Argus-10 real-time digital image processor (Hamamatsu Photonic Systems Corporation, Bridgewater, NJ) and recorded on VHS video tape. Growth rates were calculated as increases in the area of cell images displayed on a video monitor.

The Fungus Simulator, an improved version (Bartnicki *et al.*, 1994) of an earlier computer program (Bartnicki-Garcia *et al.*, 1989), was used to simulate and compare morphogenetic sequences. Simulated shapes were generated by hypothetical "vesicles" produced from a continuously advancing VSC that was programmed to follow the same path as the Spitzenkörper. The position of the Spitzenkörper and the cell profiles were both mapped as X–Y coordinates from still videotaped images of the real fungus displayed on a noninterlaced monitor screen interfaced to an Argus 10 digital image processor.

RESULTS

When growing hyphae are subjected to strong mechanical, electrical, or light stress, the Spitzenkörper typically disappear and the hyphae stop growing (Girbardt, 1957; López-Franco, 1992) but we have observed several instances in which the Spitzenkörper in mildly stressed hyphae of *R. solani* became temporarily dislodged, causing changes in growth rate and cell shape and leaving permanent alterations in hyphal morphology. One example is described below.

In an undisturbed growth hypha of *R. solani*, the characteristic Spitzenkörper is clearly visible in the apex next to the apical pole (Fig. 1) and consists of a phase-dark cup-shaped cluster of vesicles surrounding a phase-light core region (López-Franco, 1992). The center of the core of the Spitzenkörper lies about 1.1 μm from the apical pole, and the morphology of the hypha is mathematically described by the hyphoid equation (Fig. 1). This hypha was observed and videotaped continuously for about 3.5 min, during which time it behaved and elongated normally; it maintained a hyphoid shape while growing at a constant overall rate of 0.46 $\mu m^2/s$ (Fig. 2). Figure 3 illustrates the sequence of morphological changes that ensued from a transient aberrant behavior of the Spitzenkörper. Within 19 s of a slight disturbance, caused when the microscope stage was moved sideways to reposition the slide chamber, the Spitzenkörper and the surrounding cytoplasm within the apical dome momentarily increased in refractive index (became phase dark), and the Spitzenkörper began to retreat from its polar position (Fig. 3b). Most of the dark vesicle cluster became dispersed, leaving the lighter core as the visible remnant of the Spitzenkörper. For the next minute (Figs. 3c and 3d), the core of the Spitzenkörper persisted, moved around in the center of the apical dome, and became difficult

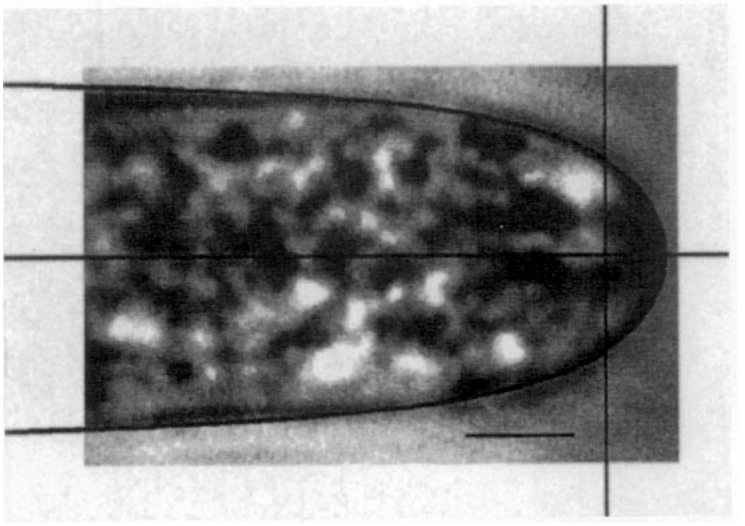

FIG. 1. Video-enhanced phase contrast micrograph of a hyphal tip of *Rhizoctonia solani* growing normally in the slide culture chamber. (This is the same hypha shown in Fig. 3 but the micrograph was recorded 14 s before the disturbance). The Spitzenkörper is clearly visible; the coordinate intersection marks the position of the Spitzenkörper core, while the dark cup-shaped assemblage to the right is the cluster of apical vesicles. The hyphal profile closely matches the superimposed hyphoid curve plotted for **d** = 1.3 μm (**d** is the distance between VSC and apical pole; Bartnicki-Garcia *et al.*, 1989). Scale bar, 2.3 μm.

to discern among the organelles. At one point, the Spitzenkörper core had moved as far as 2.6 μm from the apical pole. As the hyphal tip began to bulge, the rate of hyphal growth decreased abruptly to 0.22 $\mu m^2/s$, and the shape of the tip changed from hyphoid (Figs. 3a and 3b) to spheroid (Figs. 3c and 3d).

About 2 min after the disturbance, the Spitzenkörper began to move back towards a polar position (Fig. 3e). During the next minute (Figs. 3e–g), the Spitzenkörper became clearly visible again, with its characteristic light core and cup-shaped dark vesicle cluster, while the hypha increased its growth rate to 0.33 $\mu m^2/s$, and the tip became more tapered. During the following 2 min, the hypha regained its initial growth rate (0.48 $\mu m^2/s$), and the hyphal apex returned to its characteristic hyphoid shape (Figs. 3h–l).

The distortion of the hyphal profile observed in *R. solani* (Fig. 3) was simulated with an improved version ("Fungus Simulator") (Bartnicki *et al.*, 1994) of the original computer model (Bartnicki-Garcia *et al.*, 1989) that facil-

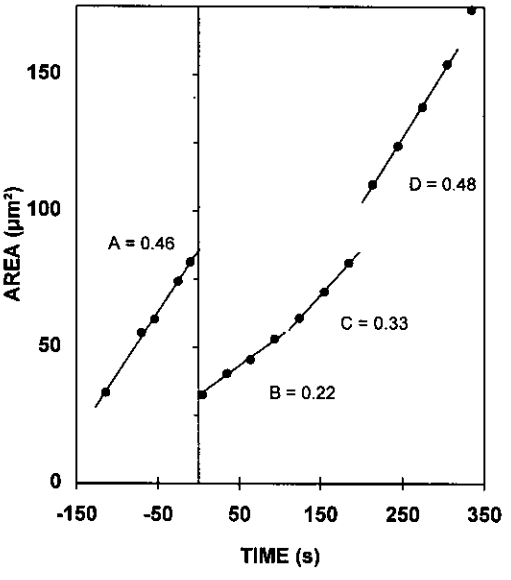

FIG. 2. Growth kinetics of *Rhizoctonia solani* during the morphogenetic sequence shown in Fig. 3. The rate of area increase was estimated from the mapped cell profiles in Fig. 4 plus additional profiles collected before zero time (not shown). The mean values ($\mu m^2/s$) depicted were calculated by linear regression.

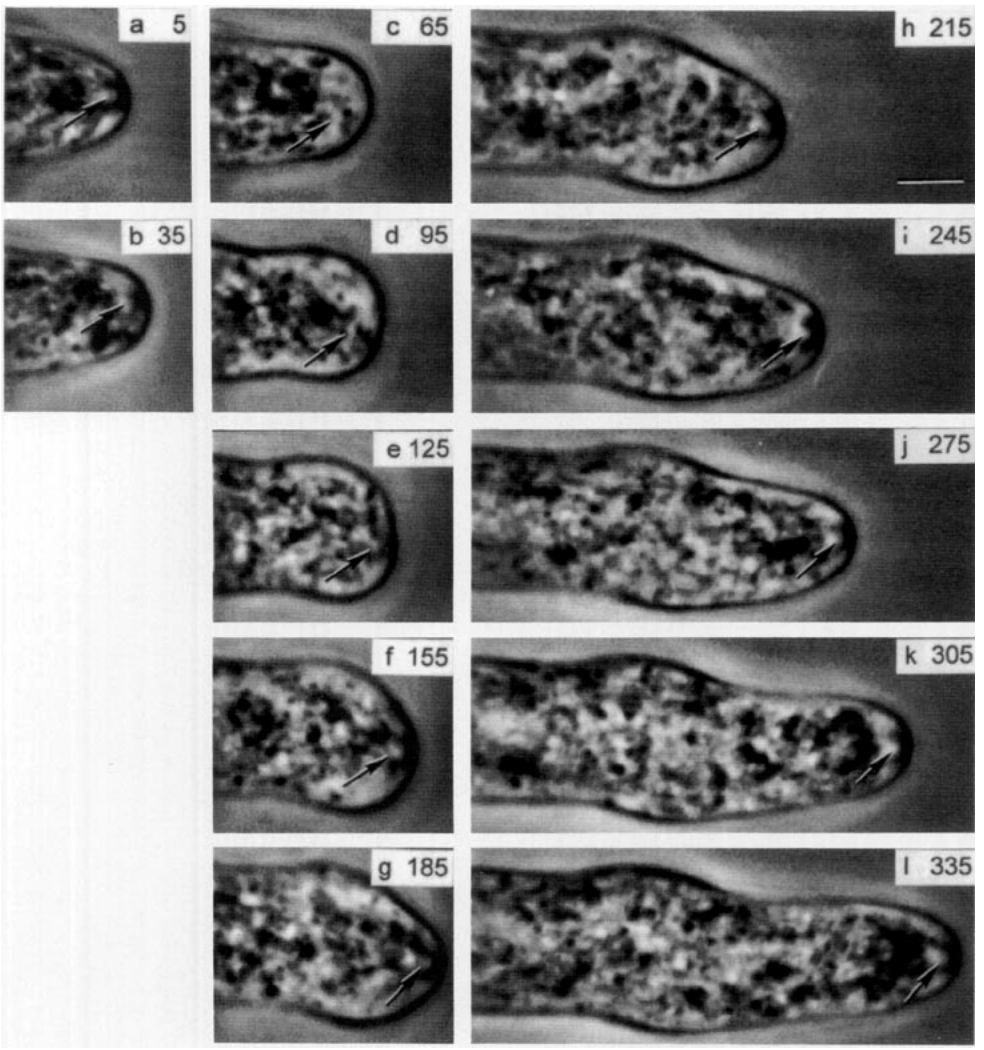

FIG. 3. Video microscopy record of a transient disturbance in the growth of the hyphal tip shown in Fig. 1. At zero time, the fungus was disturbed when the microscope stage was moved to reposition the specimen. The dark cup-shaped Spitzenkörper with its light inner core (arrows) is visible (a, b, e–l) except during the bulging phase (c,d) when the vesicle aggregate had dispersed and only the position of the light Spitzenkörper core could be determined on a video monitor. Still single-frame images were photographed on 35-mm film at the times shown in each frame (seconds from zero time). The sequence taped comprised two fields of view (a–g and h–l) and two different magnifications. All pictures are shown at the same magnification, but frames h–l were originally videotaped at half the magnification and then photographically enlarged. Scale bar, 3.8 μm.

itated programming the motion of the VSC to reproduce the movements of the Spitzenkörper and thus allowed a comparison between computer-generated shapes and profiles of the living, growing fungus (Fig. 4). The morphogenetic sequence was analyzed at 30-s intervals. At each period, the Fungus Simulator displayed the computer-generated shape plus the superimposed profile of the real cell at that time. The VSC was programmed to move according to the mapped path of the Spitzenkörper (Fig. 4, dark line) and release vesicles randomly at rates pro-

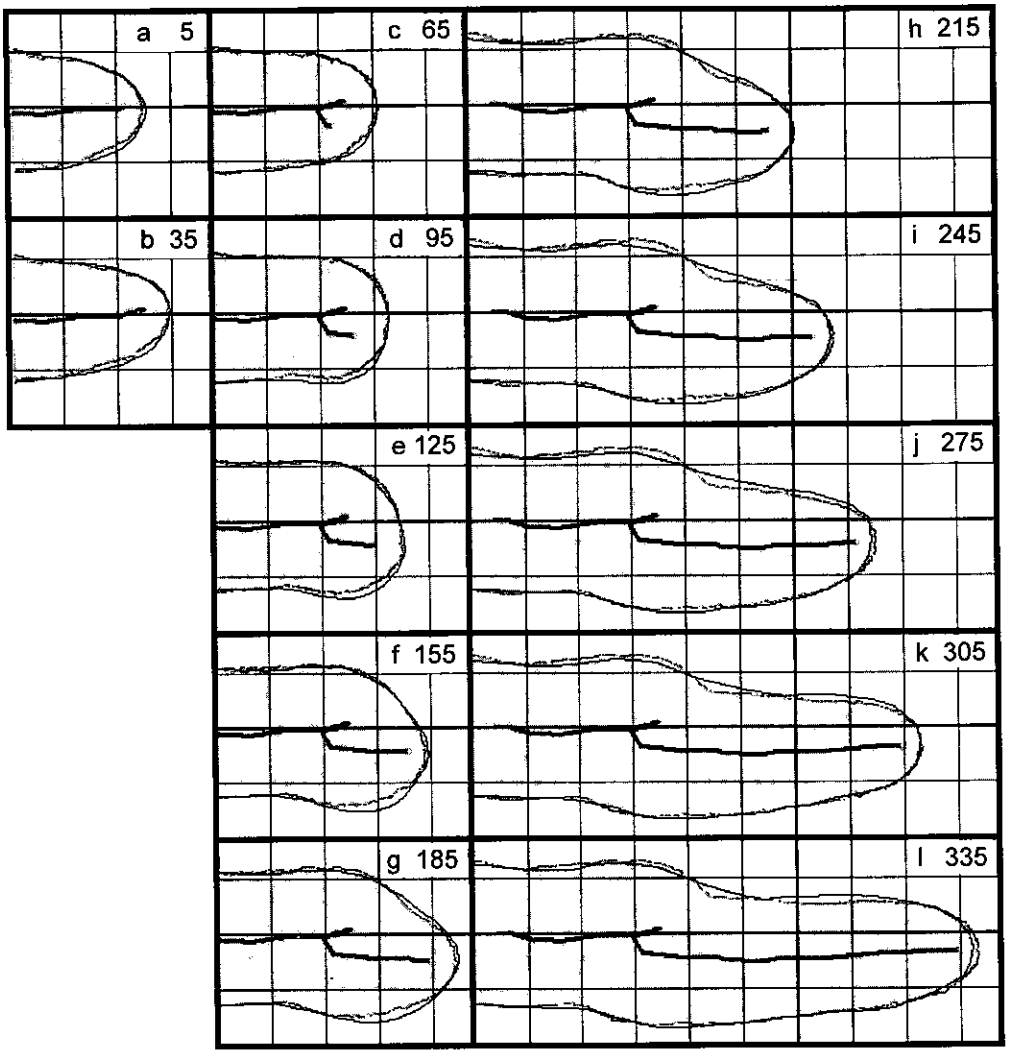

FIG. 4. Computer simulation of the growth disturbance shown in Fig. 3. The profile of the fungus was mapped at 30-s intervals and the tracings were superimposed onto the computer field of growth together with a coordinate grid. The trajectory of the Spitzenkörper in the growing hypha was mapped every 15 s (dark line in center of cell). Note that from 65 to 125 s, the precise position of the core of the Spitzenkörper was uncertain, and during this period the Spitzenkörper was assumed to advance in a straight line. The simulated shape (gray area) was generated by the Fungus Simulator from the position of the Spitzenkörper and the rate of vesicle release calculated from the rate of area increase (Fig. 2). Since the sequence in Fig. 3 was assembled from images taped in two fields of view at two different magnifications, appropriate constants were calculated to align the profiles accordingly. An additional critical correction was entered to compensate for unwarranted thermal drifting of the microscope; this amounted to a displacement of 0.22 μm (y axis) and 1.46 μm (x axis) during the shown sequence.

portional to area increase. During part of the bulging phase (65–125 s), the core of the Spitzenkörper was difficult to track because it could not be unequivocally distinguished from other cellular structures with similar appearance.

The entire sequence yielded shapes that were very similar to those of the live hypha (compare

Fig. 3 vs Fig. 4). The more typical hyphal shapes, observed before and after the bulge, are the hyphoid shapes predicted by the model from a linearly advancing Spitzenkörper that releases vesicles at a constant rate. The wider, rounded shape created during the momentary halt and subsequent reversal of the forward movement of the Spitzenkörper is what the model predicts would happen in the living cell from a Spitzenkörper that stops advancing but continues to serve as a vesicle supply center. The simulation also reproduced the downward and parallel displacement of the growth axis of the hypha after the bulging phase (Figs. 3 and 4).

A slight discrepancy between the shape generated by the computer and the actual shape of the hypha amounted to 3.8% of the mapped area within the cell profile at the end of the simulation (Fig. 4, frame 1). This discrepancy could be the result of imprecision in mapping the cell perimeters or locating the exact position of the Spitzenkörper core during its migration away from the apical pole of the hypha. Thus, while the Spitzenkörper and/or the apical pole were kept in sharp focus, parts of the highly magnified cell profile were unavoidably out of focus.

DISCUSSION

Our observations demonstrate that the Spitzenkörper can be dislodged from its polar position without affecting the viability of a hyphal tip but with major consequences to the growth rate and shape of the cell. The similarity of profiles between the stressed living hypha and the computer-generated simulation of hyphal morphogenesis supports the idea that the Spitzenkörper is the structural and functional equivalent of the hypothetical VSC. As long as the Spitzenkörper advanced linearly and maintained a close and constant distance from the apical pole (Figs. 1 and 3a, 3b, 3k, and 3l), the hypha maintained a hyphoid shape. When the Spitzenkörper retreated from its usual position, the shape of the cell changed substantially.

Our findings support the idea that the Spitzenkörper plays a central role in establishing and maintaining the highly polarized apical growth of fungal hyphae. They provide the first experimental test of the hyphoid model and reinforce the view that the Spitzenkörper is a key distribution (and probably maturation) site for the final delivery of secretory vesicles to the cell surface where they contribute to expansion of the cell wall and plasma membrane. Additional evidence in support of this concept comes from the discovery that transient "satellite" Spitzenkörper found near the hyphal apex of different fungi (López-Franco *et al.*, 1995) can produce perceptible lateral bulges in the hyphal profile. Presumably, these secondary satellite Spitzenkörper function as peripheral VSCs to produce minor distortions in hyphal shape. Although our findings pertain to fungi with a distinct Spitzenkörper, they emphasize the need to consider that fungi lacking a visible Spitzenkörper may also organize their apical vesicles in a manner that is functionally equivalent to the VSC.

ACKNOWLEDGMENTS

This work was supported in part by grants from the National Institutes of Health (GM-48257) and the National Science Foundation (IBN-9204541 and IBN-9204628). This is Journal Paper No. 14,537 of the Purdue University Office of Agricultural Research Programs.

REFERENCES

BARTNICKI, D. D., GIERZ, G., AND BARTNICKI-GARCIA, S. 1994. "Fungus Simulator": A Windows application to model fungal morphogenesis. Abs. 5th. Intern. Mycol. Congr., p. 12. Vancouver, Canada.

BARTNICKI-GARCIA, S. 1990. Role of vesicles in apical growth and a new mathematical model of hyphal morphogenesis. In *Tip Growth in Plant and Fungal Cells* (I. B. Heath, Ed.), pp. 211–232. Academic Press, San Diego.

BARTNICKI-GARCIA, S., HERGERT, F., AND GIERZ, G. 1989. Computer simulation of fungal morphogenesis and the mathematical basis for hyphal (tip) growth. *Protoplasma* **153:** 46–57.

BRUNSWIK, H. 1924. Untersuchungen über Geschlechts und Kernverhältnisse bei der Hymenomyzetengattung *Coprinus.* In *Botanische Abhandlungen* (K. Goebel, Ed.), pp. 1–152. Gustav Fisher, Jena.

GIRBARDT, M. 1957. Der Spitzenkörper von *Polystictus versicolor. Planta* **50:** 47–59.

GIRBARDT, M. 1969. Die Ultrastruktur der Apikalregion von Pilzhyphen. *Protoplasma* **67:** 413–441.

GROVE, S. N., AND BRACKER, C. E. 1970. Protoplasmic or-

ganization of hyphal tips among fungi: Vesicles and Spitzenkörper. *J. Bacteriol.* **104:** 989–1009.

GROVE, S. N., BRACKER, C. E., AND MORRÉ, D. J. 1970. An ultrastructural basis for hyphal tip growth in *Pythium ultimum*. *Am. J. Bot.* **57:** 245–266.

HEATH, I. B., GAY, J. L., AND GREENWOOD, A. D. 1971. Cell wall formation in the Saprolegniales: Cytoplasmic vesicles underlying developing walls. *J. Gen. Microbiol.* **65:** 225–232.

HOWARD, R. J. 1981. Ultrastructural analysis of hyphal tip cell growth in fungi: Spitzenkörper, cytoskeleton and endomembranes after freeze-substitution. *J. Cell Sci.* **48:** 89–103.

LÓPEZ-FRANCO, R. 1992. Organization and dynamics of the Spitzenkörper in growing hyphal tips. Ph.D. thesis, Purdue University, W. Lafayette, IN.

LÓPEZ-FRANCO, R., HOWARD, R. J., AND BRACKER, C. E. 1995. Satellite Spitzenkörper in growing hyphal tips. *Protoplasma,* in press.

MCCLURE, W. K., PARK, D., AND ROBINSON, P. M. 1968. Apical organization in the somatic hyphae of fungi. *J. Gen. Microbiol.* **50:** 177–182.

ROBERSON, R. W., AND FULLER, M. S. 1988. Ultrastructural aspects of the hyphal tip of *Sclerotium rolfsii* preserved by freeze substitution. *Protoplasma* **146:** 143–149.