ULTRASTRUCTURE OF *LASIODIPLODIA THEOBROMAE* CAUSAL AGENT OF CARIBBEAN PINE BLUE STAIN IN VENEZUELA

LUIS CEDEÑO, SARI MOHALI, ERNESTO PALACIOS-PRÜ

Lasiodiplodia theobromae (Pat.) Griff. and Maubl. (=*Botryodiplodia theobromae* Pat.), anamorph of *Botryosphaeria rhodina* (Cooke) Arx (Sutton, 1980), is a widely distributed fungus in tropical and subtropical regions where it has been reported to cause various types of damage to more than 500 plant species (Punithalingam, 1976). In Venezuela, *L. theobromae* affect important agricultural and forestry species (Cedeño and Palacios-Prü, 1992; Cedeño *et al.*, 1992; Diaz and Salas, 1980). A large number of woody species are attacked, especially when under stress (Goos *et al.*, 1961; Lewis and Van Arsdell, 1978; Mullen *et al.*, 1991; Punithalingam, 1976, 1980). The fungus is also a major cause of blue staining on fallen timber and freshly sawn lumber in tropical and subtropical areas (Findlay, 1959; Findlay and Pettifor, 1939; Lambeth *et al.*, 1989; Pawsey, 1968). Blue staining limits the industrial usefulness and greatly reduces the value of sawn wood, and increases the cost of the bleaching process in the manufacture of pulp and paper (Mancini, 1989). In temperate areas, most blue staining is caused by *Ceratocystis* spp. and related fungi (Findlay, 1959; Lambeth *et al.*, 1989; Nelson, 1934). Spread of *L. theobromae* is primarily by windborne spores, and infection usually occurs only through the ends of the logs or where the bark has been knocked off during harvest and transport (Lambeth *et al.*, 1989). *L. theobromae* occurs commonly in the Eastern part of Venezuela where several hundred thousand hectares of Caribbean pine (*Pinus caribaea* Morelet) plantations have been established over the past 25 years. The wood of cut trees is quickly colonized and heavily stained. Severe lumber degradation due to staining by *L. theobromae* also occurs if logs harvested during normal operations are not promptly processed, and if freshly sawn lumber is not treated with fungicides.

Previous work on the development of blue stained fungi in wood has dealt primarily with stain caused by *Ceratocystis* spp. and its effect on various chemical and physical wood properties (Findlay, 1959). No information exists on the effects of *L. theobromae* on wood properties of pine, nor on its development in wood, with the exception of a study demonstrating that the fibers extracted from pine wood with blue stain caused by the fungus were shorter than those from nonstained wood (Mancini, 1989). Only the pycnidiospores of *L. theobromae* have been investigated ultrastructurally (Ekundayo and Haskin, 1969; Uduebo, 1975; Yaguchi and Nakamura, 1991).

The objectives of this study were to determine, using the light microscope (LM), scanning electron microscope (SEM) and transmission electron microscope (TEM), the ultrastructure of the hyphal cells of *L. theobromae*, the development of the fungus in pine wood

KEY WORDS / *Pinus caribaea* / **Blue Stain** / *Lasiodiplodia theobromae* / *Botryodiplodia theobromae* /

L. Cedeño Mujica. **Ingeniero Agrónomo de la Universidad de Oriente, Venezuela. Maestría en Ciencias de la Universidad de Georgia. USA. Profesor-Investigador Titular del Instituto de Investigaciones Agropecuarias de la Universidad de los Andes, Mérida, Venezuela, en el área de Patología Vegetal. Centro de Microscopía Electrónica, Apdo. 163, Universidad de Los Andes, Mérida 5101-A, Venezuela. Instituto de Investigaciones Agropecuarias Apdo. 77 (La Hechicera), Universidad de Los Andes, Mérida 5101-A, Venezuela.**

Sari R. Mohali C. **Imgeniero Forestal. Msc. en Fitopatología. Profesor asistente en el Área de Biología de Vegetal de la Universidad de los Andes, Centro de Microscopía Electrónica. Área de Investigación: Patología Vegetal y Liquenología (Diagnóstico y Ultraestructura). Centro de Microscopía Electrónica, Apdo. 163, Universidad de Los Andes, Mérida 5101-A, Venezuela.**

E. Palacios-Prü. **Doctor en Medicina de la Universidad de los Andes, Mérida, Venezuela y Postdoctor en Neurociencias de la Universidad de Chicago, USA. Investigador A del Centro de Microscopía Electrónica de la Universidad de los Andes e investigador III SPI. Autor de más de 100 publicaciones científicas especializadas refrendadas, más de 180 publicaciones cortas, 2 libros de texto, 7 libros especializados y 6 capítulos de libros. Profesor Titular de la Universidad de los Andes, Mérida, Venezuela. Miembro Principal del Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICIT). Director del Centro de Microscopía Electrónica y Director del Postgrado de Ciencias Médicas Fundamentales de la Universidad de los Andes. Centro de Microscopía Electrónica, Apdo. 163, Universidad de Los Andes, Mérida 5101-A, Venezuela. Instituto Internacional de Estudios Avanzados, Caracas, Venezuela.**

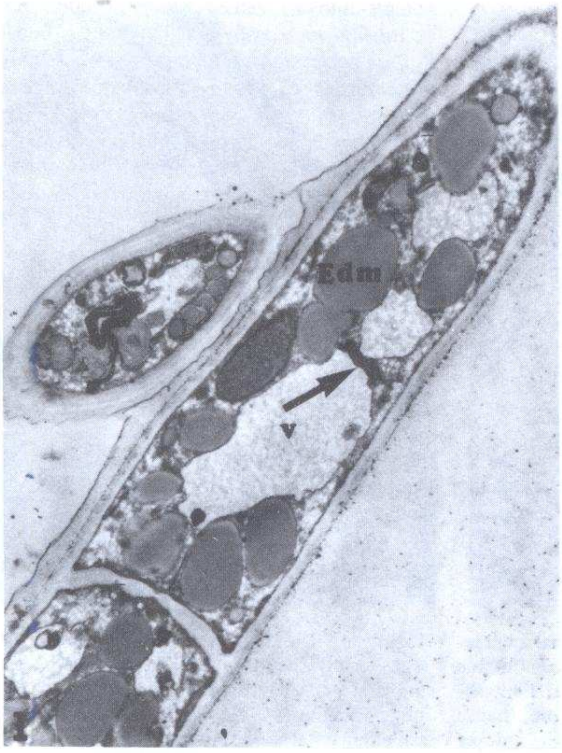

Figure 1. Longitudinal section of a hypha with abundant cytoplasmic bodies filled with electron-dense material (Edm). Note the large vacuoles (V) containing a slightly electron-dense substance. The arrow indicates a set of cisterns disposed as a stacked pile. X 9,000.

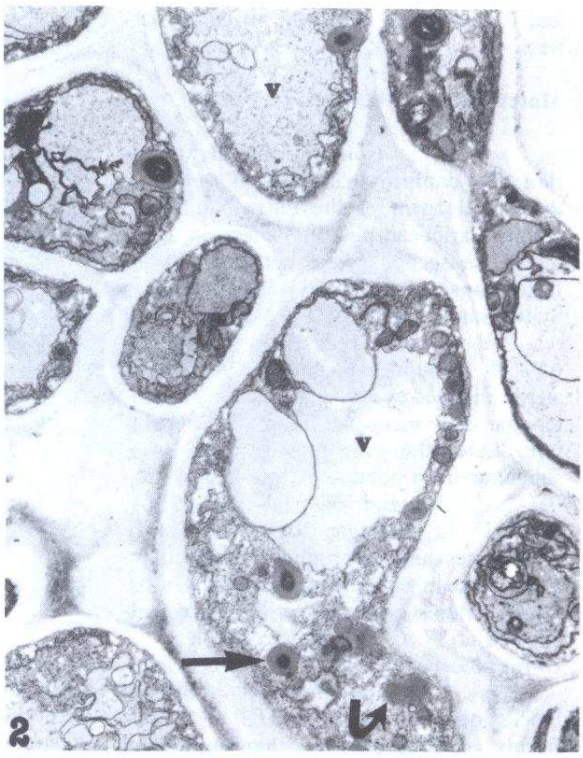

Figure 2. Section of stroma where the hyphae are cemented by slightly electron-dense material. In the hypha interior few dense bodies can be seen (curved arrow). Most of the cytoplasmic vacuoles (V) are electron-transparent. The straight arrow indicates a body with a highly electron-dense core. X 7,500.

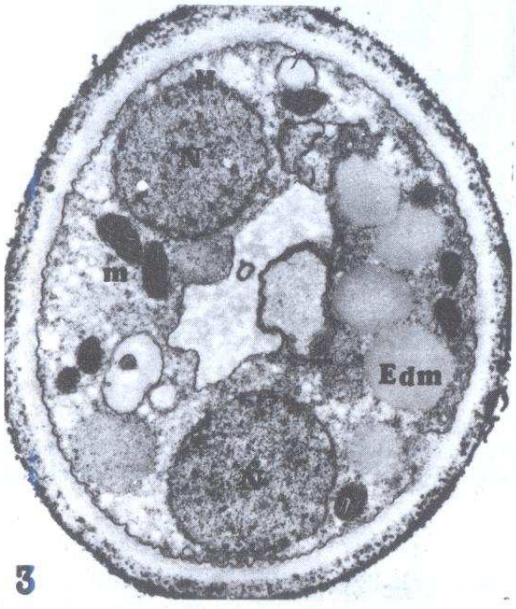

Figure 3. Transversely sectioned hypha in which two layers of cell wall can be observed, the outer one invaded by electron-dense granular material and the inner one devoid of this substance. Two nuclei (N) can be seen with uniformly distributed chromatin. Edm, electron-dense material; m, mitochondria. X 15,000.

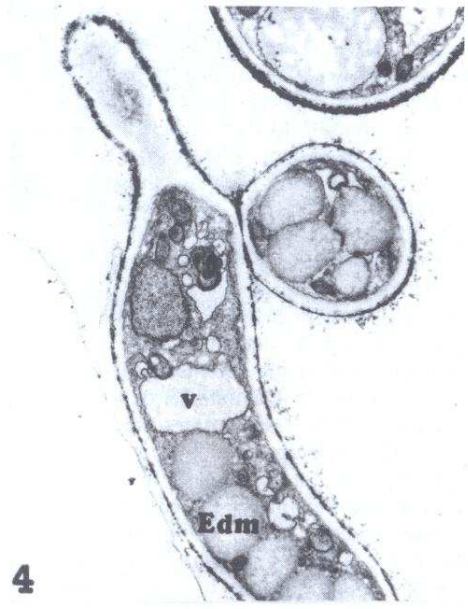

Figure 4. Longitudinally sectioned hypha showing electron-dense material (Edm) and an elongated segment of cell wall. V, vacuole. X 7,500.

and it's ability to affect the wood properties.

Materials and Methods

Isolation and identification. To confirm that ***L. theobromae*** is the causal agent of the blue staining affecting Caribbean pine in Venezuela, 2 cm transverse sections were cut from 50 infected logs collected at Uverito, Monagas State. Small pieces of blue-stained wood (ca. 2 mm^2) were treated for 5 min with 0.525% sodium hypochlorite, washed in sterile distilled water, plated onto 2% water-agar and incubated at room temperature. Later the emerging colonies were subcultured on potato-dextrose-agar (Difco PDA, 39g/L) and oatmeal-agar (OMA, oatmeal 30g and agar 20g/L) to identify the isolates and to produce the inoculum to be used in experimental infection tests. Some cultures produced on PDA were incubated at 37 °C in complete darkness.

Inoculation. Twenty blocks of pine wood (3 x 3 x 3 cm^3) freshly cut from unstained logs were used to determine the ability of the fungus to produce blue staining. Ten blocks were placed in contact with 7 day-old ***L. theobromae*** cultures grown in flasks containing PDA and then incubated at room temperature. Wood blocks used as control were placed on PDA without the fungus.

Light and Electron Microscopy:

Hyphae of *L. theobromae* from agar culture. The hyphae were ultrastructurally examined to know the features of the fungus to be observed within naturally blue-stained pine wood. The fungus was grown for 3-6 d on a thin layer (7 ml) of OMA at room temperature. Cultures were fixed for 3-4 hrs by flooding them with 3% paraformaldhyde and 3% glutaraldehyde mixed in 0.1 M sodium cacodylate buffer pH 6.3. Later, mycelial plugs, 3 mm in diameter, were cut from different sites of the colonies and postfixed overnight in 1% Osmium tetroxide in the same buffer. The samples for LM and TEM were dehydrated in a series of graded ethanol (30, 50, 70, 80, 95 and 100%), pure acetone and finally embedded in Spurr's resin (Spurr, 1969). Complete polymerization was achieved in an oven at 70°C for 48 hrs. Thick sections of Spurr-embedded specimens were treated with 2% paraphenylenediamine and the thin sections were contrasted with uranyl acetate (Gibbons and Grimstone, 1960) and lead citrate (Reynolds, 1963). Specimens were examined in Reichert Polyvar light microscope, Hitachi S-2500 scanning electron microscope and Hitachi H-7000 transmission electron microscope.

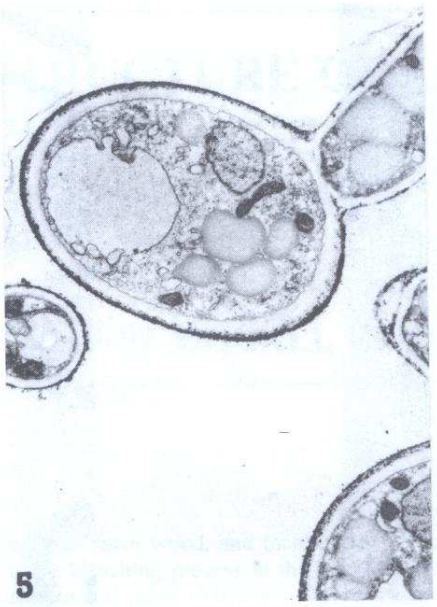

Figure 5. The hyphal variability that characterizes *L. theobromae* is clearly seen in this image in which adjacent hyphal cells of different diameters are observed. Note that a thinner hyphal cell growing from the thicker one. X 7,500.

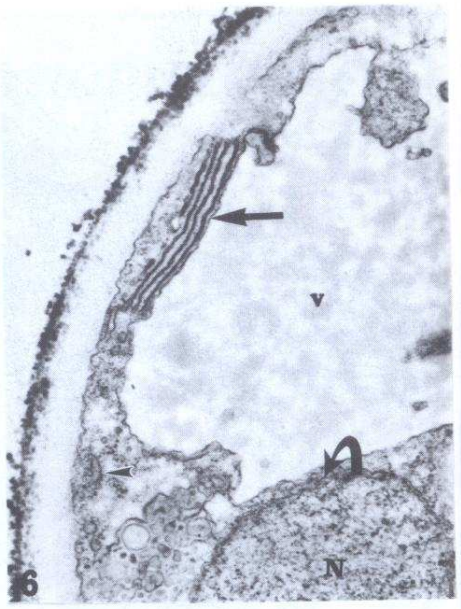

Figure 6. The hyphal segment that appears transversely sectioned in this image, possesses moderately electron-dense material within large vacuole (v). The straight arrow indicates lamellar cisterns disposed in a stacked pile. Curved arrow a nuclear pore; arrow head indicate a small rough endoplasmic reticulum cistern; N, nucleous. X 30,000.

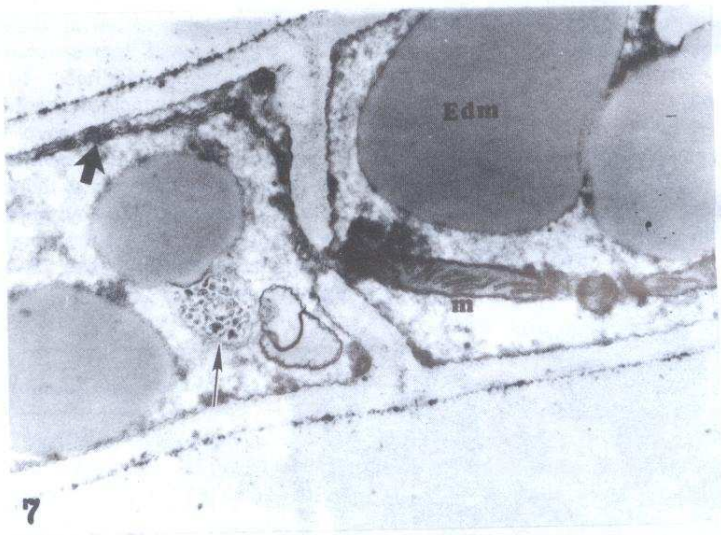

Figure 7. The septum of ***L. theobromae*** is clearly seen in this photograph. Note the lengthened mitochondrium (m) and the electron-dense material (Edm), which are associated with the septum. Arrow indicates endoplasmic reticulum; thin arrow points to a tubular membranous structure with a dense body. X 27,000.

Blue-stained pine wood. Materials to be examined in LM, SEM and TEM were freshly cut from naturally infected logs. Small pieces (ca. 2 mm^2) were fixed for one week in 3% formaldehyde and 3% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 6.3, and postfixed overnight in 1% Osmium tetroxide in the same buffer. Specimens for LM and TEM were dehydrated in ethanol and embedded in Spurr's resin. Samples for SEM were dehydrated in a series of graded acetone (30, 50, 70, 80 and 100%) and embedded in Peldrix II (Ted Pella Inc., Reading, Califor-

nia 96099). Some pieces of blue-stained wood were fixed, dehydrated in ethanol as above and then gradually embedded in 2, 8 and 10% parlodion mixed in 1:1 ethanol: ether (v/v). Thick and thin sections of Spurr-embedded material were treated as previously described. Parlodion-embedded sections were stained with 0.5% Azur II in 1% Borax.

Results

Isolation and identification. *L. theobromae* was consistently isolated from all the blue-stained tissues analyzed. On OMA, at the beginning the colonies were white, becoming later dark gray. The hyphae were initially hyaline, later turning to dark brown. After 5 days of growth at room temperature, abundant hyphal aggregates were observed on the surface of the colonies. These later developed into stromatic structures containing pycnidial locules. Pycnidiospores (conidia) grown within the locules were initially hyaline, oval-shaped, one-celled and thick-walled, but later became dark brown, two-celled and longitudinally striated on their surface. Both hyaline and pigmented pycnidiospores measured 22-29 µm in length and 12-15 µm in width. Hyaline, cylindrical and septate paraphyses were also seen within the pycnidia. The fungus produced a reddish pigment on PDA at 37°C.

Based on ***in vitro*** growth characteristics, size and morphology of the pycnidiospores, the fungus isolated from blue-stained wood is ***Lasiodiplodia theobromae*** (Pat.) Griff. and Maubl. (= ***Botryodiplodia theobromae*** Pat.), anamorph of ***Botryosphaeria rhodina*** (Cooke) Arx (Punithalingam, 1976; Sutton, 1980). Inoculation tests further confirmed the association of ***L. theobromae*** with blue staining of pine wood in eastern Venezuela.

Inoculation. Symptoms of blue stain, similar to those occurring in naturally infected logs, were observed on pine wood blocks 5d after inoculation. The blocks used as controls did not develop blue staining. ***L. theobromae*** was frequently isolated from experimentally infected tissues.

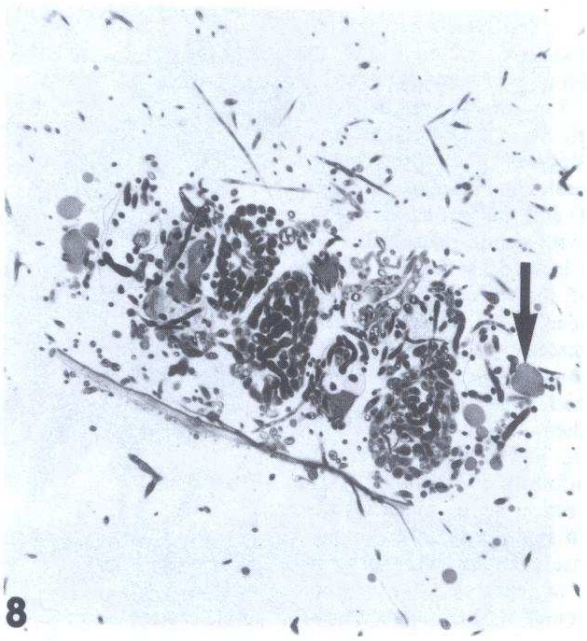

Figure 8. Light microscopy image showing accumulation of electron-dense material (arrow), around which the hyphae aggregates to form a pycnidial stroma. X 675.

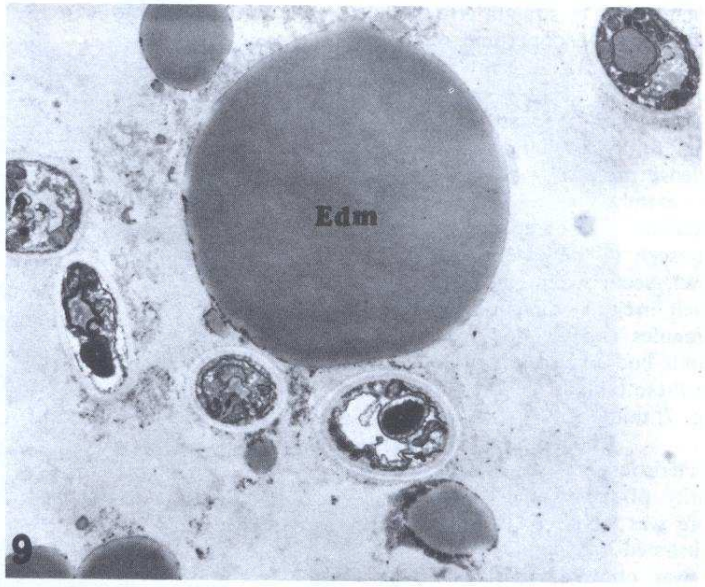

Figure 9. Transmission electron micrograph showing a large spheroidal deposit of extrahyphal electron-dense material (Edm), associated to this material some hyphae can be seen. X 6.000

Light and Electron Microscopy:

Hyphae of *L. theobromae* from agar culture. *L. theobromae* ultrastructural analysis revealed that hyphal diameter and cell wall thickness show a great variability. Furthermore, the cell wall also showed changes in the composition of its elements: in the stromatic aggregates, some hyphae had electron-dense, compact and very homogeneous cell walls, while the dispersed hyphae, showed irregular and granular cell walls. A longitudinally-sectioned hypha, whose cell wall shows electron- dense particles on its surface, can be seen in Figure 1, while in Figure 2, there are hyphae whose homogeneous cell wall is not covered by this granular, fine material. In other hyphae, such as those illustrated in Figure 3, the cell wall had two layers: the outer one containing electron-dense granular material and the inner one is totally devoid of this substance. The transverse and longitudinal sections that are shown in Figures 4 and 5, respectively, illustrate the variability in hyphal diameter of this fungus. Furthermore, it is important to note that thicker hyphae form the thinner ones. Figure 4 illustrates a longitudinally sectioned hypha with an elongated segment which lacks cytoplasmic content and is probably due to the plane in which the section was made. A transversely sectioned hypha forming another thinner hypha is shown in Figure 5.

Transverse sections revealed the cytoplasmic richness and the diameter variability of *L. theobromae* hyphae. Figure 3 shows a typical hypha with two nuclei, large amount of material with different degrees of electron density, prominent vacuoles containing heterogeneous material and numerous free ribosomes in the cytoplasmic matrix. The nuclei were often located in the most peripheral regions of the cytoplasm and were generally rounded in shape in transverse sections, and discretely elongated or fusiform in longitudinal sections (Fig. 4). These nuclei had well developed nuclear membranes; their nuclear pores were clearly seen (Fig. 6, curved arrow) and the chromatin uniformly distributed. The amounts of electron-dense material were also variable. In some cells, large amounts of electron-dense granules were observed (Fig. 7), while in other cells of the same hypha, the amount of this substance was smaller.

Some membranous structures consisting of flattened cisterns of the same length were seen associated with vacuoles (Fig. 6, straight arrow). The rough and smooth endoplasmic reticulum was rarely found (Fig. 6, arrow head and Fig. 7, arrow). The vacuoles material was homogeneous and moderately electron-dense (Fig. 6). Large amounts of similar electron-dense material was also observed free in the extrahyphal space (Fig. 2). Particular tubular membranous structures were also seen in the cytoplasm (Fig. 7, thin arrow), sectioned in different planes due to their irregular direction of growth. Dense granules and electron-dense multimembranous bodies were observed associated with these tubular membranous structures (Fig. 7, thin arrow).

The septum showed typical characteristics of Ascomycetes, but in the majority of the hyphae examined the septal pore was not seen; however, in the hypha illustrated in Figure 7, a simple septal pore was observed associated with a lengthened mitochondria and with electron-dense material that internally covers the invaginated membranes which form the septum. This material also filled the pore.

L. theobromae has the tendency to form hyphal aggregates, particularly in those sites where extrahyphal electron-dense material is deposited (Figs. 8 and 9). In Figure 9 several hyphae are shown initiating the aggregation around the electron-dense material similar to that seen within the hyphae. When the hyphae aggregate is completed, the extrahyphal compacting material contributes to the rigidity of these stromatic structures. The cell walls of the stromatic hyphae did not contain the electron-dense granular material commonly observed in the hyphae dispersed on the culture medium. On the other hand, it is important to emphasize that the cytoplasm in most of the stromatic hyphae had scanty amounts of electron-dense granules and the granules that were observed were smaller in size (Fig. 2). Large vacuoles were generally seen in the cytoplasm of stromatic hyphae (Fig. 2); some of these vacuoles contained moderately electron-dense material and others were electron-transparent. In the stromatic aggregates, the hyphae could not be followed for long distances, indicating that most of them had reduced their length to fit into the glommerulate structure that they form (Fig. 8).

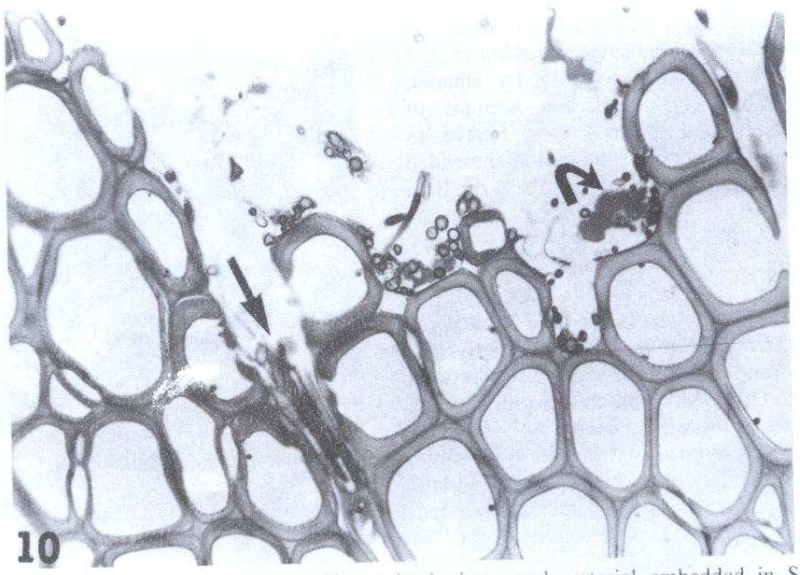

Figure 10. Transverse section of the blue-stained pine wood material embedded in Spurr's resin. The hyphae of *L. theobromae* are clearly seen within and between tracheids (straight arrow). Curved arrow indicates electron-dense material deposited in the area occupied by the fungus. Notice the wide zone invaded by the fungus. X 312.

Figure 11. Tangential section of parlodion-embedded specimen showing hyphae growing longitudinally within and between tracheids. Straight arrow indicates straight, dark, thick hypha showing regularly spaced septa from which a narrower and more hypha branches. Curved arrow shows hypha with closer septa and which grows through tracheid cell wall. X 312.

Blue-stained pine wood. The growth of *L. theobromae* could be observed within the tissues of naturally and experimentally infected wood (Figs. 10 and 11). Hyphae grew both transversely and longitudinally, and could be seen occupying an area in which the cell wall of some tracheids had disappeared, and in others showed fractures. The fun-

gus was located in the interior of tracheids as well as between tracheids (Fig. 10, straight arrow). Large masses of homogeneous and moderately electron-dense material were seen accompanying groups of hyphae (Fig. 10, curved arrow). Variation in the hyphal morphology was observed in the histological sections of parlodion-embedded material (Fig. 11). Hyphae of different diameters were found showing both regular and irregular septal arrangements. Regularly disposed septa were observed in long, straight hyphae growing within and between tracheids (Fig. 11, straight arrow), but this regularity was not observed in hyphae of irregular direction of growth which showed constrictions probably because they grow through cell walls (Fig. 11, curved arrow).

SEM revealed in better detail the variation in thickness and in trajectory of the hyphae of ***L. theobromae***. The straight arrow in Figure 12 shows a thin, transversely oriented hypha branching from a thicker one. However, not all the hyphae that were transversely oriented with respect to the tracheids are thin since, in the same image, a thick hyphae was also growing transversely (Fig. 12, curved arrow). Some hyphae having irregular trajectory (Fig. 12, asterisk), were longitudinally oriented in relation to the main axis of the tracheids. Figure 13, shows the hyphae abundance in the interior of a ray. These hyphae have different diameters and growth direction. Rays are made up of parenchymatous cells and, in the majority of the observed sections, a large amount of hyphae could be seen sending branches toward the tracheids. This means that rays are invaded first and then the tracheids.

TEM showed more information about the relationship existing between ***L. theobromae*** hyphae and pine wood elements. Figure 14, shows a transversely sectioned hyphae whose cytoplasm has degenerated and only few structures appear adhered to the cytoplasmic membrane. A large vacuole containing moderately electron-dense granular material can be observed in the interior of this hyphal cell (Fig. 14, asterisk). The hyphae within the wood had heterogeneous cell walls formed by two layers (Figs. 14 and 15): the outer one containing electron-dense granular material giving it a mineralized-like aspect, and the inner one without this material, as usually observed in fungal cell walls. The electron-dense substance forms part of the complex that adheres the hyphae to the wood cells (Fig. 14). In some sections several hyphae were seen aggregated by the electron-dense material and the same substance adhere the hyphal aggregates to the wood elements (Fig. 15).

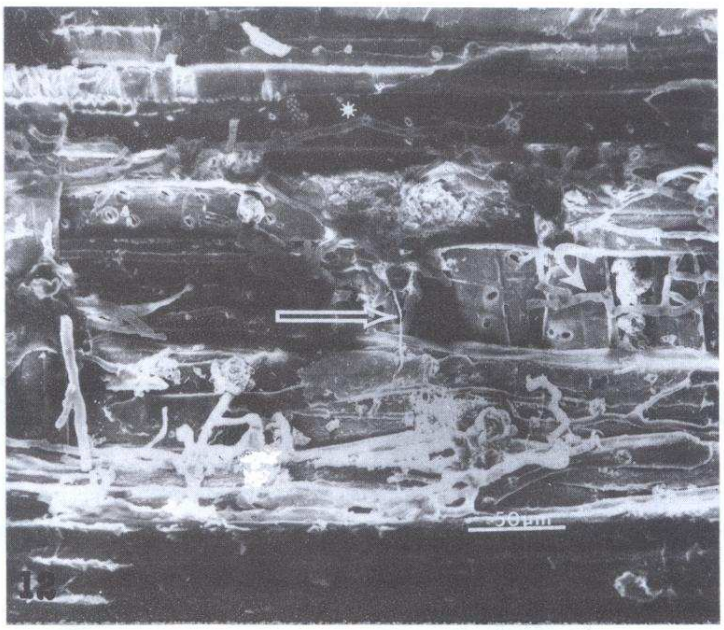

Figure 12. The variability demonstrated by the hyphae of *L. theobromae* in thickness and in direction of growth, can be clearly seen in this scanning electron microscope photograph. Asterisk indicates a moderately thick hypha located within the lumen of a tracheid. Transversally orientated thick hypha (curved arrow) and thinner hypha (straight arrow) are also seen.

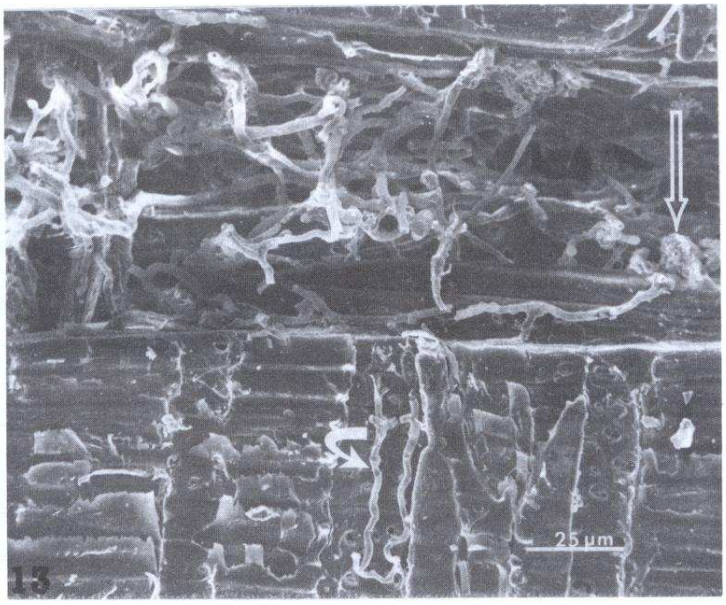

Figure 13. Scanning electron microscope image of a ray invaded by hyphae having different directions of growth. Straight arrow indicates part of the electron-dense material deposited in the ray. Curved arrow shows an undulated hypha.

Figure 16, shows a hypha in the intercellular space of the wood, and in this case, the electron-dense material could be observed filling the intercellular space around the hyphae.

Discussion

This ultrastructural study contributes to the information relative to the structure and dynamics of ***L. theobromae*** hyphae (Cedeño *et al.*, 1992). It is important to note that this fungus possesses a significant variability as shown by it's growth on OMA, it's diversity in cytoplasmic content and it's wide range of hyphal diameter. During the first growth stages, the fungus produced a white mycelium that later turned dark-gray and then formed hyphal aggregates. Within these

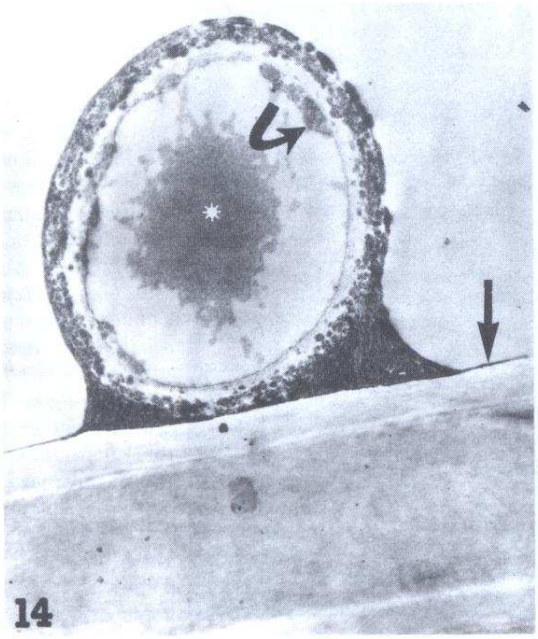

Figure 14. Hypha adhered to the cell wall of a tracheid by the electron-dense granular material produced by the fungus. Straight arrow shows a thin layer of this material covering neighboring host cell wall. Asterisk indicates the electron-dense substance deposited within a large cytoplasmic vacuole. The substance can also be seen attached to the inner face of the vacuole membrane (curved arrow). X 19,000.

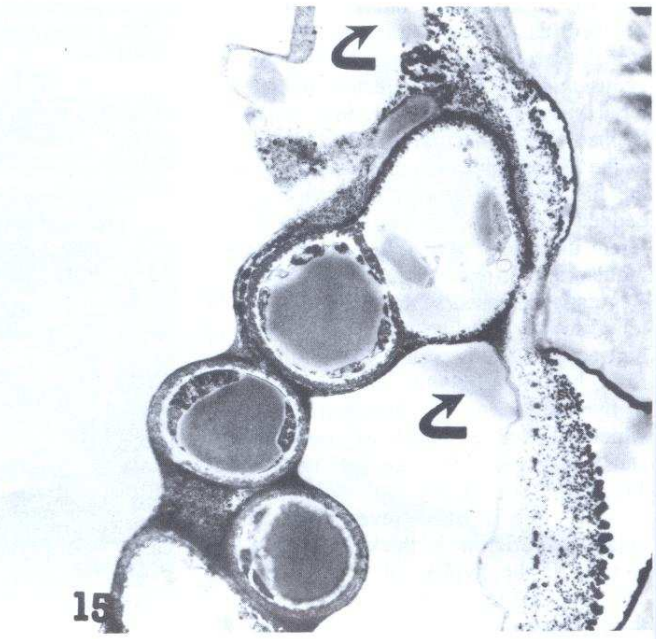

Figure 15. Hyphae aggregated by electron-dense material. The same substance adhered the hyphal aggregate to the tracheid cell wall. Curved arrow indicate extracellular material of medium electron-density. X 9,500.

aggregates, large, spheroidal deposits of a homogeneous and moderately electron-dense substance were observed; this material seems to contribute to organize the hyphal stromatic structures. Pycnidial locules developed within these structures.

The variation in the hyphal diameter has been interpreted as evidence that ***L. theobromae*** produces two kinds of hyphae (Olofinboba, 1974). However, our observations indicate that the fungus does not form different hyphae, but simply that thin hyphae are branched from the thick ones. This observation is supported by the fact that the ultrastructure of both thin and thick hyphae was similar. The hyphae forming part of stromatic structures showed homogenous cell walls, while those dispersed on the substrate had two layers, the outer one, surrounded by a highly electron-dense, heterogeneous material and a homogeneous inner layer. Cell walls structured by two layers have also been observed in pigmented pycnidiopores (Ekudayo and Haskin, 1969; Uduebo, 1975; Yaguchi and Nakamura, 1991). This electron-dense material is similar to the substance deposited in the cell walls of mature pycnidiospores (Yaguchi and Nakamura, 1991).

Similar electron-dense material was also seen scattered on the culture medium (Figs. 7, 9 and 10), indicating that the fungus contributes to the precipitation of some salts that became adhered to the outer layer of the cell wall. This mechanism is favored by the fact that in a given moment of the hyphal development, the cell wall may be punctured, facilitating the aggregation of the electron-dense material that is deposited on the surface of dispersed hyphae. On OMA-medium, grown samples a colored material was seen after the third day of growth. At this time, the colonies turned

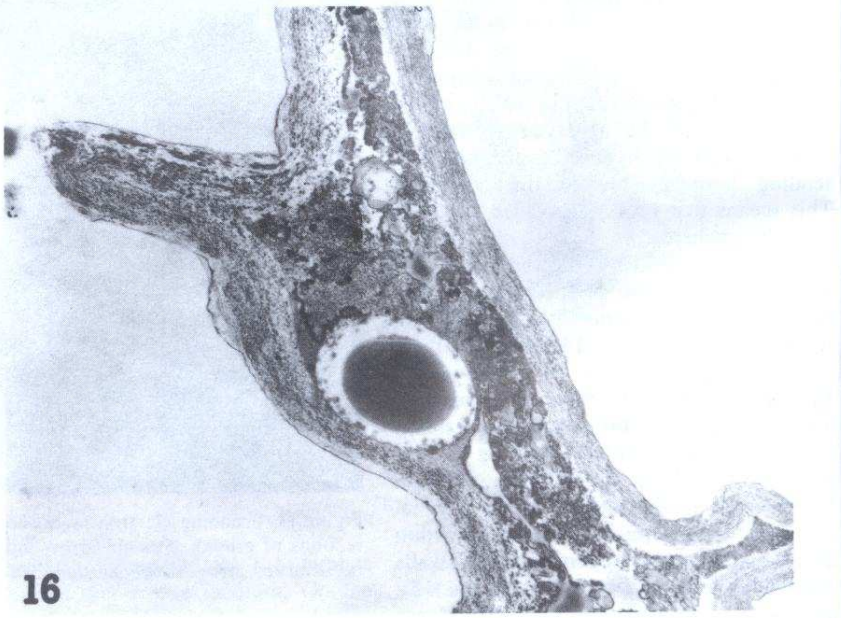

Figure 16. Hyphae of *L. theobromae* were also found between tracheids, as can be observed in this image. Notice the large amount of electron-dense material deposited in the space around the hypha. X 19,000.

from white to a bluish color similar to that seen on blue-stained pine wood. With respect to the ***in vitro*** behavior, Alasoadura (1976) demonstrated that, during the first 72 h of growth on OMA, the hyphae of ***L. theobromae*** remained in a vegetative stage with hyaline hyphae, but later cells became dark-brown due to deposits of melanin-like pigment. Hyphal cells, whose cytoplasm appeared almost totally occupied by vacuoles containing electron-dense material, were seen in thin sections prepared from 3-6 day-old cultures. As in mature pycnidiospore (Yaguchi and Nakamura, 1991), the hyphae became dark pigmented due to deposits of electron-dense material that this fungus produces in large amounts.

The hyphae of ***L. theobromae*** are multinucleate in longitudinal sections, more than two nuclei were consistently observed. Due to the abundance of organelles, these nuclei were laterally located and in no case nucleoli were observed. The chromatin was uniformly distributed, in contrast to other fungi, e.i. ***Rhizoctonia solani*** Kuhn (Cedeño and Palacios-Prü, 1990), where the chromatin forms central and peripheral clumps. Abundant membranous elements were found in the cytoplasm, such as vacuoles and vesicles, all of which were related to the formation of electron-dense material, which in some vacuoles were seen fragmented and coagulated but preserving their homogeneity and low electron density. Cytoplasmic organelles, such as membranous bodies composed of three to six flattened cisterns in a stacked pile, and tubular membranous structures, were observed in the hyphae and are similar to those reported in ***R. solani*** (Cedeño and Palacios-Prü, 1990). The stacked pile of cisternae is similar to the non-identified lamellate structure reported in pycnidiospores (Yaguchi and Nakamura, 1991).

LM, SEM and TEM revealed that ***L. theobromae*** invaded all the elements of Caribbean pine sapwood, but it grew more abundantly in the rays. A large amount of thin hyaline hyphae, as well as thick, dark brown pigmented hyphae, were observed within the rays. These observations differ from those of Olofinboba (1974), who reported that ***L. theobromae*** produced two kinds of hyphae in ***Antiaris africana***: one thin and hyaline hyphae mainly located in the medullary rays and a second thick, dark pigmented hyphae growing predominantly in vessels, tracheids and fibers. Our investigation showed that this fungus developed only one kind of hyphae, since thinner, hyaline hyphae appeared to be terminal or lateral branches of thicker, dark brown hyphae. The fungus adapted it's direction of growth according to it's location in the wood. In those elements where ***L. theobromae*** grew abundantly, such as in the rays, numerous, longitudinally orientated hyphae were observed. The infection by ***L. theobromae*** caused staining and mechanical damages, such as distention and breakage, which seemingly weakened the anatomical structures. These results partially support those published by Findlay and Pettifor (1939) and Findlay (1959), who showed that ***L. theobromae*** caused a reduction in strength and the toughness, and decreased the bending strength in tropical hardwood of low density. However, these findings are opposed to those of Lambeth *et al.* (1989) who published that the blue discoloration induced by fungi usually does not affect the strength and the mechanical properties of lumber. The deep-seated penetrating stains are caused by fungi having dark-coloured hyphae which produce staining pigments that diffuse into the cell walls of the wood (Findlay, 1959). Our observations suggest that the blue staining symptom is due to the dark- coloured hyphae invading the host tissues plus the electron-dense substance deposited on the wood cell wall. Brown hyphae of ***Ceratocystis*** spp. also produce a bluish staining in conifers (Nelson, 1934).

ACKNOWLEDGMENTS

This work was partially financed by CDCHT-ULA grant M-400-92. We express our gratitude to Mauro Nieto, José Ramírez and Francisco J. Durán for their technical assistance during this investigation.

REFERENCES

Alasoadura, S.O. (1970): Culture studies on ***Botryodiplodia theobromae*** Pat. *Mycopath. Mycol. Appl. 42*: 153-160.

Cedeño, L., and Palacios-Prü, E. (1990): Ultraestructura de ***Rhizocotonia solani*** Kuhn patógeno de ***Phaseolus vulgaris*** L. var. 'seminole' en Venezuela. *Turrialba 40*: 356-367.

Cedeño, L., and Palacios-Prü, E. (1992): Identificación de ***Botryodiplodia theobromae*** como la causa de lesiones y gomosis en cítricos. *Fitopatol. Venez. 5*: 10-13.

Cedeño, L., Mohali, S., and Palacios-Prü, E. (1992): Ultraestructura del hongo ***Botryodiplodia theobromae*** causante del manchado azul del pino caribe. Pag. 30. In: *Resúmenes de la XXXII Reunión Anual de la Sociedad Americana de Fitopatología, División del Caribe,* Septiembre 20-24, Mérida, Venezuela.

Díaz, C., and Salas, G. (1980): ***Lista de patógenos de las plantas cultivadas en Venezuela.*** *Centro de Investigaciones Agropecuarias de la Región Centro-Occidental, Araure, Portuguesa, Venezuela. 62 p.*

Ekundayo, J.A., and Haskin, R. H. (1969): Pycnidium production by ***Botryodiplodia theobromae***. II Development of the pycnidium and fine structure of the maturing pycnospore. *Can. J. Bot. 47*: 1423-1424.

Findlay, W.P.K. (1959): Sap-stain of timber. *Forestry Abstracts 20*: 1-7.

Findlay, W.P.K., and Pettifor, C.B. (1939): Effect of blue stain on the strength of Obeche (***Triplochiton scleroxylon***). *Emp. For J. 18*: 259-267.

Gibbons, I.R., and Grimstone, A.V. (1960): On flagellar structure in certain flagellate. *J. Biophys. Biochem. Cytol. 7*: 697-716.

Goos, R.D., Cox, E.A., and Stotzky, G. (1961): ***Botryodiplodia theobromae*** and its association with ***Musa*** species. *Mycologia 53*: 262-277.

Lambeth C., Osorio, G., and Osorio, L.F. (1989): Blue stain incidence in commercial plantations species in Colombia: Effects of storage time and debarking. Smurfit Cartón de Colombia. *Investigación Forestal. Research Report N° 122.* 6 p.

Lewis, R., Jr., and Van Arsdell, P.E. (1978): Vulnerability of water-stressed sycamores to strains of ***Botryodiplodia theobromae***. *Plant Dis. Rep. 62*: 62-63.

Mancini, R.F.N. (1989): Determinación del efecto del manchado azul sobre el blanqueo y la longitud de rotura de las pulpas al sulfito de ***Pinus caribaea*** var. ***hondurensis***. *Tesis de grado. Universidad de Los Andes, Facultad de Ciencias Forestales, Mérida, Venezuela. 69 p.*

Mullen, J.M., Gilliam, C.H., Hagan, A.K., and Morgan-Jones, G. (1991): Canker of dogwood caused by ***Lasiodiplodia theobromae***, a disease influenced by drought stress or cultivar selection. *Plant Dis. 75*: 886-889.

Nelson, R.M. (1934): Effect of blue stain fungi on southern pine attacked by bark beetles. *Phytopathol. Z. 7*: 327-353.

Olofinboba, A.O. (1974): Sap stain in ***Antiaris africana***, an economically important tropical white wood. *Nature (UK) 249*: 860.

Pawsey, R.G. (1968): Observations on blue-stain and pin-hole beetles, and their control, in unpeeled logs of ***Pinus caribaea*** in Trinidad. *Commonwealth Forestry Review 47*: 211-224.

Punithalingam, E. (1976): ***Botryodiplodia theobromae***. Descriptions of pathogenic fungi and bacteria N° 519. *Commonw. Mycol. Inst.*, Kew, England.

Punithalingam, E. (1980): Diseases attributed to ***Botryodiplodia theobromae***. Pat. *Bibl. Mycol.* 123 p.

Reynolds, E.S. (1963): The use of lead-citrate stain in electron microscopy. *J. Cell Biol. 17*: 208-212.Spurr, A.R. (1969): A low viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastr. Res. 26*:31-43.

Sutton, B.C. (1980): The Coelomycetes: Fungi Imperfecti with pycnidia, acervuli and stromata. *Commmonw. Mycol. Inst.*, Kew, England. 696 p.

Uduebo, A. E. (1975): Fine structural studies on the pycnidiospores of ***Botryodiplodia theobromae*** Pat. *Ann. Bot. 39*: 605-610.

Yaguchi, Y., and Nakamura, S. (1991): Fine structure of walls of pycnidiospores in ***Lasiodiplodia theobromae*** Pat. Tokio University of Agriculture. *Scientific Report 35*:283-289.