Conidiogenesis of Marssonina panattoniana and its Potential as a Serious Postharvest Pathogen of Lettuce

H. E. Moline and Flora G. Pollack

Research Plant Pathologist, Horticultural Crops Marketing Laboratory, Agricultural Marketing Research Institute, Agricultural Research Service; and Mycologist, Plant Protection and Quarantine Programs, APHIS, respectively, U.S. Department of Agriculture, Beltsville, Maryland 20705.

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ABSTRACT

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The symptoms of a destructive leaf disease of lettuce (*Lactuca sativa*) which appeared in marketing channels, and the morphological characteristics of the causal fungus (*Marssonina panattoniana*) on the host and in culture are described. The disease is characterized by yellow to brown sunken lesions on the veins, and shot-hole lesions on the leaf blades. Harvested mature lettuce inoculated with a conidial suspension showed symptoms of infection after 10 to 17 days at 7 to 18 C. Light and scanning electron microscope studies revealed the absence of acervuli and the presence of annellations on the conidiogenous cells (conidiophores), raising doubt as to the taxonomic position of the fungus in the genus *Marssonina*.

Additional key words: Fungi Imperfecti, annellations, taxonomy.

Since its description in 1895 (5), epiphytotics of the fungal disease of lettuce caused by *Marssonina panattoniana* (Berl.) Magn. have caused extensive losses (3, 10, 13). During cold and wet weather, the fungus can severely damage young seedlings or mature plants in the field. The disease is widespread in temperate areas around the world. Its common names are "anthracnose", "shot-hole", "leaf perforation", or "rust" in the United States and "ring spot" in England, Australia, and New Zealand.

The etiology of the disease and methods for its control have been investigated (6, 8, 18). We undertook to study the behavior of an isolate of *M. panattoniana* under storage conditions on mature heads of harvested lettuce and to study the morphology of the causal fungus. The impetus for this study was a shipment of Romaine lettuce from Salinas, California, which was destined for the Washington, D.C., area fresh markets. The material was intercepted by agents of the Fresh Fruits and Vegetables Branch, Agricultural Marketing Service, U.S. Department of Agriculture (USDA) forwarded to us at Beltsville.

MATERIALS AND METHODS

Samples of decayed lettuce from a rail shipment that contained 30-40% unmarketable heads were received for diagnosis. Leaves were covered with lesions bearing masses of white conidia.

Conidia from these lesions were transferred to potato-dextrose agar (PDA) plates, which were then placed in incubators at 2, 7, 13, and 18 C in continuous darkness, and at room temperature under normal light conditions. Leaf tissue containing lesions was free-hand sectioned, stained, and examined with the light microscope. Examinations were made in lactophenol-cotton blue, KOH-phloxine, and distilled H_2O. The isolate was compared with the fungus in collections of lettuce anthracnose in the herbarium of the National Fungus Collections, USDA.

In the first test, Florida-grown Romaine lettuce was inoculated with suspensions of conidia washed from infected leaves following the procedure of Couch and Grogan (8). The spore suspensions (30-40 ml) were atomized onto each of 12 heads of lettuce. Three heads were sprayed with distilled water as uninoculated checks and three unsprayed heads served as dry checks. All were stored at 7 C for 1 week in perforated plastic bags which maintained a saturated environment and then moved to 18 C.

Thirty heads of California-grown Romaine, iceberg-type, head lettuce, and Florida-grown endive were inoculated with a conidial suspension that was prepared from mycelial mats grown on PDA. The mats were chopped in distilled water, and the mixture was strained through cheesecloth. Iceberg lettuce was split in half so that leaves of different ages would be exposed to the pathogen. Lettuce and endive were placed in perforated plastic bags, two heads per bag, and three bags of each kind of produce were stored at 18, 13, and 7 C; six bags of each were stored at 2 C. One lot of each type of produce stored at 2 C was removed to 18 C at 10 days, and each week thereafter, for observation of symptoms.

Three detached leaves of lettuce cultivar Great Lakes were wrapped around each of six infected heads of Romaine lettuce. Rubber bands were used to assure good contact. These were also stored at 18, 13, 7, and 2 C. Six

noninoculated checks also were stored.

Growth characteristics of the fungus were evaluated on PDA, Tochinai agar (21), and lettuce agar. To prepare the latter, we ground 500 g of Romaine lettuce in distilled water, expressed the sap through cheesecloth, added 15 g of Difco Bacto agar, and enough water to bring the liquid to 1 liter, then sterilized the mixture.

Lesions were cut from midribs of infected leaves and prepared for examination by scanning electron microscopy. Tissue blocks 2-3 mm square were fixed in aqueous 4% glutaraldehyde overnight, rinsed with two changes of distilled water, and dehydrated through a graded ethanol series. The tissues were then critical-point dried (2), coated with gold-palladium, and examined with a Hitachi SEM 2 scanning electron microscope.

Another group of lesions removed from midrib tissue and blocks of agar containing the fungus were fixed in 4% glutaraldehyde buffered with sodium cacodylate (0.01 M, pH 7.2), rinsed, postfixed in 2% aqueous osmium tetroxide, and embedded in Spurr plastic. Sections 1 μm thick were cut with glass knives, stained with Paragon stain, and examined with the light microscope.

RESULTS AND DISCUSSION

Pathogenicity tests.—Initially, lesions on the leaf midrib were small, water-soaked sunken areas which elongated to 5-8 mm in the direction of the long axis of the leaf. As lesions enlarged they became elliptical, straw-yellow to bright-yellow to brown, and eventually coalesced to form a continuous series of lesions along the midrib (Fig. 1). The older lesions became white with a distinct brown margin as conidiophores and conidia formed. The margin was beyond the outermost region of mycelial growth indicating that the fungus may ramify through cells killed by fungal metabolites or by host response to fungal infection. Lesions were circular or angular on interveinal areas of the leaf blade. Finally, these areas dried and dropped out, leaving a perforated leaf with the shot-hole symptoms that have been described (6, 10). Shot-hole symptoms may be indicative of a host reaction that limits the advance of the pathogen by death of infected cells.

Abundant conidial production was associated with lesions on major veins and the midrib. This abundance contrasted with the sparsity of conidial development reported earlier (18); however, other reports (6, 8) indicate that conidia were recovered from shot-hole areas after incubation of infected leaves in moist chambers. We also recovered conidia from such areas, but found that the large midrib lesions were a better source.

Symptoms of lettuce anthracnose were observed on 11 of 12 heads in the first test of Romaine lettuce after 11 days. The Romaine and Iceberg lettuce stored at 18 and 13 C in the second test had symptoms on inner leaves after 10 days, whereas those heads stored at 7 C developed symptoms 17 days after inoculation. In all instances, symptoms included numerous elliptical lesions on midribs and veins, and shot-hole perforations of leaf blades.

Fig. 1. *Marssonina panattoniana* lesions on the midrib and blade of Romaine lettuce.

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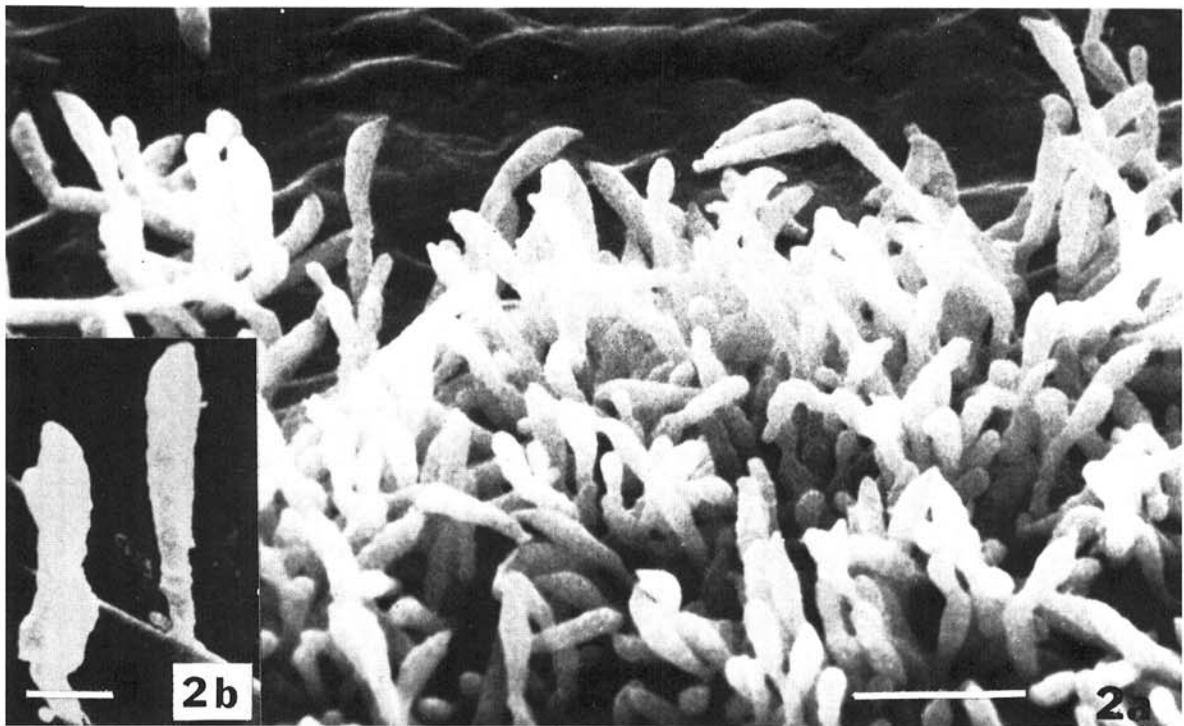
Fig. 2-(a, b), 3, 4-(a to c), 5, 6. Scanning electron micrographs of *Marssonina panattoniana* lesions on Romaine lettuce midrib tissue. **2a)** Mass of conidiogenous cells and conidia produced above the cuticle (bar = 10 μm). **2b)** Conidiogenous cell bearing a conidium has grown through the cuticle (bar = 5 μm). **3)** Low magnification micrograph of lesion showing border of lesion (arrows), cracking of dead host tissue at center of lesion, and a white mass of conidiogenous cells bearing conidia on the surface of the cuticle (bar = 50 μm). **(4a-c)** Annellations on conidiogenous cells (bar = 1 μm). **4a)** A single distinct annellation (A) at the tip of a conidiogenous cell with a very young developing conidium (C) at its apex. **4b** One distinct annellation (A) and another developing as a conidium (C) enlarges at the apex of the conidiogenous cell. **4c)** Conidiogenous cell showing two annellations, conidium (C) partially detached, and new growth of the conidiogenous cell (CG). **5)** Cluster of conidia produced from a single conidiogenous cell (CG); note the truncate bases of conidia (arrows), their distinct pointed apices, and appearance of their being held together by a mucilagenous exudate (bar = 2 μm). **6)** Truncate apex (arrow) of a conidiogenous cell with a dome-shaped protrusion bearing the conidium (C) (bar = 1 μm).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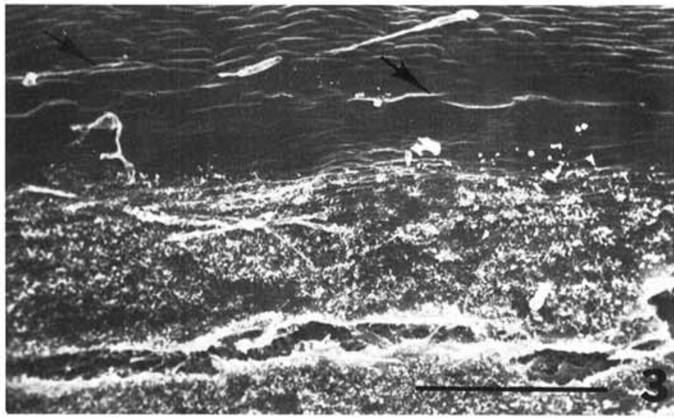
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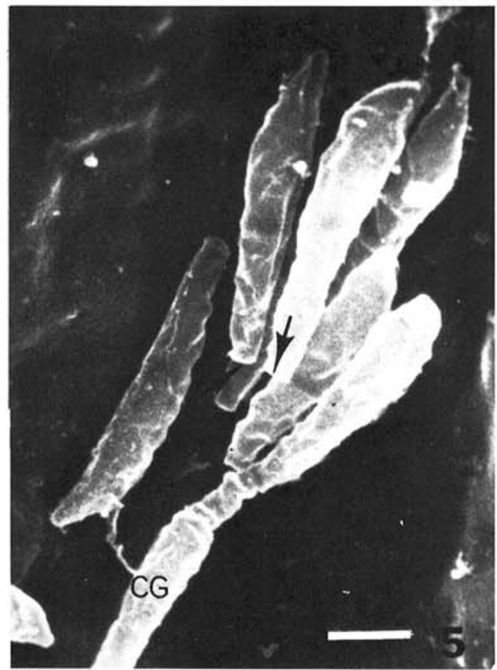
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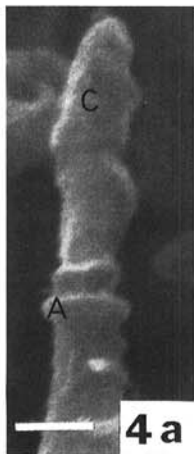
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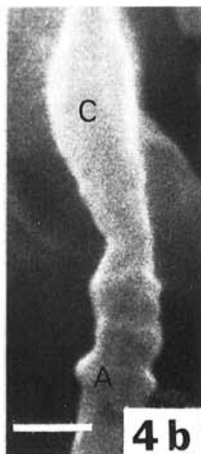
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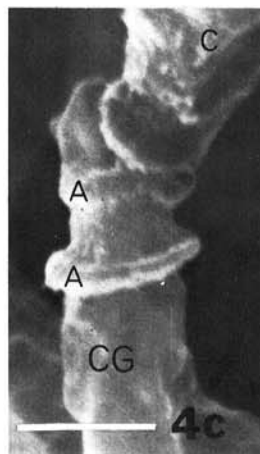
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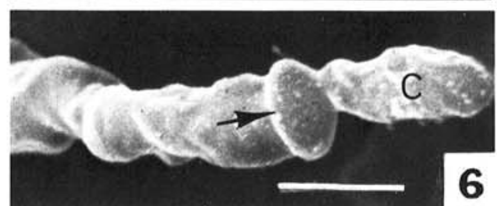
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Lettuce held at 2 C had symptoms of infection after 6 weeks of storage. Those heads that had been moved from 2 to 18 C at 10 days after inoculation and those moved from 2 C at weekly intervals thereafter developed symptoms after 4 days at 18 C. No lesions were found on any uninoculated checks.

Appearance of the disease symptoms on previously healthy leaves that had been placed adjacent to lesions on infected heads showed that infection may spread to healthy heads in transit. Outer leaves infected in this manner could be removed, but the possibility remains that once established infection can spread from leaf to leaf throughout the head.

Lettuce anthracnose infection of endive was not observed at any of the incubation temperatures. Field infection of endive has been reported, but not confirmed (10). Numerous brown lesions were seen on young leaves of inoculated endive, but became water-soaked and indistinct spreading over entire leaves, and only bacteria could be isolated from them. Stevenson (18) warned of the similarity of anthracnose lesions to other types of injury. We concluded, on the basis of the 24 heads of endive inoculated, that this isolate does not infect *Cichorium endivia* L.

Description and taxonomic status of the fungus.—The most rapid growth of the fungus in culture was at 18 C and at room temperature, with growth limited to 30- to 35-mm radius after 2 months. This slow growth habit was consistent with that reported for the organism (8, 18). Colony color varied from pale flesh pink on lettuce and Tochinai agar to Japan rose on PDA (Plate VIII of 16). Colonies on PDA were compact, wrinkled, and puckered with no aerial mycelium. Conidia were produced in great abundance, and formed moist masses over the surface and throughout the agar. Growth habit on lettuce agar was similar, with abundant conidial production. Growth on Tochinai agar was of the spreading type. Budding conidiophores produced long chains of bulbous cells throughout the agar, but did not produce typical conidia. A culture, designated ATCC 32199, has been deposited in The American Type Culture Collection.

The fungus on lettuce showed heavy, but diffused, sporulation over the lesions (Fig. 2). Conidiophores and conidia formed on the surface of the lesions singly (Fig. 2-a), in small clusters, or in large white masses. Other workers (6, 11, 13, 18) have reported pink masses of conidia. Appel and Laibach (3) reported that groups of conidiophores broke through the epidermis and formed whitish centers in the lesions. Brandes (6) described acervuli; Dandeno (9) conversely called the fungus a hyphomycete because of the lack of organization of the fructification into a structure that could be called an acervulus. Scanning electron micrographs (Fig. 2, 3) show that conidiophores and conidia formed on the surface of collapsed tissue rather than by rupturing through the epidermal layer. Ruptures in the lesions (Fig. 3) resulted from modifications of the tissue due to dying, collapsed cells and invading hyphae. Hyphal strands parallel to the surface grew through the cuticle and epidermis (Fig. 7-b) and formed a hyphal weft, or eustroma (Fig. 8) consisting entirely of fungal hyphae (20). Any cell in such strands was able to produce conidiophores regardless of its position in the host. The hyphae finally penetrated the deeper tissue, and strands appeared to follow the cell walls of the mesophyll (Fig. 9). These submerged hyphal cells also produced conidiophores and conidia.

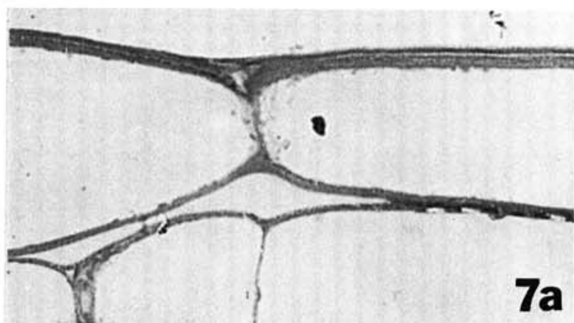
Conidiophores and conidia on the host and in culture were similar. Conidiogenous cells were ampulliform (1) having short tapered apices (Fig. 10) which became elongated and annellated (14) (Fig. 4, 11-a, b) with the production of successive conidia. Up to four annellations (Fig. 11-a) were seen, indicating that four conidia had been formed and had seceded. The apex of the conidiogenous cell elongated as it produced a sequence of conidia (Fig. 5). Conidiogenous cells were 3.0-3.5 μm wide at their largest diameter and up to 12 μm long. We observed, on the host and in culture, many conidiophores which consisted of two or three segments, each shaped like a conidium which had not seceded, through which hyphae had grown and which then functioned as other conidiogenous cells (Fig. 14).

Conidia were hyaline, smooth-walled, and curved, each had an apical bend or small hook and a truncate base (Fig. 12), which was widest in the first conidium formed on the conidiophore. Subsequent conidia had narrower bases. Conidia were formed singly from successive apices, which were produced by growth of the conidiogenous cell through its apex. Mature conidia consisted of two cells (Fig. 13); the upper cell was broader and slightly longer than the lower, which was tapered and connected with the truncate conidiogenous cell (Fig. 6). Detached conidia also appeared to be capable of replicating conidia. They were often constricted at the septum, and had up to four guttules. A few conidia had two septa. One-celled conidia were 8-11 $\times$ 2-3 μm, whereas two-celled conidia were 12-18 $\times$ 3-5 μm.

In 1895, Berlese (5) named the organism *Marsonia panattoniana* and in 1896 Selby (17) sent a specimen from Ohio to Ellis and Everhart who, unaware of the earlier name, called the fungus *Marsonia perforans*. In 1906, it was renamed *Didymaria perforans* (Ellis and Everhart) Dandeno (9). Finally it was named *Marssonina panattoniana* (Berl.) Magn. (15) when it was discovered that the original generic name *Marsonia* had been used for a plant genus and was not available for the fungus genus.

Observation by both the light- and the scanning electron microscopes, showed our isolate to have the characteristics of a hyphomycete. Fructifications were →

Fig. 7-(a, b), 8, 9, 10, 11-(a, b), 12, 13, 14. Light micrographs of *Marssonina panattoniana*. **7a)** Noninfected Romaine lettuce epidermal tissue showing normal cuticle ($\times$ 1,520). **7b)** Infected epidermal tissue; longitudinal section through midrib showing hyphal penetration of cuticle (arrows) ($\times$ 1,520). **8)** Transverse section through leaf midrib showing hyphal growth in cuticle and fruiting on the surface ($\times$ 3,000). **9)** Transverse section through mesophyll cell showing hyphal growth along cell walls ($\times$ 1,520). **10)** Young ampulliform conidiogenous cell (arrow) with another developing laterally ($\times$ 4,000). **11a)** Conidiogenous cell that has shed its conidium, with attenuated tip bearing annellations (arrow) ($\times$ 4,500). **11b)** Annellated conidiogenous cell bearing a developing conidium (arrow) ($\times$ 5,000). **12)** Septate conidia showing truncate base and beaked apex ($\times$ 3,800). **13)** Septate hyaline conidia ($\times$ 1,800). **14)** Cluster of conidiogenous cells showing percurrent growth habit observed when conidia do not dehisce ($\times$ 5,000).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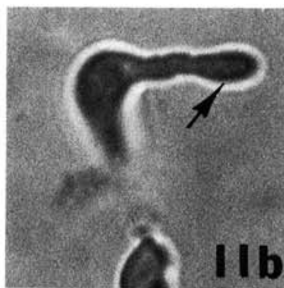
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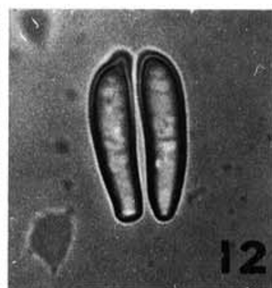
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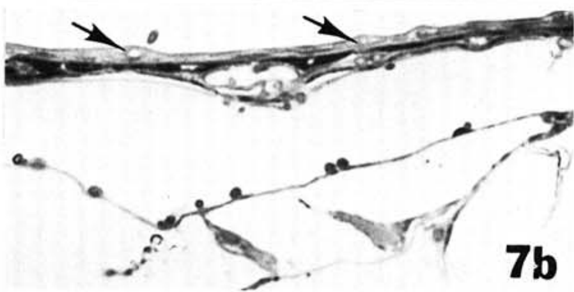
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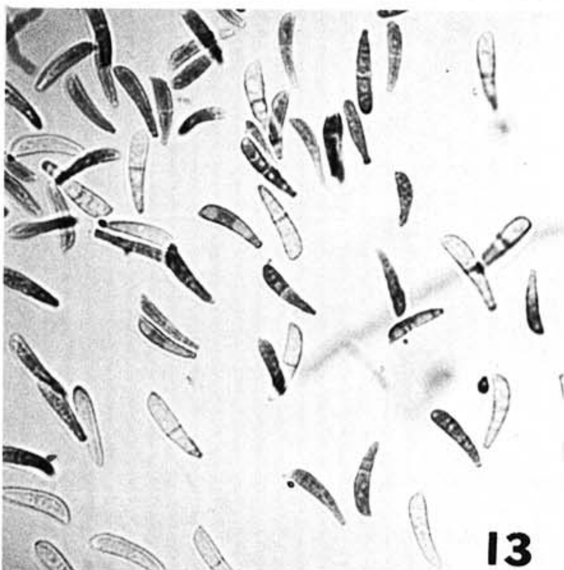
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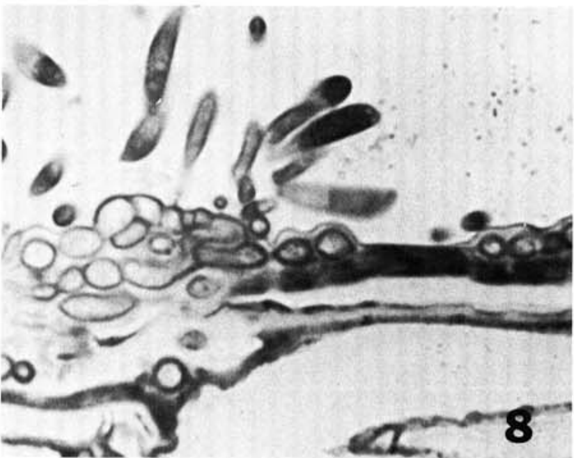
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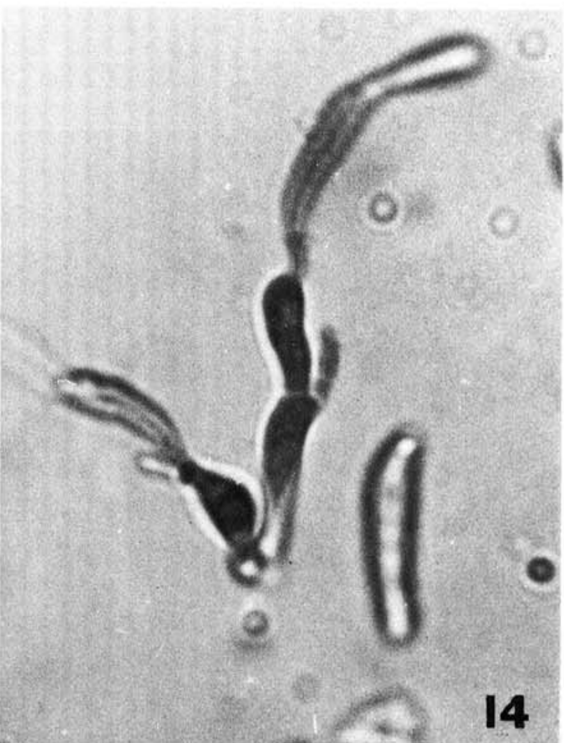
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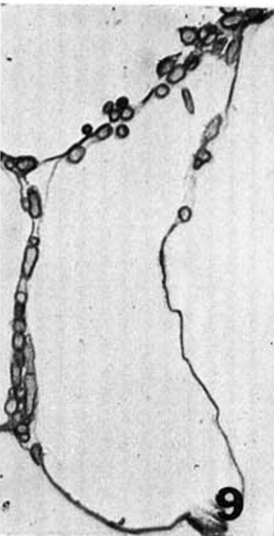
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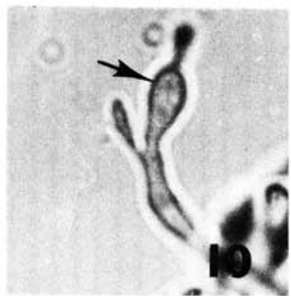
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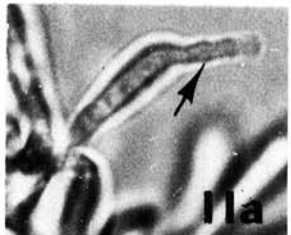
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too diffuse to be considered acervular, not typical of the Melanconiales as reported by Brandes (6). Conidiophores occurred singly, in small clusters, or in masses. Hyphal cells ramified through the cuticle, epidermis, and mesophyll, and any of these cells appeared to be capable of producing conidiophores in the same way as *Rhynchosporina* von Arx (4) and *Rhynchosporium* (7). Sutton (19, 20) and Hughes (12) emphasized that the taxonomic value of fructifications in the Deuteromycetes is less significant than formerly believed and have placed greater emphasis on conidiogenesis. The conidiogenous cells of our isolate of *Marssonina panattoniana,* were annellidic and not phialidic as reported for other species of *Marssonina.* Sutton (19) reported that conidia of *Marssonina* spp. are produced from simple phialides, and von Arx (4) supported this, saying *Marssonina* conidia are borne on rounded or conical phialides. The morphological characteristics, thus, raise doubts about the proper placement of this species in the genus *Marssonina.*

If scanning electron microscope studies provide support for the light microscope studies of Sutton (19, 20) and von Arx (4) on the presence of phialides in the genus *Marssonina, Marssonina panattoniana* will have to be placed in another genus.

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