Parasitic fungi Sclerotiniaceae: morphology and ultrastructure

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The parasitic fungi Sclerotiniaceae Botrytis cinerea from Rosa spp. flowers, Botrytis paenaeae from Paeonia officinalis flowers, Botrytis tulipae from Tulipa gesneriana leaves, Dumontinia tuberosa from Ranunculus ficaria root, Monilinia fructigena from Malus domestica fruits, Monilinia laxa from Prunus domestica fruits, Monilinia linhartiana from Cypripedium oblonga fruits and Sclerotinia sclerotiorum from Daucus carota ssp. sativus root were studied regarding morphological and ultrastructural characteristics of sporulation and/or sclerotia.

The Botrytis species cause grey mould of plants and produce abundant grey mycelium and long, branched conidiophores that have ovoid and one-celled conidia with protuberances on cell wall. The cell wall of conidia has two layers and appears dark until the sclerotia are formed from hyphae surrounded by a matrix of glucan.

The Dumontinia tuberosa species has large black sclerotia with 1-2 (3) black, stipitate and cupulate apothecia. The asci are cylindrical and contain eight unicellular, uniseriate, hyaline, ellipsoid and smooth ascospores.

The Monilinia species are the cause of brown fruit rot of apples, pears, plums, quinces etc. and these species produce pustules of conidia formed in chains.

Sclerotinia sclerotiorum causes white mould in numerous cultivated and wild plant species and produces mycelium and sclerotia formed by the hyphae components embedded in an extracellular glucan matrix.

Keywords: electron microscopy; light microscopy; nutritive medium; sporulation; sclerotia; ultrastructure

1. Introduction

The Sclerotiniaceae family comprises pathogenic or saprobic fungi on various plant parts [1] and these species form sclerotia and produce stalked apothecia which grow from stromata (sclerotium) located within the colonized host plant tissue. The sclerotium is a food storage organ and is usually differentiated into two parts, a rind (cortex) of dark, thick-walled cells and a medulla of hyaline cells [2]. Apothecia are black, stipitate and cupulate and contain a hymenial layer with rows of asci and paraphyses. Ascospores are large or small, ellipsoid, usually asperate, hyaline, ellipsoid and smooth ascospores.

The Botrytis genus comprises over 20 species [3] which produce gray mold on vegetables, ornamentals, fruits, and some field crops worldwide [4]. The members of this genus include Botrytis cinerea Pers., Botrytis allii Munn, Botrytis fabae Sardina, Botrytis paenaeae Oudem., and Botrytis tulipae (Lib.) Lind [5]. All species of Botrytis form sclerotia which may, depending on the isolate and cultural conditions, differ in size and shape [6]. Moreover, Botrytis species form gray mycelium and long and branched conidiophores, that have ovoid and one-celled conidia [2,7]. The Botrytis conidia appear dark because of melanin, which protects the spores against enzyme action and probably UV [8].

The Dumontinia genus is a member of Sclerotiniaceae family and it comprises only one species: Dumontinia tuberosa (Bull.) L.M. Kohn [1, 9]. This species is rare [10] and is associated with black rot disease [11,12].

Monilinia species are widely distributed plant pathogens and are classified among economically the most important pathogens [13]. The Monilinia genus comprises important species such as Monilinia fructigena Honey, Monilinia laxa (Aderh. & Ruhlland) Honey, Monilinia linhartiana (Prill. & Delacr.) Dennis. These pathogens are the cause of brown fruit rot of apples, pears, plums, quinces etc. The Monilinia species produce pustules of conidia formed in chains [2,4,14].

The genus Sclerotinia is the designated type genus of the Sclerotiniaceae family and it includes widespread and important phytopathogenic species such as Sclerotinia sclerotiorum (Lib.) de Bary, Sclerotinia minor Jagger and Sclerotinia trifoliorum Eriks. [1]. The species of Sclerotinia readily produce sclerotia on infected plant material and in culture. A variety of morphological and molecular characters are allow to differentiate the three plant pathogenic species of Sclerotinia: Sclerotinia sclerotiorum, Sclerotinia minor, and Sclerotinia trifoliorum [15].
2. *Botrytis cinerea*

*B. cinerea* causes serious pre- and postharvest diseases named gray mold in more than 200 plant species, including agriculturally important crops and harvested commodities, such as grapes, tomatoes, strawberries, cucumbers, bulb flowers, cut flowers and ornamental plants [16]. The broad host range of *B. cinerea* results in great economic losses, not only during growth but also during storage and transportation of products [17]. Necrotrophs kill their host cells by secreting toxic compounds or lytic enzymes and also produce an array of pathogenic substances that can subvert host defenses [18].

Rose gray mold occurs on leaves or flower buds of plants. The *B. cinerea* fungus isolated from *Rosa* spp. flowers produces gray mycelium with long conidiophores and numerous one-celled conidia (Figure 1A). The mycelium grows and invades the tissues which become covered with a whitish-gray mold. The surfaces of dry *B. cinerea* conidia and other *Botrytis* spp. had many short protuberances (Figure 1B). Hydration and redrying causes the disappearance of these protuberances [6, 19-21].

The *B. cinerea* conidium ultrastructure presents a regular cell wall with a two-layer structure, plasmalemma, and cytoplasm matrix with nucleus, mitochondria and vacuoles. The cell wall external layer is thin and electron dense and the inner one is thick, uniform and less electron dense [20-22]. Among cell organelles, mitochondria are numerous, usually ovoid and medium electron dense. Vacuoles are similar in size to mitochondria [6, 23].

![Figure 1](image1.png)

**Fig. 1** *Botrytis cinerea* conidium: A. Light microscopic view of a conidiophore (cp) with conidia (c); B. Scanning electron micrograph showing randomly positioned surface protuberances.

![Figure 2](image2.png)

**Fig. 2** *Botrytis cinerea* sclerotium: A. Scanning electron micrograph showing cortex (a) and medulla (b); B. Transmission electron micrograph of a cross section showing hyphal cells from the medulla embedded in a polysaccharide matrix (CW. cell wall; N. nucleus; C. cytoplasm; L. lipids; PM. polysaccharide matrix).

The *B. cinerea* fungus frequently produced black and irregular sclerotia with distinct layers at the surface of infected tissues and the fungus overwinters in this form. Transverse sections of *B. cinerea* sclerotia obtained from culture showed a cortex more compact than the medulla, with less extracellular matrix between the cells (Figure 2). The rind
cells had darkly pigmented septa. The medullary cells were embedded in a continuous polysaccharide extracellular matrix of β-glucans, uninterrupted by lacunae [7].

*B. cinerea* is difficult to control because it has a variety of modes of attack, diverse hosts as inoculum sources, and it can survive as mycelia and/or conidia or as sclerotia for extended periods in crop debris. In addition, *B. cinerea* produces a range of cell wall-degrading enzymes, toxins, and other low-molecular-weight compounds such as oxalic acid. New evidences suggest that the pathogen triggers the host to induce programmed cell death as an attack strategy [24]. *B. cinerea* strains are highly genetically and physiologically variable and several strains developed resistance to most of the fungicides used to control them [25-27].

### 3. *Botrytis paeoniae*

The *B. paeoniae* fungus produces gray mold on *Paeonia* plants [14], and it is widely distributed in Europe and America [28]. This parasitic fungus can survive as mycelia and/or conidia and sclerotia in peony plants [29].

The *B. paeoniae* fungus isolated from *Paeonia officinalis* flowers presents ovoid conidia with numerous, randomly positioned protuberances on the cell wall’s surface (Figure 3A) similar to those of *B. cinerea* [20-21]. The conidia showed the following features: a regular cell wall with a two-layer structure; plasmalemma; cytoplasm matrix with nucleus and nucleolus; mitochondria, etc. The cell wall’s external layer was thin and electrondense; the inner wall was thick, uniform, and less electrondense. The cytoplasm matrix (cytosol) was uniformly distributed, and the nucleus was ovoid or spherical. The mitochondria were numerous and usually ovoid and had an average electron density. The intracellular nutrient reserves were glycogen and lipids [29].

![Fig. 3](image-url) Scanning electron micrograph showing randomly positioned surface protuberances on *Botrytis* conidium: A. *B. paeoniae*; B. *B. tulipae*.

The *B. paeoniae* sclerotia showed the medullary hyphae components (cell wall, plasmalemma, cytoplasm, nucleus, lipid bodies, and glycogen) surrounded by a continuous polysaccharide extracellular matrix of β-glucans [29]. The internal structure and histochemistry of *B. paeoniae* sclerotia are similar to those of *B. cinerea* and *B. fabae*. The rind walls contain melanin pigments, the medullary hyphae are surrounded by a continuous matrix of β-glucans, and the intracellular nutrient reserves are protein, glycogen, polyphosphate and lipids [30].

### 4. *Botrytis tulipae*

Tulip fire or tulip gray mold is the most common and destructive disease to tulips, and is caused by the fungus *B. tulipae* [31,32]. The fungus attacks all parts of the tulip and can rapidly kill its host's tissue and continue growing on the dead remains [2]. *B. tulipae* produces abundant gray mycelium and long, branched conidiophores with one-celled, ovoid conidia. The conidiophores and clusters of conidia form a grape-like cluster [2,4].

The *B. tulipae* fungus isolated from *Tulipa gesneriana* leaves presented unicellular and ellipsoidal or obovoid conidia [31] with numerous randomly positioned protuberances (Figure 3B); however, these protuberances are fewer than those present in *B. cinerea* conidia [20, 21] and *B. paeoniae* [29]. The *B. tulipae* conidia present a regular cell wall with a two-layer structure; plasmalemma; cytoplasm matrix with nucleus and nucleolus; mitochondria, etc. The cell wall’s external layer was thin and electrondense; the inner wall was thick, uniform, and less electrondense (Figure 4). The cell wall of the conidia had 2 layers and appeared dark because of melanin (Epton and Richmond, 1980).

*B. tulipae* fungus penetrates tulip leaves and produced irreversible ultrastructural changes in epidermal and mesophyll cells. [33]. The infection of host plants by *B. tulipae* is mediated by numerous extracellular enzymes and
metabolites. Several cell wall-degrading enzymes contribute to the conversion of host tissue into fungal biomass, and other enzymes such as laccases and proteases are involved in pathogenesis [34].

**Fig. 4** *Botrytis tulipae* conidium: A. Transmission electron micrograph of an oblique section; B. A portion of an oblique section (detailed): CW. cell wall; C. cytoplasm; P. plasmalemma; M. mitochondrion; N. nucleus; Nu. nucleolus; L. lipids; ECW. external layer of cell wall; ICW. inner layer of cell wall.

5. **Dumontinia tuberosa**

*D. tuberosa* is a rare species [10] and it is pathogenic on rhizomes of *Anemone* [9,35], on *Hepatica nobilis* Schreb. var. *japonica* Nakai f. *magna* (M.Hiroe) Kitam. [12] and occasionally on *Ranunculus* plants [10].

**Fig. 5** *Dumontinia tuberosa*: A. Sclerotium (s) with apothecium (a); B. Colony on potato dextrose agar (PDA) with mycelium (m) and black sclerotia (s).

The *D. tuberosa* sclerotia found on *Ranunculus ficaria* L. root were black and formed 1-3 apothecia which were black, stipitate and cupulate (Figure 5A) and had about 2 cm in diameter [9]. On nutritive medium the fungus *D. tuberosa* formed unicellular microconidia, branched and multicellular hyphae and sclerotia which are white at first and later became black (Figure 5B).

The hymenial layer of apothecium has rows of asci and paraphyses (Figure 6). Asci were cylindrical (120-175 x 8-10 µm) and contain eight ascospores uniseriate, hyaline, smooth, ellipsoid, biguttulate, bi-nucleate to tetra-nucleate, of 11-16 (18) x 5-8 µm (Kohn, 1979).
Fig. 6  *Dumontinia tuberosa*: A. Light microscopic view of an ascus (a) with 8 ascospores; B. Scanning electron micrograph showing the smooth surface of an ascospore.

6. *Monilinia fructigena*

*M. fructigena* causes a common brown rot on apples, pears and the disease appear when the fruit approaches maturity as small, circular, brown spots that spread rapidly in all directions [4,36].

The mycelium produces chains of elliptical conidia on hyphal branches arranged in tufts (sporodochia) often in concentric zones on apples and pears [2,4]. The conidia are blastoconidia formed in chains which extend in length at their apices by budding of the terminal conidium. Occasionally more than one bud is formed, and this results in branched chains [2]. Conidia are hyaline and unicellular, elliptical to oval, arranged in chain in which the youngest conidium is at the chain base [14,37].

The *M. fructigena* sporodochia were isolated from *Malus domestica* fruits and its ultrastructural characteristics were studied. The conidium ultrastructure presented a regular cell wall with a two-layer structure, plasmalemma, and cytoplasm matrix with nuclei, mitochondria and vacuoles (Figure 7).

Fig. 7  *Monilinia fructigena* conidium: A. Transmission electron micrograph of an oblique section; B. A portion of an oblique section (detailed): CW. cell wall; C. cytoplasm; N. nucleus; P. plasmalemma; M. mitochondrion; L. lipids; ECW. external layer of cell wall; ICW. inner layer of cell wall.

The *M. fructigena* pathogen overwinters as mycelium in mummified fruit on the tree and in cankers of affected twigs or pseudosclerotia in mummies in the ground [4]. Brown rot is transmitted by conidia because the apothecial state is only rarely formed [2].

7. *Monilinia laxa*

*M. laxa* produces a brown rot of fruits and the disease occurs on a range of host plants, such as sweet cherry, plum, peach, nectarine, apricot and almond, as well as on some pome fruits [36,38]. The plums can be infected in all developmental phases as well as during transport and storage. The parasite penetrates into fruits through wounds on the fruit epidermis but infection can also occur at the point of contact between healthy and diseased fruits. The mycelium develops in fruit and produces sporodochia on their surface [37,39].

Our study revealed structural characteristics of sporodochia obtained from *Prunus domestica* fruits. The *M. laxa* conidia are arranged in chains on short conidiophores, have ellipsoidal shape (12-15x10 μm), are hyaline and unicellular.
and this conidial form is called *Monilia laxa* [14,36]. At ultrastructural level, the *M. laxa* conidia and conidiophores presented a regular cell wall with a two-layer structure, plasmalemma, and cytoplasm matrix with nuclei, mitochondria and vacuoles. The nuclei were spherical in shape and the cytoplasm matrix was uniformly distributed. Besides, the cell wall external layer is thin and electron dense and the inner layer was thick, uniform and less electron dense (Figure 8).

![Fig. 8](image)

*M. laxa* overwinters as a mycelium and conidia in attacked organs of the plant [14] and the disease determined by this fungus has a worldwide distribution [37, 40-42].

### 8. Monilinia linhartiana

The *M. linhartiana* species produces a brown rot on quince and the disease is specific only for this fruit species [43-45]. Symptoms caused by *M. linhartiana* are observed very early in spring mainly on leaves that are next to the flower. Infected leaves and fruits start to wilt and die, and the infection readily spreads on young fruits. Dried fruits turn into mummies that remain to hang on the branches [44].

The *M. linhartiana* mycelium develops in fruit and produces sporodochia with conidiophores and conidia arranged often in concentric zones on fruits of *Cydonia oblonga*. The conidia are one-celled, hyaline, limoniform, have smooth walls and disjunctors and are arranged in chains of up to 30 conidia [43]. The *M. linhartiana* fungus isolated from *Cydonia oblonga* fruits presented conidia with a regular cell wall with a two-layer structure, plasmalemma, and cytoplasm matrix with nuclei, mitochondria and vacuoles. The cell wall external layer was thin and electron dense and the inner layer was thick, uniform and less electron dense (Figure 9A).

The life cycle of monilia disease of quince established that the primary infection of young leaves is caused by ascospores from mummified fruit on the soil and secondary infection (flower infection) due to conidia produced on infected leaves [43]. The brown rot on quince is mentioned in different countries [36-37, 43-45].

### 9. Sclerotinia sclerotiorum

This species causes a range of diseases (*Sclerotinia* rot, white mould, stalk break) of numerous succulent plants, particularly vegetables and flowers resulting in significant economic loss in the field and postharvest. The most important crop plants affected are sunflower, soybean, oilseed rape, lettuce, cabbage, carrots etc. [2,4]. The white mould disease occurs worldwide and affects plants in all stages of growth (seeds, young plants, mature plants and harvested products) and the main symptom of disease is the appearance on the infected plant of a white fluffy mycelial growth in which soon afterwards develops sclerotia [4]. The *S. sclerotiorum* fungus may enter into plant cells through direct penetration, enzyme and toxin effects, mechanical pressure, or indirectly through wounds or natural openings such as lenticels or stomata [46]. The pathogen’s invasion mechanisms include producing the cell wall and middle lamella dissolving enzymes, toxins, enzymes to degrade host tissue and defense substances, and rapidity in host plant infection. *S. sclerotiorum* facilitates penetration and colonization of host plant tissues by secreting multiple pectinolytic enzymes [47]. Different studies present enzymes secreted by *S. sclerotiorum* and the role of oxalic acid as an important factor of pathogenicity in the infected plant tissues [48-51].
The S. sclerotiorum fungus isolated from Daucus carota ssp. sativus root was cultivated on a nutritive medium and was studied regarding ultrastructural characteristics of sclerotia (Figure 9B). The sclerotia obtained in culture were white at first but later became black [14]. The micrographs from the internal zone of S. sclerotiorum sclerotia showed the hyphae components (cell wall, lipid bodies, mitochondria, nucleus, plasmalemma, and vacuoles) connected by glucan and interhyphal space [22,52-53]. The fungus Sclerotinia sclerotiorum overwinters as sclerotia on or within infected tissues, as sclerotia in soil, and as mycelium in dead and living plants [4].

10. Conclusions
The parasitic fungi Sclerotiniaceae Botrytis cinerea, B. paeoniae, B. tulipae, Dumontinia tuberosa, Monilinia fructigena, M. laxa, M. linhartiana, and Sclerotinia sclerotiorum have specific morphological and ultrastructural characteristics of sporulation and sclerotia. This structural data may be used for disease diagnosis and evolutive therapy effect.

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References


