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# The interaction apparatus of *Asteridiella callista* (Meliolaceae, Ascomycota)

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***Abstract:*** We document here for the first time ultrastructural details of the cellular interaction of *Asteridiella callista* and its host *Stachytarpheta mutabilis* var. *violacea* from Costa Rica. *A. callista* attaches to the host with appressoria, invades the epidermal cell wall and forms an apoplastic complex cisternal net, presumably for nutrient uptake from its host. This unique structure, called an interaction apparatus (Ia), consists of cisternae surrounded by a membrane continuous with the fungal cytoplasmic membrane. Subsequently the apoplastic trunk of the Ia extends into the host epidermal cell wall and contacts the host cytoplasmic membrane. Electron-opaque material, probably of fungal origin appears at the host cytoplasmic membrane. Finally these electron-opaque deposits are encased by host material. Functional and systematical aspects of this interaction scenario are discussed.

***Key words:*** appressorium, cellular interaction, cisternal tubules, concentric bodies, Costa Rica, *Stachytarpheta mutabilis* var. *violacea*, Verbenaceae

## Introduction

Species of the Meliolaceae (Meliolales, Ascomycota), also known as black or dark mildews, are parasitic fungi of mainly tropical distribution. They infect foliage of members of numerous plant families. Species of this group are characterized by superficial, dark, thick-walled, branching hyphae with appressoria (capitate hyphopodia) and conidiogenous cells (mucronate hyphopodia); superficial, dark, perithecia containing clavate asci with thin walls; and asci typically containing 2–4 brown ascospores with four septa each (Hansford 1961, Kirk et al. 2008).

Communication between fungi and plants was the focus of numerous papers (see e.g. Yi and Valent 2013 and references therein). However, in Meliolaceae studies have focused predominantly on morphological characteristics and consequently few studies have analyzed details of their parasitic interactions. For example, Luttrell (1989) described microscopic structures of the interaction between *Meliola floridensis* Hansf. and *Persea borbonia* (L.) Spreng. (Lauraceae) and Mueller et al. (1991) reported on ultrastructural details of the interaction between *Meliola sandwicensis* Ellis & Everhart and the host *Kadua acuminata* Cham. & Schltdl., syn. *Hedyotis acuminata* (Cham. & Schltdl.) Steud. (Rubiaceae).

The objective of the present study therefore was to examine the ontogeny of structural characteristics in the cellular interaction between *A. callista* and its host. Our results provide evidence for the evolution of a unique interaction process in this biotrophic plant pathogen.

## Materials and methods

*Materials.*—Colonies of *Asteridiella callista* (Rehm) Hansf. were collected on leaves of *Stachytarpheta mutabilis* (Jacq.) Vahl var. *violacea* Moldenke (Verbenaceae) in Costa Rica, Puntarenas, Cantón Garabito, Distrito Tárcoles, National Park Carara, 9°46′N, 84°36′W, 30–50 m, 5 Jul 2011, by Carlos O. Morales & Délfida Rodríguez J.2324 (TUB, USJ). For accuracy the material was compared with the type material of *Asteridiella callista* (Rehm) Hansf. (BPI 693091). This species is recorded here for the first time for Costa Rica. It is known to infect members of Verbenaceae from Guyana, China, Ecuador, Grenada, Indonesia, Java, Philippines, Puerto Rico, Trinidad and Virgin Islands (Hansford 1961, Farr et al. 2013).

*Morphological analyses.*—Hyphae with appressoria of *A. callista* were observed with a Zeiss Axioskop 2 Plus microscope. Small sections of the colonies were cut with aid of dissecting microscope, and preparations were examined as slide mountings in water.

*Ultrastructural analyses.*—The ultrastructure was studied with a Zeiss EM 109 transmission electron microscope at 80 kV with a transfaseroptic camera. Sections of leaves 2 $mm^2$ with colonies of *A. callista* were cut in the field and immediately placed in vials containing 2% of the fixative solution of Karnovsky (1965) and stored until further processing. In the laboratory samples were transferred six

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times in 0.1M sodium cacodylate buffer, post-fixed in 1% osmium tetroxide in the same buffer for 1 h in the dark, washed in distilled water and stained in 1% aqueous uranyl acetate 1 h in the dark. After five washes in distilled water, samples were dehydrated in acetone, with 10 min changes at 25%, 50%, 70%, 95%, and three times in 100% acetone, embedded in Spurr's plastic (Spurr 1969) and sectioned with a diamond knife. Serial sections were mounted on formvar-coated, single-slot copper grids, stained with lead citrate at room temperature for 5 min and washed with distilled water.

## RESULTS

Hyphae and ascomata of *A. callista* grow only on the upper epidermis of the leaves of *S. mutabilis* var. *violacea* (FIG. 1). Our morphological and ultrastructural analyses of the interaction between the pathogen and the host showed that initially appressoria attached to epidermal cell walls (FIG. 2A). Within the appressoria, a complex structure developed (FIG. 2B–F) which is designated here as an interaction apparatus (Ia). This structure, which resembles roughly the interaction apparatus described by Bauer et al. (1997) for the Exobasidiales and by Simon et al. (2004) for the ascomycete *Cymadothea trifolii*, consisted of elongate cisternae that fused to form a trunk (FIG. 2A, B, E, F). Opposite the epidermal cell wall the trunk was limited by an electron-opaque line (FIG. 2B, D, E). Mitochondria were numerous in the vicinity of the Ia (FIG. 2C–F), and sometimes concentric bodies of unknown origin and function could be observed (FIG. 2F). The tubular system of the Ia was continuous with the fungal cytoplasmic membrane (FIG. 2B, D). Thus, the intracisternal space represented an apoplastic compartment. Within the apoplastic compartment of the Ia, electron-opaque granular particles were visible (FIG. 2D).

At this stage of the infection, structural changes were observed at the contact zone of the appressorium and the epidermis cell wall of the host. The apoplastic trunk of the Ia, accompanied by partial desintegration of the basal appressorial wall, formed a penetration pore (FIG. 2A, B, E) that extended through the cuticle and the thick cell wall of the host (FIG. 3A). Thus, the apoplastic trunk of the Ia contacted directly the host cytoplasmic membrane (FIG. 3D). Within the resulting apoplastic tubule, designated here as Ia-canal, membranes or components of the host cell wall could not be observed (FIG. 3A–D).

An electron-opaque tubular structure (in three-dimensional reconstruction from serial sections) within the epidermal cell wall finally surrounded the Ia-canal (FIG. 4A, B). Between the apex of the Ia canal and the host cytoplasmic membrane, electron-opaque deposits appeared (FIG. 3B). As a consequence, the host cytoplasmic membrane invaginated at this area

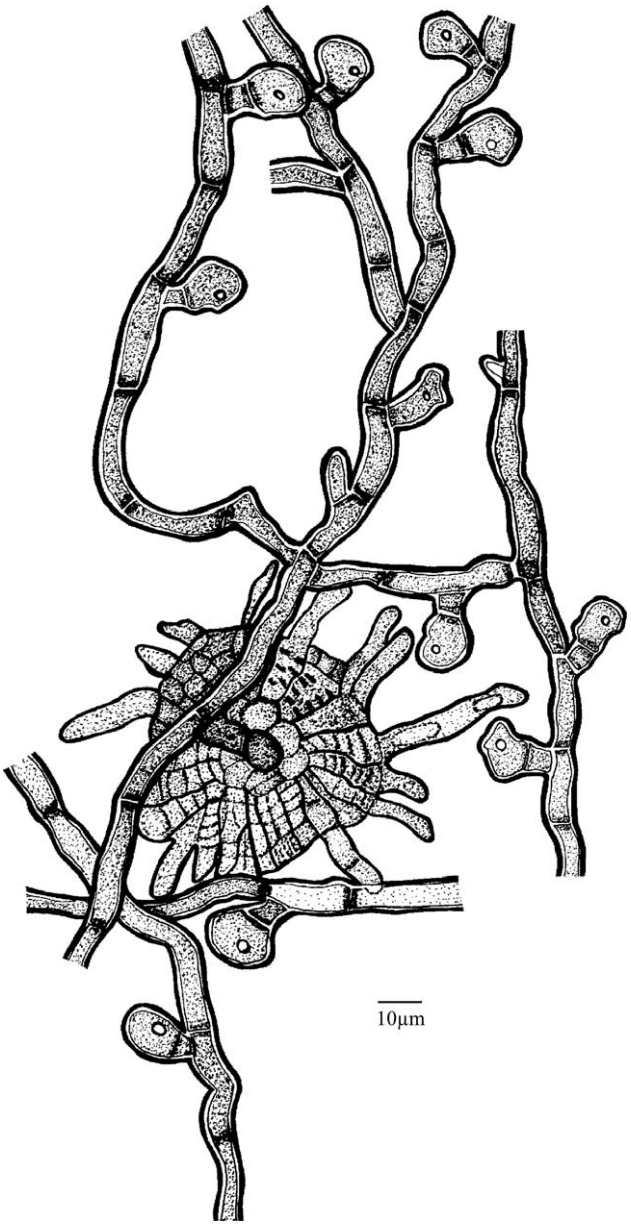


FIG. 1. Colonies of *Asteridiella callista* showing hyphae and appressoria. Bar = 10 µm.

(FIG. 3B, C). Finally, the host cell added wall appositional material of different electron density and unclear substructure to the site of interaction in response to the fungal attack (FIG. 3D). This appositional material led to the encasement of the original electron-opaque deposition, which thereafter separated from the cytoplasm and collapsed (FIG. 4A, B).

## DISCUSSION

Species of Meliolaceae have been considered fungi that inflict little damage on their hosts (Wellman 1972) and probably this is the reason that most

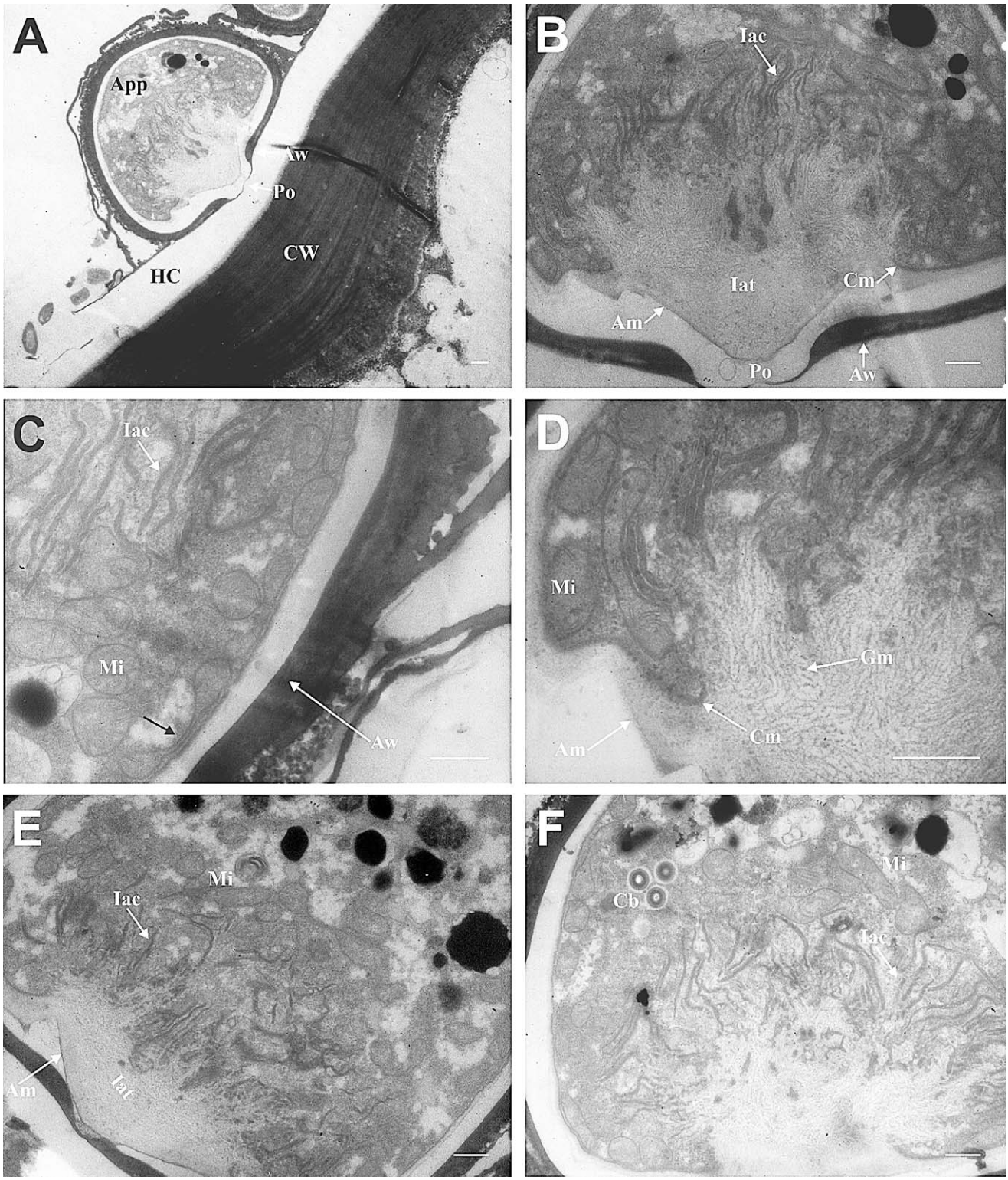


FIG. 2. Sections showing ultrastructural details of the interaction apparatus of *Asteridiella callista*. A. Overview of an attached appressorium (App) of *A. callista* located on the leaf surface of *Stachytarpheta mutabilis* var. *violacea* with an initial stage of a penetration pore (Po), appressoria wall (Aw), host cuticle (HC) and host cell wall (CW). B. Detail from A showing the interaction apparatus. Note that the Ia consists of a cisterna net (Iac), which is continuous with the cytoplasmic membrane (Cm) of the fungus. Penetration pore (Po), appressoria wall (Aw) and the trunk of the Ia are visible. Note that in direction to the host the trunk is limited by an electron-opaque line (Am). C. Enlargement of an appressorium showing the cisternal net (Iac) of the interaction apparatus. Note that numerous mitochondria (Mi) are located in the vicinity of the Ia. The appressoria wall is visible at Aw. D.

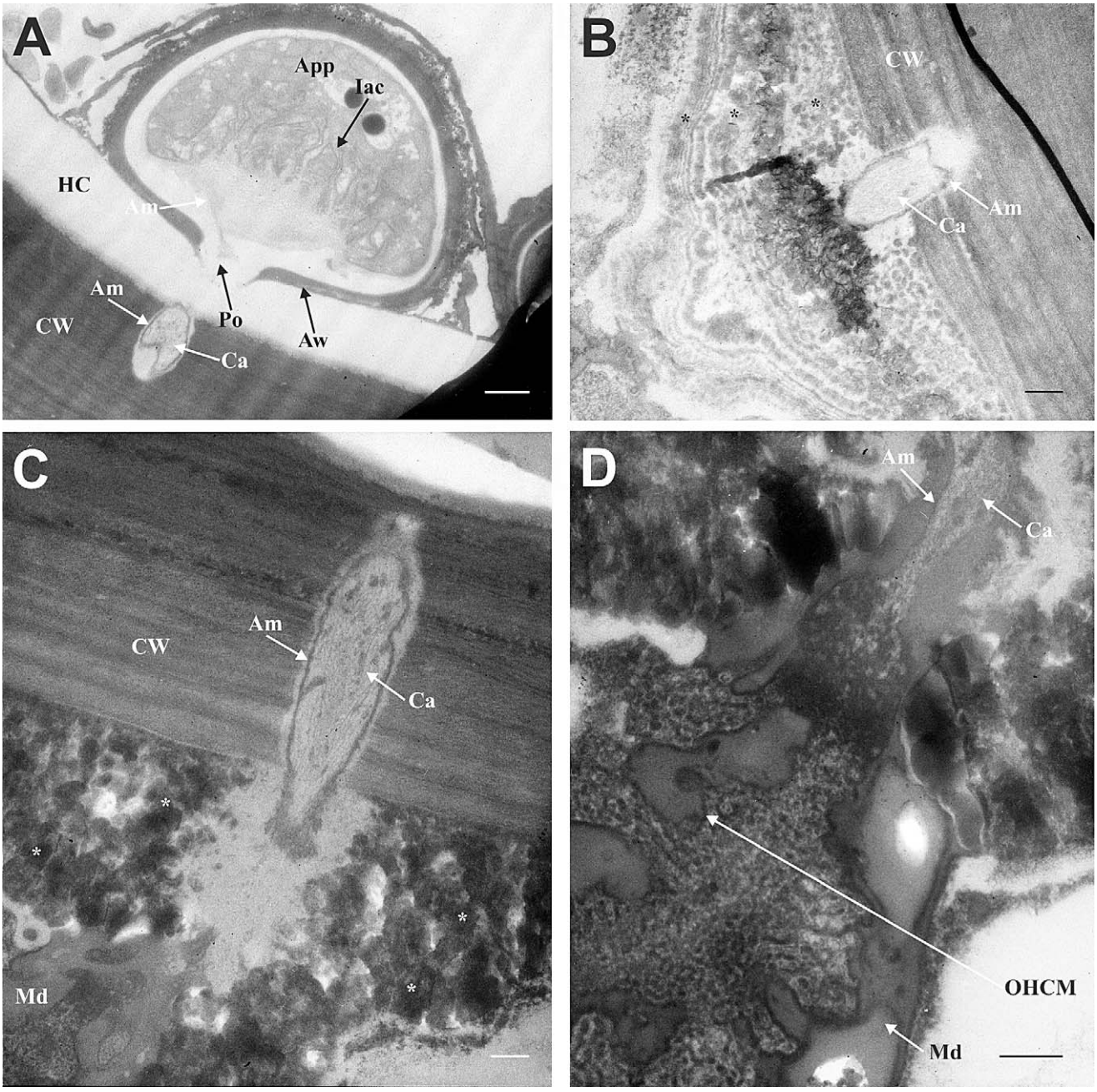


FIG. 3. Penetration of the host epidermal cell wall. A. Attached appressorium (App) showing the complex cisterna net (Iac), the apoplastic trunk (Iat) and the penetration pore (Po) of the Ia. The electron-opaque line limiting the apoplastic compartment of the Ia opposite the host is visible at Am. Note the canal (Ca) penetration the host cuticle (HC) and epidermal cell wall (CW). Note also that the canal is devoid of membranes. B. Terminal part of an interaction canal (Ca) with the electron-opaque margin (Am) within the host cell wall (CW). Note the electron-opaque material at the apex of the canal. Host response is visible at *. C. Terminal part of an interaction canal (Ca) within the host cell wall (CW) showing presumed fungal material (Md) at the vicinity. Host response is visible at regions marked with asterisks. D. Section showing the contact zone between the interaction canal (Ca) and the host cytoplasm. Note the presumed fungal material (Md) encased by the original host cytoplasmic membrane (OHCM). Note that the canal is limited by a fine electron-opaque line (Am). Bars: A = 1 μm, B–D = 0.5 μm.

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Apoplastic part of the Ia showing the granular material (Gm) within the Ia. Note that the Ia is continuous with the fungal cytoplasmic membrane (Cm). Mitochondria are visible at Mi. E. Detail of appressorium showing the apoplastic trunk (Iat) of the Ia. Mitochondria (Mi) are located in the vicinity of the Ia. Note that the trunk is limited by a fine electron-opaque line (Am). F. Section through an Ia showing the tubular net (Iac), concentric bodies (Cb) and mitochondria (Mi) in the vicinity of the Ia. Bars: A = 1 μm, B–F = 0.5 μm.

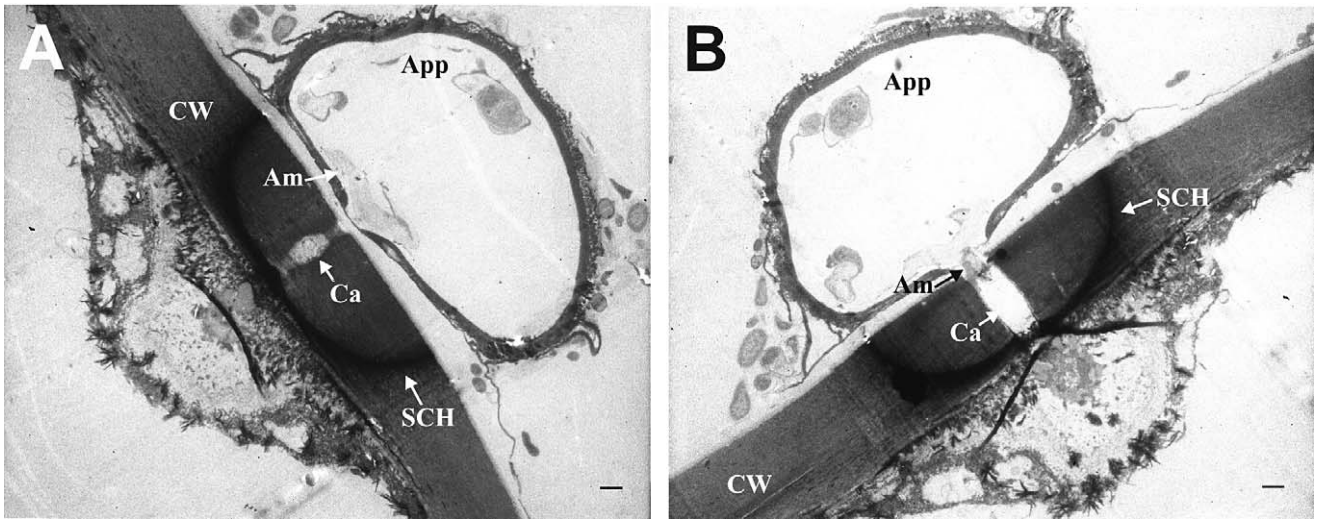


FIG. 4. Final step of the cellular interaction. A, B. Sections showing the structural change (SCH) in the host cell wall (CW) around the canal (Ca). Note that the appressoria (App) are empty at this stage. Bars: A, B = 1 μm.

studies of this group were restricted to hyphal and ascocarp morphology. Therefore, the goal of our study is to document the cellular interaction between a Meliolaceae species and its plant hosts.

Our results are diagrammed (FIG. 5) and they are discussed as follows.

*Asteridiella callista* invades only the host epidermal cells but not the host cytoplasm. The interaction apparatus (Ia) consists of branched and often net-like cisternae that protrude deeply into the fungal cell and that are continuous with the fungal cytoplasmic membrane. It is considered to be involved in the transfer of nutrients. Thus, the resulting apoplastic compartment, with its greatly enlarged surface area, resembles the wall ingrowths of transfer cells as illustrated by Pate and Gunning (1972). Mitochondria found in large numbers around the cisternae might provide the energy required for transport processes, a role suggested for the numerous mitochondria present near the wall ingrowths in transfer cells (Pate and Gunning 1972).

To our surprise, similar interaction apparatuses have been reported for the members of the basidiomycetous order Exobasidiales (Bauer et al. 1997) and the ascomycetous dothideomycete *C. trifolii* (Simon et al. 2004). In *C. trifolii* the Ia is located only in intercellular hyphae (Simon et al. 2004), whereas in the Exobasidiales species it is located in intercellular hyphae as well as in haustoria (Bauer et al. 1997). The fact that similar interaction structures are found in distantly related fungi may indicate their efficient funtional properties in nutrient transfer from the hosts to the parasites and thus explain why convergent processes have occurred. Although the function of the Ia in *A. callista* has not been studied exclusively, the enormous surface area of the cisternae that would allow a high rate of nutrient transfer supports the suggestion that this structure plays a dominant role in nutrient transfer. In addition, in *A. callista* the apoplastic trunk of the Ia forms a penetration pore that extends into the epidermal cell wall and directly contacts the host cytoplasmic membrane. Although the two interacting cells are in close contact, the cytoplasm of the appressorium remains well separated from the host cytoplasm. Thus, the pathogen may avoid attacks by the host's intracellular defense mechanisms. The only visible response of the host to the fungal attack is the encasement of the interaction area by appositional material. Apparently, *A. callista* successfully balances transferring nutrients from the host and avoiding host defense responses.

In *A. callista* an apoplastic Ia canal, extending from the trunk of the Ia through the epidermal cell wall,

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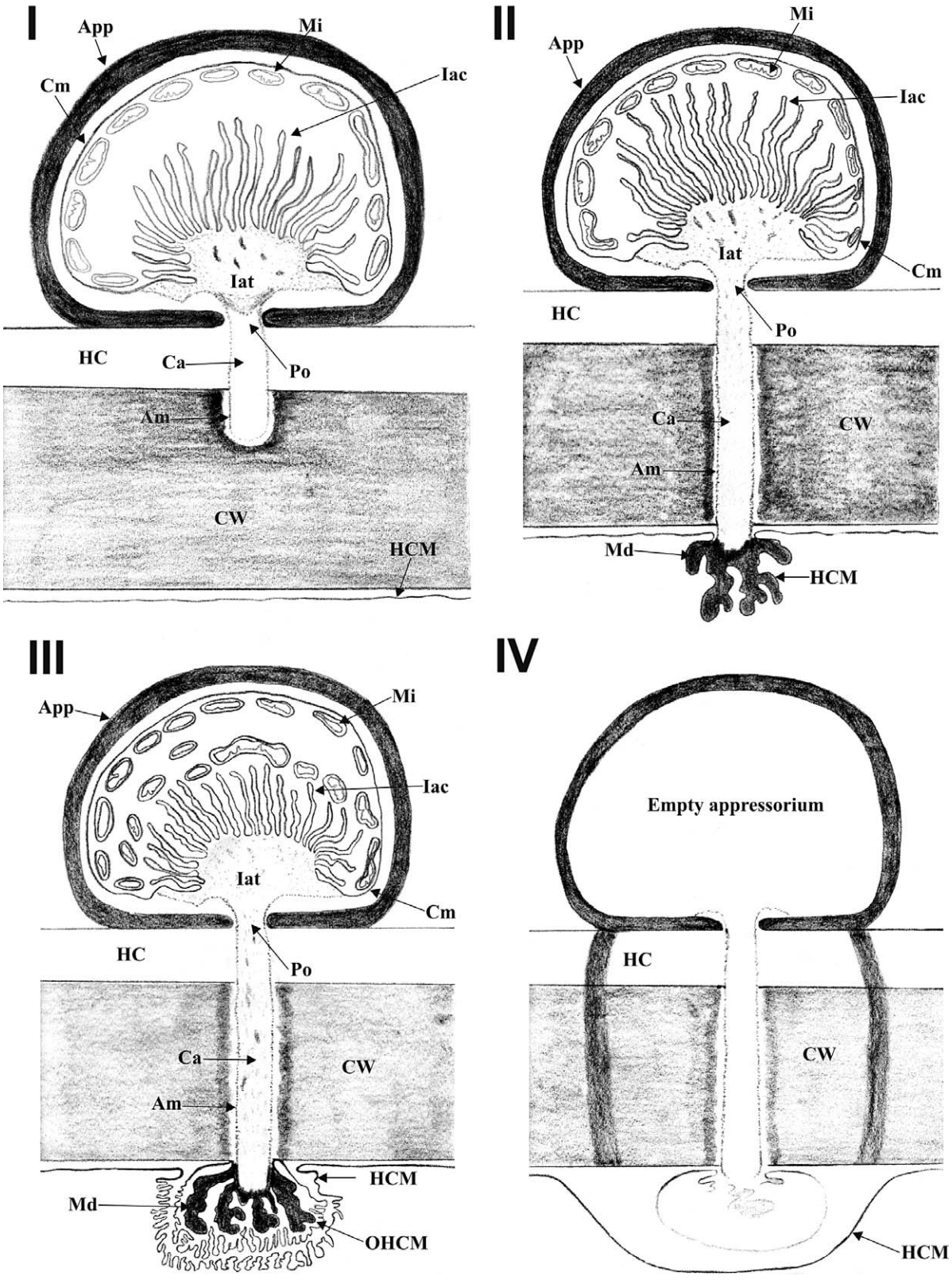
FIG. 5. Synthesized scheme resulting from analyses of serial sections. Four different steps in the cellular interaction between *A. callista* and *S. mutabilis* var. *violacea* from Costa Rica are recognized: (I) formation of interaction apparatus and canal, (II) deposition of material at the host cytoplasmic membrane, (III) encasement of the infection area and (IV)




degeneration. Abbreviations: Am = margin of the apoplastic compartment of the Ia; App = appressorium; Aw = appressoria wall; Ca = canal; Cm = fungal cytoplasmic membrane; CW = host cell wall; HC = host cuticle; HCM = host cytoplasmic membrane; Ia = interaction apparatus; Iac = cisterna net of the Ia; Iat = trunk of the Ia; Md = deposition of presumed fungal material; Mi = mitochondria; OHCM = original host cytoplasmic membrane; Po = penetration pore.

links the Ia and the host cytoplasmic membrane. This Ia canal may be analogous to the tubes connecting the Ia and the host cytoplasm in *C. trifolii* and the Exobasidiales. In *Cymadothea* and the Exobasidiales, the host cell wall is chemically altered within the tubes (Bauer et al. 1997, 2001; Begerow et al. 2002; Simon et al. 2004). It is known at least for *Cymadothea* that within the tubes the pathogen degrades pectins but not cellulose or xyloglucan (Simon et al. 2005). In contrast, in *A. callista* it appears that within the canal the host cell wall is dissolved. Thus, the canal in *A. callista* can act perfectly as an intake tube for the nutrient transfer.

So-called concentric bodies have been documented by Griffiths and Greenwood (1972) in 43 species of lichens and two non-lichenized fungi. To our surprise, these bodies also have been observed in the vicinity of the Ia in *A. callista*, however their functional role in the cellular interaction remains unclear.

The apoplastic electron-opaque deposit created at the apex of the Ia canal and the host cytoplasmic membrane may be analogous to the host bubble reported by Simon et al. (2004) for *C. trifolii*, and similar interaction structures in the Exobasidiales (Bauer et al. 1995, 1997; Begerow et al. 2002). As discussed by Bauer et al. (1997) for the Exobasidiales in detail, these deposits may originate from exocytosed fungal material by transfer toward the host cell. The tubular electron-dense structure formed around the Ia-canal in *A. callista* within the epidermal cell wall might be equivalent to the haustorial neckband found in the rusts or to the Casparian strip of endodermis cells (Heath 1976), probably preventing the leakage of solutions that are transferred from the host to the parasite.

In conclusion, the interaction structure in *A. callista* resembles the Ia of *C. trifolii* and the Exobasidiales. At least, the interaction structures of the Exobasidiales are of considerable taxonomic value for delimiting genera and higher taxa within this order and types of cellular interaction represent the backbone of the new system of the Ustilaginomycotina in general (Bauer et al. 1997, 2001; Begerow et al. 2006). Because little is known regarding the cellular interactions of the Meliolales (Luttrell 1979, Mueller et al. 2001), one of our future projects is to examine other species of this order to test whether these interaction structures also can be used as systematic markers for specific groups within the Meliolales.

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