ULTRASTRUCTURE OF THERMOPHILIC FUNGI
V. CONIDIAL ONTOGENY IN HUMICOLA GRISEA VAR. THERMOIEDEA AND H. INSOLENS

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It is proposed that *Humicola grisea* var. *thermoidea* is a synonym of *Humicola insolens*. *H. insolens* is characterized by the development of two distinct spore types, Type 1 conidia representing typical aleuriospores, and Type 2 conidia representing typical chlamydospores. Conidial ontogeny demonstrates a developmental plasticity not seen in other species, e.g. Type 1 conidia are produced both holoblastically and holothallically.

Cooney & Emerson (1964) described five thermophilic hyphomycetes: *Humicola grisea* var. *thermoidea* Cooney & Emerson, *H. insolens* Cooney & Emerson, *H. lanuginosa* (Griffiths & Maublanc) Bunce, *H. stellata* Bunce, and *Torula thermophila* Cooney & Emerson. *Humicola lanuginosa* and *H. stellata* possess characteristic terminal conidia formed on short lateral conidiophores. Both these species are taxonomically distinct and have since been transferred to the genus *Thermomyces* (Ellis, 1981c). *Torula thermophila* produces characteristic thick-walled chlamydospores (Ellis & Griffiths, 1976) and has recently been reclassified by Austwick (1976) as *Scytalidium thermophilum* (Cooney & Emerson) Austwick.

The three thermophiles that form the *Humicola-Scytalidium* complex have often been confused in the literature because it is not easy to differentiate between them. All produce dark-coloured conidia singly and/or in chains. In a discussion of thermophilic fungi, Emerson (1968) stated that 'In *Humicola grisea* Traaen var. *thermoidea* Cooney and Emerson chlamydospores are, again, borne singly on short lateral aleuriophores, but they are always smooth-walled and lack any attachment piece. Instead, the spore nearly always shows a flattened apiculus where it was attached to the aleuriophore. Only very rarely are intercalary chlamydospores to be found in this species. *Humicola insolens* Cooney and Emerson besides bearing single terminal spores on short lateral aleuriophores, as in the three previous species, regularly produces intercalary chlamydospores singly, in pairs, or in short chains. Whether terminal or intercalary, the spores are smooth-walled and brown. Finally, in *Torula thermophila* Cooney and Emerson where the chlamydospores are again smooth and brown, virtually all spores are formed in longer or shorter chains which are usually intercalary but may be terminal.' From this discussion it is obvious that within the *Humicola-Scytalidium* complex the species described are quite similar and tend to intergrade: in fact, Awao & Otsuka (1974) described *H. insolens* as a probable synonym of *H. grisea* var. *thermoidea* and ecological considerations led Hedger (1975) to describe *S. thermophilum* as *H. insolens*.

It should be noted that when describing the spores of the above fungi many authors have added to the confusion by the indiscriminate use of the term chlamydospore and aleuriospore. All chlamydospores so far described in the literature are characterized by the presence of a secondary cell wall deposited internal to the original hyphal wall and the presence of hydrophobic substances within the secondary wall indicating that chlamydospores function primarily as organs of perpetuation rather than dissemination, a criterion Griffiths (1974a) considered to be basic for delimiting chlamydospores from other spores. In this respect chlamydospores differ from aleuriospores, which are clearly organs of dissemination, and which are characterized by the absence of a secondary cell wall (Griffiths, 1974b). However, the major distinction between these two spore types is a developmental one. The Kananaskis Conference (Kendrick, 1971) rejected the term aleuriospore and replaced it with holoblastic conidium. Therefore, as chlamydospores are produced by the structural modification of hyphal segments (Griffiths, 1974a) they are representatives of the thallic mode of conidiogenesis, whereas aleuriospores, being holoblastic conidia, are representatives of the blastic mode of conidiogenesis.

This paper reports ultrastructural details of conidial ontogeny of *H. grisea* var. *thermoidea* and
H. insolens. Special emphasis is given to speciation in the *Humicola-Scytalidium* complex.

**MATERIALS AND METHODS**

Cultures of *Humicola grisea* var. *thermoidea* (ATCC16453) and *H. insolens* (ATCC16454) were maintained in 9 cm polystyrene Petri dishes on yeast-starch agar (Emerson, 1941) and incubated at either 40 or 52 °C. To study successive stages in the development of the spores on each of the two species, portions of agar bearing sporulating mycelium were removed from the edge and centre of the colony and prepared for transmission and scanning electron microscopy as described previously (Ellis, 1981).

**RESULTS**

*Humicola grisea* var. *thermoidea*

Mature conidia were solitary, dark brown, single-celled, smooth-walled structures that were generally globose to sub-globose in shape, 6–(9)–11 μm, although atypical ovate, elliptical or pyriform conidia (6–8 × 7–11 μm) were also observed. Conidia were formed either singly at the apex of short lateral conidiophores, or intercalarily, usually singly, but also in chains of two, three or four cells. Ultrathin sections through conidia showed the presence of two structurally distinct spore types.

Type 1 conidia were found in both terminal and intercalary positions and were characterized by a cell wall, 0.4–0.6 μm wide, differentiated into an outer, melanized, electron-dense layer and an inner, hyaline, electron-transparent layer of equal thickness (Figs 1, 2). In old conidia the outer melanized, electron-dense layer was often extended to almost the entire width of the conidial wall (Fig. 3). Another distinctive feature of Type 1 conidia was the often peripheral arrangement of cytoplasmic organelles leaving a noticeably less dense central, coarsely granular, cytoplasmic matrix containing numerous lipid droplets (Fig. 3). The peripheral cytoplasm contained one or more lobed nuclei each with an electron-dense granular nucleolus, numerous ovate to elongate mitochondria with well defined cristae, endoplasmic reticulum and numerous ribosomes (Fig. 3).

Terminal Type 1 conidia arose after the holoblastic evagination of the electron-transparent, apical, cell wall region of a conidiophore to form a conidial initial (Fig. 5). Conidia were delimited from the conidiophore by transverse septa which were characterized by a simple pore capped by amorphous electron-dense material similar in appearance to the adjacent Woronin bodies (Fig. 7). At this stage the young conidia were bounded by a thin, electron-transparent, cell wall (Fig. 5) which, during its continued growth, became progressively melanized (Fig. 6) until the cell walls of the maturing conidia exhibited the two-layered electron-dense and electron-transparent morphology described earlier. At maturity conidia were subtended by conidiophore cells which had undergone cytolysis, presumably to aid in dehiscence of the conidia. Unfortunately, in the many sections examined, no developmental stages of intercalary Type 1 conidia were observed, however, as will be seen later, indications are that development may be thallic.

Type 2 conidia were also found in both terminal and intercalary positions and were characterized by a much thicker cell wall, 0.8–1.1 μm wide, consisting of a thin, electron-dense, outer layer and a much wider electron-transparent, inner layer, the outer region of which often became pigmented to varying degrees by the deposition of electron-dense, melanin-like granules (Fig. 8). The finely granular, electron-dense cytoplasm typical of Type 2 conidia contained the same array of organelles as those seen in Type 1 conidia. However, the following differences were noted: (a) the organelles did not generally occur around the periphery although there was a tendency for some mitochondrial accumulation there, presumably owing to greater availability of oxygen at the periphery; (b) there was almost a complete absence of lipid droplets; (c) the nuclei were more irregular in outline; (d) the mitochondria appeared to be more spherical, and (e) there was noticeably more endoplasmic reticulum present (Fig. 8).

*Humicola insolens*

Structurally, conidia of *H. insolens* were indistinguishable from those of *H. grisea* var. *thermoidea*. Typical Type 1 and Type 2 conidia were observed in both terminal and intercalary positions (Figs 9–12).

Terminal Type 1 conidia arose in an identical manner to those of *H. grisea* var. *thermoidea*; i.e. by the holoblastic evagination of a conidial initial from near the apex of a conidiophore (Fig. 13). Young conidia were subsequently delimited by the development of transverse septa and further enlargement and development produced typical Type 1 conidia exhibiting the characteristic two-layered cell wall morphology previously described (Figs 9, 10).

Intercalary Type 1 conidia arose after the swelling of short individual, hyphal segments, i.e. conidial development was holothallic (Fig. 14). Young conidia increased in volume until they became globose. The thin, electron-transparent
Fig. 1. Type 1 conidium of *H. grisea* var. *thermoidea*. Note the inner, electron-transparent layer and outer, melanized layer of the cell wall, nuclei (N) and mitochondria (M).

Fig. 2. Intercalary Type 1 conidium of *H. grisea* var. *thermoidea*. Note junctions (arrows) between the melanized cell wall of the conidium and the subtending hyphal strand (H).
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*Humicola* spp.
septa were traversed by a simple pore near which were Woronin bodies (Fig. 14). At this stage of development conidia were bounded by a thin, electron-transparent, cell wall analogous to the original hyphal cell wall. During subsequent development and maturation deposition of melanin occurred until the spore wall consisted of two distinct layers: an outer, electron-dense layer and an inner, electron-transparent layer (Figs 10, 15). All Type 1 conidia contained similar cytoplasmic organelles, i.e. one or more nuclei with conspicuous electron-dense nucleoli, many ovate to elongate mitochondria, small amounts of endoplasmic reticulum, numerous ribosomes and lipid droplets (Figs 4, 9, 10). Cytoplasmic organelles were often arranged peripherally leaving a central organelle-free, coarsely granular cytoplasmic matrix (Fig. 4).

Terminal and intercalary Type 2 conidia (Figs 11, 12) were structurally identical to those observed in H. grisea var. thermoidea. Note: (a) their characteristic thick cell walls differentiated into a thin, electron-dense, outer layer, and a much wider, electron-transparent, inner layer, the outer region of which often became pigmented by the accumulation of electron-dense melanin-like granules, and (b) their more finely granular cytoplasm, which contained the same array of organelles. Once again, no early developmental stages of Type 2 conidia were observed, but indications favour a thallic mode of development.

An ultrastructural feature of both H. grisea var. thermoidea and H. insolens, perhaps related to growth at high temperatures, was the presence of numerous cytoplasmic vacuoles containing electron-dense deposits in both hyphae and conidia grown at 52°C (Figs 4, 12). These dense body vacuoles were not observed in hyphae and conidia grown at 40°C (Figs 3, 8, 11). However, Type 1 conidia grown at 40°C did contain abundant neutral lipid droplets which were virtually absent in similar conidia grown at 52°C (Figs 3, 4).

**DISCUSSION**

Evidence is presented in this paper for the synonymy of *Humicola grisea* var. *thermoidea* and *H. insolens*. Firstly, the obvious morphological similarity between type strains representing *H. grisea* var. *thermoidea* and *H. insolens* indicates the possibility that one is dealing with the same fungus. In fact, ultrastructurally, conidia of these two species are indistinguishable. Secondly, when comparing a large number of isolates, as was done by Ellis (1981a) for Australian isolates and by Awao & Otsuka (1974) for Japanese isolates, one observes a marked gradation and overlapping in the ability of isolates to produce intercalary conidia, the primary character for the separation of the two species (Emerson, 1968). This finding prompted Awao & Otsuka (1974) to describe *H. insolens* as a probable synonym of *H. grisea* var. *thermoidea*. Thirdly, the cultural differences that do occur between isolates are probably no greater than that normally expected among different strains of a single species. In this respect it is significant that Bertoldi, Lepidi & Nuti (1973) found that different isolates of *H. grisea* var. *thermoidea* had a difference in GC content of up to 13%, which was more than the variation found between all four species of the genus *Thermomyces* (10%). Further, on the basis of GC%, *Humicola grisea* Traaen was not closely related to its varietal form, *H. grisea* var. *thermoidea* (Bertoldi et al., 1973). Thus it appears that *H. grisea* var. *thermoidea* is a separate taxon which shows considerable genetic variation between strains, and the following nomenclatural changes are proposed:


This system was chosen (1) because of the thermophilic nature of the species, (2) to separate the species from *H. grisea*, and (3) to allow for differentiation between the extreme ranges of this species at the variety level if necessary (although the author doubts the merits of such a taxonomic distinction).

Recently, Subrahmanyam (1980) has described a new thermophilic mould as *Humicola grisea* var. *indica*. However, his description clearly falls within the species range of *H. insolens* and the present author doubts the acceptability of this taxon.

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Fig. 3. Type 1 conidium of *H. grisea* var. *thermoidea* grown at 40°C. Note melanized cell wall, peripheral arrangement of nuclei (N), central lipid droplets (L) and Woronin body at the conidiophore attachment point (arrow).

Fig. 4. Type 1 conidium of *H. insolens* grown at 52°C. Note nucleus (N), mitochondria (M) and dense body vacuoles (V).
Fig. 5. Young terminal Type 1 conidium (CON) in *H. grisea* var. *thermoidea* showing thin unmelanized cell wall. Note cell wall of a nearby Type 2 conidium.

Fig. 6. Young Type 1 conidium of *H. grisea* var. *thermoidea* showing cell wall differentiation into an outer, electron-dense melanized layer and an inner, electron-transparent layer.

Fig. 7. Delimiting septum and central pore complex (arrow) between a mature terminal Type 1 conidium and subtending conidiophore in *H. grisea* var. *thermoidea*. 
Fig. 8. Type 2 conidium of *H. grisea* var. *thermoidea* grown at 40°. Note thick cell wall with a thin, electron-dense, outer layer (arrow), nuclei (N), mitochondria (M) and endoplasmic reticulum (ER).

*Humicola insolens* was characterized by the development of two types of conidia. Both types were found in terminal and intercalary positions and externally they were of similar shape, size and texture; i.e. neither light microscopy or scanning electron microscopy could distinguish the different conidial types. Further, it must be stressed that Type 1 and Type 2 conidia were morphologically distinct from one another and that they did not represent different developmental stages of a common conidial form.

Type 1 conidia were similar to the aleuriospores (holoblastic conidia) observed by Griffiths (1974b) in *H. grisea*, especially in their cell wall structure and in distribution and appearance of cytoplasmic organelles. The ontogeny of Type 1 conidia demonstrates the kind of developmental plasticity that is characteristic of this species. Terminal Type 1 conidia were formed holoblastically in an identical manner to those of *H. grisea* (Griffiths, 1974b). However, intercalary Type 1 conidia were formed holothallically after the compartmentalization of a hyphal strand. This variation in the kind of conidiogenesis from a holothallic mode to a holoblastic mode of one conidial type represents an anomalous situation previously observed only in imperfect yeasts (Cole, 1975).

On the other hand, all Type 2 conidia, regardless of their position, possessed a characteristic thick cell wall similar to that seen in the chlamydospores of *Scytalidium thermophilum* (Ellis & Griffiths, 1976). According to the criteria used by Griffiths (1974a) to distinguish between aleuriospores and chlamydospores, Type 1 conidia of *H. insolens* would represent typical aleuriospores while Type 2 conidia would represent typical chlamydospores. However, as pointed out before, the Kananaskis Conference (Kendrick, 1971)
Fig. 9. Type 1 conidium of *H. insolens*. Note the inner, electron-transparent layer and the outer, melanized, electron-dense layer of the cell wall, nuclei (N) and mitochondria (M).

Fig. 10. Intercalary Type 1 conidium of *H. insolens* grown at 52°. Note distinctive melanization of the outer layer of the cell wall, junction (arrows) of the melanized conidial cell wall with the subtending hyphal strand (H), nuclei (N) and dense body vacuole (V).
Fig. 11. Type 2 conidium of *H. insolens* grown at 40°. Note thick cell wall with distinctive, thin, electron-dense, outer layer (arrow), peripheral arrangement of mitochondria (M) and endoplasmic reticulum (ER).

Fig. 12. Mature Type 2 conidium of *H. insolens* grown at 52°. Note thick cell wall, nucleus (N), mitochondria (M) and dense body vacuoles (V).
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rejected the term aleuriospore and replaced it with holoblastic conidium. Clearly, Type 1 conidia of *H. insolens* cannot be classified solely as holoblastic conidia.

Although Type 1 conidia cannot be satisfactorily classified by means of developmental ontogeny they are clearly organs of dissemination (Griffiths, 1974a) and as such will be termed simply as conidia in the present study. It should be noted that in most isolates of *H. insolens* there is a predominance of Type 1 conidia which results in cultures appearing jet black in colour. However, some cultures were light in colour, this being due to the predominance of the less pigmented Type 2 conidia.

In conclusion, the present study has resolved many of the problems associated with the *Humicola-Scytalidium* complex. It is suggested that all isolates belonging to this complex can now be placed into two distinct species: *Humicola insolens* Cooney & Emerson and *Scytalidium thermophilum* (Cooney & Emerson) Austwick.

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**REFERENCES**


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Fig. 13. Holoblastic conidiogenesis of Type 1 conidium of *H. insolens*. Note thin, single-layered cell wall (arrow), nuclei (N) and endoplasmic reticulum (ER).

Fig. 14. Holothallic conidiogenesis of Type 1 conidium in *H. insolens*. Note thin, electron-transparent, cell wall (arrow), delimiting septa with central pore and associated Woronin body (W), mitochondria (M) and subtending hyphal strand (H).

Fig. 15. Young intercalary Type 1 conidium of *H. insolens*. Note distinctive melanization of the outer layer of the cell wall (arrow).