

## THE ASCOCARPS OF ASPERGILLUS ALLIACEUS

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(WITH 11 FIGURES)

*Aspergillus alliaceus*, described in 1926 by Thom and Church (4) as a new sclerotial species in the *A. wentii* Group, was based upon two strains: C.T. 4660, isolated by Eddy from a decaying garlic bulb sometime prior to 1924 when it was discussed without name by Walker and Lindgren (6) as a wound parasite of onions; and C.T. 4656, isolated from a dead blister beetle (*Macrobasis albida*) by Cohen and High of the Bureau of Chemistry and submitted to Thom for identification in 1922. Thom and Church recognized that *A. alliaceus*, as exemplified by these strains, might develop a perfect stage, and in their original description reported unsuccessful attempts "to induce the development of an ascogenous phase in these sclerotia whose appearance is so suggestive of perithecia."

The discovery by Raper, Fennell, and Tresner (3) of an ascosporic stage in the sclerotoid parenchymatous structures of *Aspergillus citri-sporus* and *A. ornatus*, members of the "sclerotial" *A. tamarii* section of the genus, further suggested that the development of a perfect stage by other sclerotium-producing species of *Aspergillus* could be anticipated. Although careful examination of sclerotia from numerous cultures of the *A. wentii* and *A. flavus* Groups over a period of many years had failed to disclose a single ascosporic strain, these authors recognized the possibility of their existence and concluded that "extension of our search for additional forms, and the introduction of new procedures for isolating them, may in time justify our optimistic hope."

During an investigation of the fungous flora of Australian soils by one of us (J. H. W.), many sclerotial species of *Aspergillus* were isolated. All such isolates were held for extended periods and, following publication of the description of *A. ornatus*, these sclerotial forms were re-examined for the presence of a perfect stage. The sclerotia of two isolates, SA 15 and SA 117, in cultures about a year old were discovered to be full of spores. In order to determine their nature, new cultures were prepared and examined at regular intervals; after three months,

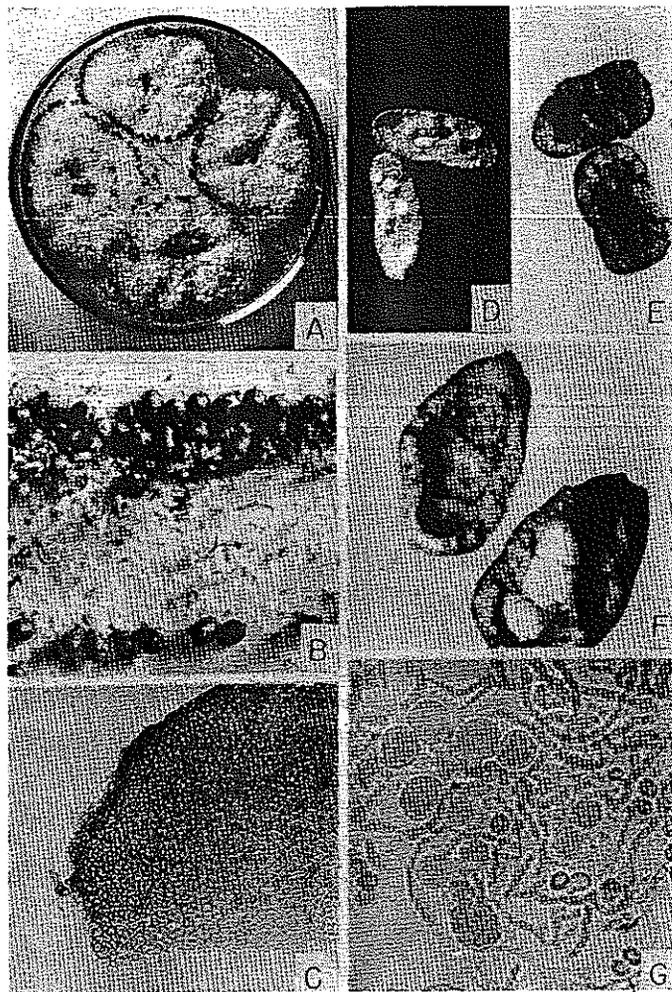


FIG. 1. A-G.

FIG. 1. *Aspergillus alliaceus* Thom and Church. A. Colonies developed from single ascospores of QM 1892, steep agar, 10 days. B. Stromatic bodies of QM 1892,  $\times 5$ . C. Hand-section of stromatic body of QM 1892, showing internal sclerenchymatous tissue as it appears just prior to the appearance of recognizable

asci and ascospores were seen in SA 117 and somewhat later in SA 15. The latter strain, although it continues to produce abundant sclerotia, has failed to develop ascospores in more recent tests.

The two isolates, SA 15 and SA 117, were forwarded in September 1954, to K. B. Raper, University of Wisconsin, for examination and for his opinion regarding the possibility that they represented a new species. Raper immediately recognized their cultural similarity to *Aspergillus alliaceus*, and microscopic examination of the conidial structures verified this relationship. However, the isolates from Australia consistently produced larger and more elongate sclerotia than those of the type strains studied by Thom and Church, (4).

The two ascosporic strains from Australia were then sent to the Quartermaster Laboratories, where they were grown in comparative culture with one of the co-types of *Aspergillus alliaceus* (C.T. 4656; QM 1885). Once again SA 117 produced ascospores at three months and SA 15 failed to do so at any age up to two years. The representative strain of *A. alliaceus*, examined at lengthening intervals, finally was observed to produce the ascigerous stage at ten months, although it had been regarded as a strictly sclerotial culture for thirty years. Cleistothecia, asci, and ascospores of the Thom strain and of those from Australia are identical.

The discovery of a delayed ascogenous stage in the presumed "sclerotia" of *Aspergillus alliaceus* necessitates an enlargement of the original diagnosis and the following emended description is proposed:

*ASPERGILLUS ALLIACEUS* Thom and Church emend.

Colonies on Czapek's-solution agar growing rapidly, 6.0-7.0 cm in ten days at 25° C, composed of a compact, white basal felt with loosely floccose aerial mycelium about 1 mm deep, developing abundant silvery gray to black "sclerotia" in more or less concentric zones, producing a limited number of upright conidiophores bearing radiate conidial heads which rarely affect the colony appearance; no characteristic odor noted; exudate fairly abundant, clear, collecting in droplets on the sclerotia, upon evaporation leaving slight depressions or circular areas of slightly different color (FIG. 1, B); reverse white to cream to yellow with the black sclerotia easily visible through the mycelium. Conidial heads

cleistothecia,  $\times 110$ . D. Stromatic body from QM 1892 laid open to show four randomly situated immature cleistothecia,  $\times 7.5$ . E. Stromatic body from QM 1885 showing a single cleistothecium,  $\times 25$ . F. Stromatic body from QM 1892 containing three mature cleistothecia, somewhat shrunken during photographing. Their imprint is shown plainly in the empty half on the left,  $\times 25$ . G. Fertile hyphae, asci, and ascospores of QM 1892,  $\times 560$ .

radiate, splitting into divergent columns in age, at first yellow, becoming dull golden brown to buff, variable in size, 100–1000  $\mu$  in diameter, smaller heads borne on short conidiophores from aerial mycelium, larger structures on long conidiophores from substrate hyphae; conidiophores smooth, yellow, sinuous, variable in size, 0.2–3 mm  $\times$  7.5–15.0  $\mu$ , with walls up to 2.0  $\mu$  thick; vesicles globose, thick-walled and showing

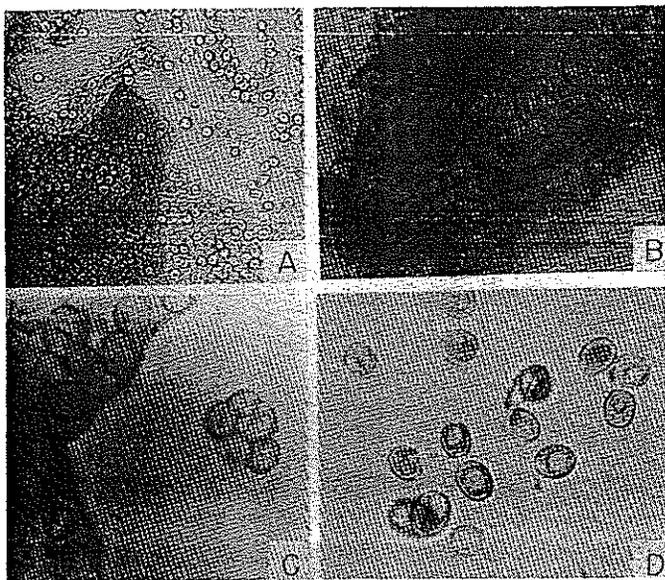


FIG. 2. *Aspergillus alliaceus* Thom and Church. A. Fragment of cleistothecial wall,  $\times$  245. B. As A, in greater detail, showing flattened, irregular, elongate cells composing the cleistothecial walls,  $\times$  1000. C. A single ascus and ascospores visible through the extremely thin wall of a completely mature cleistothecium, from QM 1885,  $\times$  1025. D. Mature ascospores of QM 1885, showing eccentric oil droplets and equatorial lines,  $\times$  1025.

pores after the sterigmata are detached, fertile over their entire surface, variable in size, 15–75  $\mu$  in diameter but mostly 50–60  $\mu$ ; sterigmata in two series, extremely crowded, primaries 7–13  $\times$  3–5  $\mu$ , each bearing 6 to 8 secondary sterigmata; secondaries mostly 9.0–10.0  $\times$  1.5–2.2  $\mu$  but varying from 7.0–13.0  $\mu$  in length, with sides usually parallel but sometimes inflated with age; conidia smooth-walled, yellow, oval to subglobose, 3.0–4.0  $\times$  2.5–3.5  $\mu$ . Sclerotia erect, sessile or borne on a suggestion of a stalk, horny, ovate to elliptical, 1–3 mm in vertical axis by

500–700  $\mu$  in diameter, at first white, then gray-black and white-tipped, finally becoming black in age, composed of greenish, thick-walled, sclerenchymatous tissue throughout (FIG. 1, C). Initiation of the ascogenous phase has not been observed but may be evidenced by the “channelling” of the stromatic tissue (FIG. 1, C) which precedes the appearance of independent ascocarps at one to seven or eight loci randomly scattered throughout the tissue (FIG. 1, D and E), the number of these locules appearing to be directly correlated with the size of the stromatic body. Cleistothecia (FIG. 1, F) globose, variable in size, consisting of ascogenous hyphae, asci, and ascospores (FIG. 1, G) surrounded by thin sheath-like membranes composed of single layers of irregular, flattened cells (FIG. 2, A and B); maturing extremely slowly, requiring from three to ten months depending upon the strain; at maturity completely filling the stromatic body except for a tough outer wall usually 150–200  $\mu$  thick (FIG. 1, F). In age the thin walls of the separate cleistothecia rupture and the entire cavity is filled with free ascospores; viewed at this stage the stromatic bodies appear to be single heavy-walled ascocarps. Asci (FIGS. 1, G; 2, C) 8-spored, oval to globose, 15–18  $\mu$  in diameter, evanescent after maturation of the ascospores; ascospores (FIG. 2, C and D) uncolored, smooth, thin-walled, elliptical, variable in size, mostly 5.5–9.0  $\times$  5.0–7.0  $\mu$ , with younger spores showing a fine equatorial furrow that becomes less evident in age; mature spores containing an eccentric oil droplet. Ascospore germination commences in the equatorial region but in most instances is preceded by simple swelling rather than by the valvular separation that is characteristic of ascospore germination in other ascigerous *Aspergilli*. Colonies developing from single ascospores (FIG. 1, A) are entirely typical of the parent strains.

#### Cultures examined:

- SA 15 (QM 1891)—Isolated by J. H. Warcup, 1952, from soil (red-brown earth), Waite Institute, Adelaide, S. Australia.  
 SA 117 (QM 1892)—Isolated by J. H. Warcup from a sandy loam soil from Mt. Riddock, central Australia.  
 SA 122—Source as SA 15.  
 C.T. 4656 (QM 1885)—Isolated by Cohen and High from a dead blister beetle (*Macrobasis albida*) at the Pharmacognosy Laboratory of the Bureau of Chemistry, Washington, D. C.

#### DISCUSSION

In the belief that the members of the genus *Aspergillus* (including those species which develop a perfect stage) form a natural and cohesive entity, Thom and Raper (5) recognized three ascosporic sections: the *A. glaucus*, the *A. fumigatus*, and the *A. nidulans* Groups; and, as in

earlier publications, they reduced to synonymy other names of generic rank which had been used for these groups. With the subsequent description of the ascigerous stages of *A. ornatus* and *A. citrisporus* (3), the *A. tamaritii* section was recognized as a fourth ascospore group. More recently, Benjamin (1) has advocated the re-establishment of the genera *Eurotium*, *Sartorya*, and *Emericella* to cover the first three of these ascospore sections but has failed to provide a suitable disposition of *A. ornatus* and *A. citrisporus*. The ascospore stage of *A. alliaceus*, described in the present paper, differs so markedly from that known in any other *Aspergillus* that its placement in one of the previously known ascigerous sections of the genus is untenable. It is likewise impossible to place this species in either *Eurotium*, *Sartorya*, or *Emericella* and, if one followed this system, it would be necessary to establish not only a fourth genus to cover *A. alliaceus* but also a fifth to provide for *A. ornatus* and *A. citrisporus*. Since we believe, with Thom and Raper (5) and Raper (2), that such fragmentation of the genus *Aspergillus* is undesirable, we prefer simply to recognize that a fifth major section, the *Aspergillus wentii* Group, has been added to the list of ascigerous *Aspergilli*.

In all of the ascospore species thus far recognized in *Aspergillus* the cleistothecia show a marked degree of similarity in the structure of their walls, which at maturity consist of a thin (usually single) layer of flattened cells. In members of the *A. glaucus* Group these cells generally are unobscured by enveloping hyphae and the yellow cleistothecia appear naked. In *A. fischeri* the ascocarp initially appears to have a cottony consistency and one gains the impression of a truly fibrous outer wall; at maturity, however, most of this outer covering can be removed, revealing the thin wall of cells to which a portion of the enveloping hyphae still may be attached. In the *Aspergillus nidulans* Group the cleistothecial wall appears somewhat fibrous in the early stages but at maturity consists of a thin layer of flattened angular cells. Cleistothecia in this group are characterized particularly by a loose envelope of thick-walled hülle cells. In *A. ornatus* the cleistothecium initially suggests a developing sclerotium and when broken open is seen to consist of a mass of angular parenchymatous cells. With further development, cells in the central area of the ascocarp are digested by the developing ascogenous tissue and finally a thin outer layer of flattened polygonal cells remains. In *A. alliaceus* the multiple cleistothecia develop within a persistent, indurate mass of sclerenchymatous tissue. Considered as individual structures, however, the ascocarps are not basically different from those in the groups previously studied. Here also the outer covering of a

mature cleistothecium consists of a thin layer of irregularly elongate, flattened cells.

## SUMMARY

The description of *Aspergillus alliaceus* Thom and Church, a member of the *A. wentii* Group, is emended to include a perfect stage which differs from all other ascospore *Aspergilli*. Multiple discrete thin-walled cleistothecia develop within a sclerotoid stromatic body which retains a heavy outer wall even when the ascocarps are completely mature.

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