ASPERGILLUS ROBUSTUS, A NEW SPECIES IN THE *A. OCHRACEUS* GROUP

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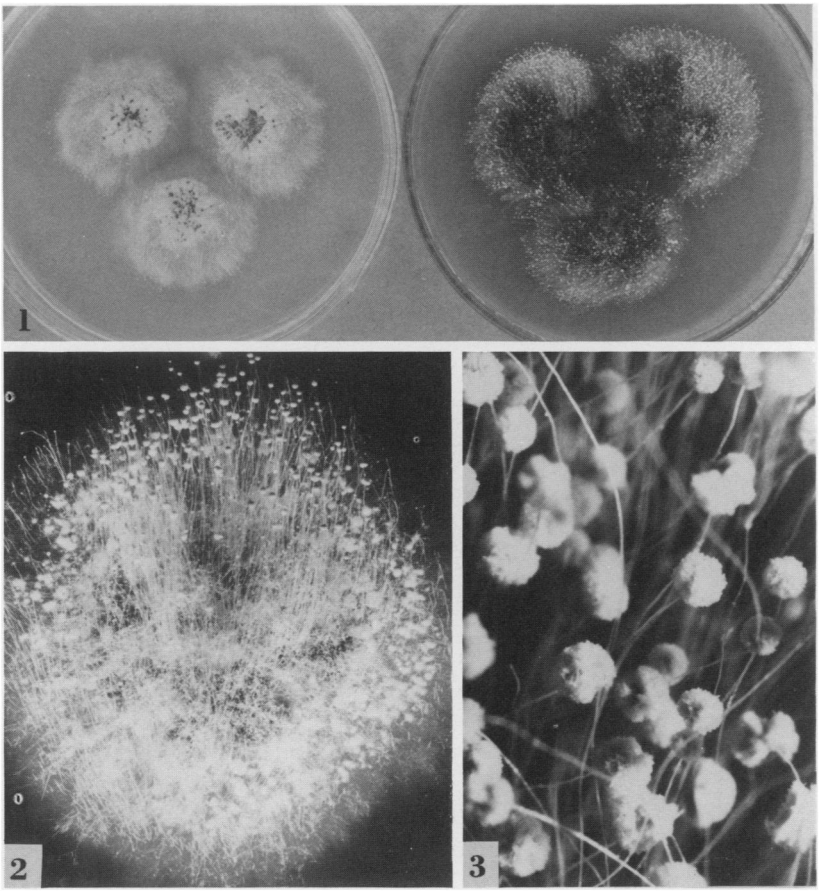
The species herein described was isolated in 1966 from native forest soils collected that same year at two sites in Kenya and Tanzania. It is characterized particularly by its tall conidiophores (commonly 4 mm, rarely 8–9 mm) bearing yellow-buff radiate heads and its irregular black sclerotia, 250–900 μm in diam. The following description is drawn primarily from the type culture, WB 5286.

Aspergillus robustus Christensen & Raper, sp. nov. FIGS. 1–9

Coloniae in agaro Czapekii 3–4 cm diam quatuordecim diebus 24–26 C crescentes; capitula conidica ochroleuca (**4**, XXX) vel lutea (**4**, XVI); facies reversa lutea-aurantiaca (**4**, III) deinde roseo-fusca vel umbrina (**4**, XXIX); sclerotia nigra, abundantia, forma enormi, 250–900 μm diam, non crescunt per tempus. Capitula conidica radiantia, plerumque 250–750 μm diam, biseriata; conidiophorae plerumque 750–5,000(–8,000) μm × 10–21 μm, vertentes ad lucem, succineae ad apices, membranis 2.5–4.5(–7.0) μm crassis, enodes vel subasperae; vesiculae globosae vel subelongatae, 35–90 μm diam et prope 40–60 × 34–45 μm, membranis crassis; metulae elongatae, ad tempus conidiogenesis latiores et saepe septatae, 11–21 × 4.5–7.0 μm; phialides 9.0–12.6 × 3.4–4.5 μm; conidia eleganter rugosa, ellipsoidea, plerumque 3.5–4.5 × 2.8–3.4 μm.

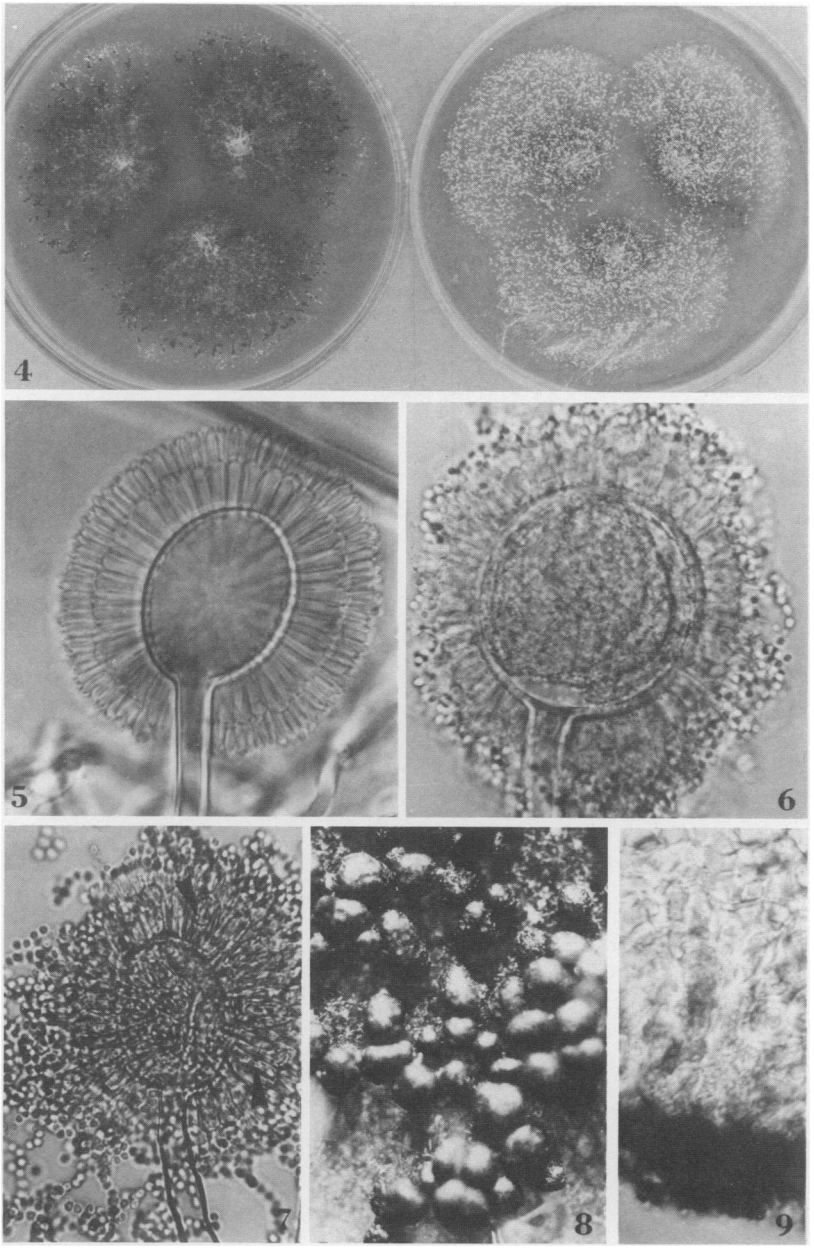
Typica cultura: WB 5286, in anno 1966 collecta et isolata e solo silvae acacialis in colle mensiformi prope Mombasa, Kenya, Africa; in herbario NY deposita.

Colonies on Czapek's solution agar attaining diam of 3–4 cm in 14 da at 24–26 C, substrate mycelium thin, mostly submerged, somewhat raised and buckled centrally with dense development of black sclerotia on the substrate surface beneath the much taller-growing conidiophores (FIGS. 1–2); exudate clear to pale amber in droplets on conidiophore bases and sclerotia; reverse antimony yellow to ochraceous orange (**4**, XV) or orange (**4**, III) with surrounding agar yellow-tinged at 7–10 da shading to pinkish buff, pinkish cinnamon and sepia (**4**, XXIX) in age, green brown to dark brown beneath sclerotia; odor faint, musty. *Conidial heads* radiate (FIG. 3) splitting into columns in age, cream buff (**4**, XXX) to naples yellow and mustard yellow (**4**, XVI), 250–



FIGS. 1–3. *Aspergillus robustus* WB 5286. 1. Colonies on Czapek and malt agars, respectively, grown at 24–26 C for 14 da. 2. Positively phototropic conidiophores, on Czapek agar, incubation at 24–26 C for 6 da. × 3.8. 3. Characteristic radiate, cream-buff conidial heads borne on tall-growing conidiophores. × 20.

750 μm in diam, biseriate; *conidiophores* smooth to very slightly roughened, upper portion brownish or becoming faint amber in age, uniform in diam or somewhat narrowed near the base, mostly 750–5,000 × 10–21 μm, rarely to 8,000 μm, positively phototropic (FIG. 2), wall commonly 2.5–4.5 μm thick to 6.0–7.0 μm near the base; *vesicles* globose to somewhat elongate (FIGS. 5–7), thick walled, fertile over the entire surface, often brown tinged especially in age, variable in size, when globose 35–90 μm in diam, mostly 40–70 μm, or elongate 34 × 30 μm to 63 × 57 μm mostly 40–60 × 34–45 μm; *metulae* elongating and widen-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ing in early stages of conidium formation and frequently becoming septate (FIG. 7), finally nearly twice the length of the phialides, 11–21 × 4.5–7.0 μm; *phialides* 9.0–12.6 × 3.4–4.5 μm; *conidia* delicately echinulate, thin walled, ellipsoidal, mostly 3.5–4.5 × 2.8–3.4 μm, separated by prominent connectives. *Diminutive conidiophores,* mostly 50–300 μm long, common in Czapek's and malt extract agar cultures from apparently thigmotactic trailing hyphae which coil around larger conidiophores; biseriate, conidia as described, vesicles and appendages reduced in size. *Sclerotia* irregular in shape (FIG. 8), determinate, about 250–900 μm mostly 400–700 μm in longest dimension, first white and rapidly shading to yellow brown then black, enveloped in a closely adherent sparse weft of brown vegetative hyphae and in young cultures commonly with pale amber exudate; clusters of sclerotia in old cultures apparently resulting from continuous development adjacent to existing sclerotia; constructed of sclerenchyma-like isodiametric cells 10–16 μm in diam, surface two to three layers of cells dark pigmented, interior cells hyaline (FIG. 9); no evidence of asci through 18 wk on Czapek's and malt extract agars.

Colonies on malt extract agar 3.5–4.0 cm in diam in 14 da at 24–26 C; surface characterized by very tall phototropic conidiophores arising from a thin substrate mycelium with sclerotia prominent centrally and subcentrally; sclerotium production enhanced by dark incubation (FIG. 4). Conidiophores only sparsely formed in the dark at room temperature, mostly less than 3.5 mm tall. Colony reverse finally near mars brown to mummy brown (**4**, XV), greenish black beneath sclerotia, with limited production and diffusion of pigment; odor not pronounced. Conidiophores on malt extract agar somewhat larger than on Czapek's agar, vesicles 46–95 μm in diam mostly 50–80 μm or near 60 × 48 μm when elongate and conidiophores up to 9 mm, otherwise with conidial structures equivalent to those described above.

Growth on Czapek's agar at both 30 C and 12 C slower than at 24–26 C; colony predominately sclerotial with conidiophores few in number and short after 3 wk at 30 C, entirely conidial at 12 C.

The WB 5286 isolate of *A. robustus* was isolated from surface soil of a thorn forest situated on a plateau above Mombasa, Kenya. Two

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FIGS. 4–9. *Aspergillus robustus* WB 5286. 4. Colonies on malt agar, 4 wk old, grown under dark and light conditions, respectively, at 24–26 C; showing dark-incubation enhancement of sclerotium development and repression of conidial structure formation. 5. Very young elongate vesicle, with radiately arranged, crowded metulae. × 535. 6. Globose vesicle and thick-walled conidiophore. × 410. 7. Older head, with enlarged and occasionally once-septate metulae. × 410. 8. Sclerotia on Czapek agar in surface view to show the characteristic irregular form of these black, determinate structures. × 15. 9. A portion of a sclerotium in cross section, showing thick-walled, hyaline interior cells. × 425.

additional isolates, WB 5369 and WB 5370, were obtained from native forest soils collected at Lake Manyara in the Rift Valley area of Tanzania. WB 5369 and WB 5370 are very similar culturally and morphologically to WB 5286, differing only in relative abundance of conidiophores and sclerotia and intensity of amber color in the upper portion of the conidiophore. In WB 5370, for example, young as well as older conidiophore apices are conspicuously amber-tinged.

The HOLOTYPE for the species, a dried plate culture of WB 5286, has been placed in the Cryptogamic Herbarium of The New York Botanical Garden. Living cultures of *A. robustus* WB 5286 are on deposit in the American Type Culture Collection, Rockville, Maryland (ATCC 36106), at the Commonwealth Mycological Institute, Kew, Surrey, England (IMI 216612), and with the Centraalbureau voor Schimmelcultures, Baarn, Netherlands (CBS 428.77).

Aspergillus robustus is clearly related to species in the *A. ochraceus* group as defined by Raper and Fennell (3). Whereas the combination of features including tall conidiophores, relatively long and occasionally septate metulae, globose to subglobose vesicles and globose to radiate heads, sclerotia, and secondary production of diminutive heads is exhibited by species in three sections of the genus (*A. candidus*, *A. niger* and *A. ochraceus* groups), the form of the vesicle as well as the color of the conidial heads, and the pattern, structure and color of the sclerotia all indicate a primary alliance with the nine taxa comprising Raper and Fennell's *A. ochraceus* group. Within that group, *A. robustus* resembles *A. alliaceus* Thom in conidiophore and vesicle dimensions and in its production of black sclerotia, but differs from that species in sclerotium form and in color of interior cells, and in head color, metula size (7–13 μm in *A. alliaceus* versus 11–21 μm in *A. robustus*), vesicle shape, and conidium size and shape. The sclerotia in *A. alliaceus* are silvery gray to black, white tipped, vertically elongate, and macroscopically are more uniform in shape and size (500–700 μm diam) than are those in *A. robustus*, the sclerotia of which are characteristically irregular in form and size (250–900 μm diam), lack white tips, and are covered with a closely adherent sparse weft of brown hyphae. Additionally, the interior tissue in *A. alliaceus* sclerotia is greenish and develops cleistothecia tardily whereas in *A. robustus* interior sclerenchymatous cells are hyaline and the tissue is sterile even after 4 mo.

Considering the two *A. ochraceus* group members described since 1965, *Aspergillus dimorphicus* Mehrotra & Prasad (2) differs from *A. robustus* in its much smaller dimensions, occasionally branched

conidiophores and absence of sclerotia; like *A. robustus* and *A. alliaceus,* it commonly develops secondary diminutive conidiophores. *Aspergillus lanosus* Kamal & Bhargava (1) resembles *A. robustus* in its formation of very tall conidiophores (to 5 mm) and somewhat elongate vesicles, but differs in the absence of sclerotia, has relatively short metulae, has small, smooth, globose conidia, and rarely forms branched conidiophores. It is of interest that all known isolations of *A. robustus* and *A. lanosus* have been from forest soils in Africa and India respectively.

Dr. James C. Cavender collected the African soils which have yielded *A. robustus* and Dr. Dorothy B. Prest obtained all isolates of the new species while at the University of Wisconsin in 1966; we gratefully acknowledge their contributions to this study. Miss Dorothy I. Fennell kindly examined WB 5286 and confirmed our interpretation of its taxonomic status and Dr. Herbert M. Howe, Miss Ann C. Worley, Miss Paula Simms and Mr. Richard Heinzen have helped prepare the Latin diagnosis and illustrations. The senior author expresses sincere appreciation to the College of Agricultural and Life Sciences and the Graduate School, University of Wisconsin-Madison, for the funds, provided under a Department of Bacteriology Visiting Professorship, which have supported this study.

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